Geoffrey I. Hackett • Michael Kirby

Testosterone in cardiometabolic and other diseases

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Dedication

This book is dedicated to Dr. Graham Jackson, who truly believed that bettering the human condition is the greatest good that any individual can achieve, we hope that this book will follow in that tradition.

Two interests took precedence over all others; his family and his medical practice. Graham's warm, personal touch and intelligence, seemed to be coupled with an inexhaustible supply of energy and enthusiasm, that made him a superb teacher and physician.

He seemed to find 36 hours in each of his days, and for his colleagues and patients, there was a limitless amount of time. He was a mentor for other doctors and a role model of both compassion and propriety.

He was a real pioneer, particularly in the area of erectile dysfunction, demonstrating the pathophysiological links with atherosclerosis and cardiovascular disease.

He was unusual as a cardiologist, in appreciating the importance of healthy sexual function.

Discussion of this taboo subject was enabled for his patients by his ability to listen and ask, the motto being 'If you don't ask, patients don't tell'.

The key that turned the lock was the discovery of Viagra. Remarkably, although the drug modestly improved blood flow through the coronary arteries, it had a dramatic effect on erectile function, which opened up a whole new field of sexual medicine in the context of heart disease.

The men in the early trials apparently refusing to return the active medication at the end of the trial because of the beneficial effects they had seen in erectile function.

In 2001, Graham set up a male cardiovascular health clinic, at Guy's and St Thomas'. This was innovative and unique, since it was the only cardiac clinic taking an interest in treating erectile dysfunction in cardiac patients.

He lectured both nationally and internationally at numerous conferences attended by cardiologists, GPs and urologists, all over the world.

His spellbinding lectures were always superb and they were delivered with a wonderful sense of humour and with great poise and charisma.

His research was of great interest, not only to cardiology, but also to those working in the field of diabetes, endocrinology, urology and psychosexual medicine.

It is now widely recognised that erectile dysfunction is a marker of underlying cardiovascular disease and Graham would often make the point that a patient with ED was a cardiovascular patient until proved otherwise. ED=ED, he would say, although it means 'erectile dysfunction', it is caused by 'endothelial dysfunction'.

Graham's pioneering work clearly demonstrated the safety of the PDE5 inhibitors, which rather than being dangerous, were actually beneficial, as long as they were not co-prescribed with nitrate drugs.

Much of his work was published in the International Journal of Clinical Practice; where, he was the editor for 22 years.

He would have enjoyed reading this book about testosterone and the adverse effects of low levels of the hormone on erectile function, premature death from cardiovascular disease, body composition, metabolic syndrome and diabetes, and the progress that has been made in diagnosis and management in the last 7 years.

Foreword

Geoffrey I. Hackett, Michael Kirby

This book is timely, as the accumulated evidence included regarding the benefit of testosterone replacement in men with classical symptoms and a documented low level of the hormone can no longer be regarded as controversial and calls for further research before prescribing the medication are unjustified. The negative impacts of testosterone deficiency (TD) on human health have been well documented. We need to put behind us the anxiety produced by the reported association between testosterone use and increased occurrence of myocardial infarction and stroke which prompted the FDA to issue a safety bulletin in 2014.[1]

There is no doubt that some inappropriate prescribing and marketing has happened in the US which led to the FDA taking a further view in 2015.[2]

The result of the marketing effort led to a fourfold increase during 2003 to 2013 in the rate of TRT in men aged 18 to 45 years.[3]

The US Food and Drug Administration (FDA) has opposed testosterone therapy in men with age-related hypogonadism but not in men with classical hypogonadism despite the acknowledgement that TD does indeed merit treatment. In reality, most of the published research relates to age related hypogonadism with few trials conducted in exclusively classical aetiologies.

This identification of an age-related category who according to the FDA do not merit treatment has caused great confusion to prescribers on both sides of the Atlantic as the body of evidence for benefit reflects prescribed usage in these cases..

This situation was taken to task by Abdulmaged Traish in 2020, concluding that TD is a pathophysiological condition that merits T treatment, irrespective of the underlying causes, or the historical terms to define it.[4]

The key issues are that there is no evidence that the response to testosterone therapy of age-related hypogonadism occurs via different physiological or biochemical mechanisms than primary or secondary hypogonadism, and the beneficial response to treatment and the risks and benefits are no different.

Ageing alone does not cause a significant decline in T levels if men stay healthy, and the predominant form of TD, in aging men, is mixed with primary and secondary hypogonadism components, attributed to varying pathophysiology and

important comorbidities such as diabetes and the metabolic syndrome, all of which are explored in this book.[5]

It was demonstrated many years ago that luteinizing hormone(LH) levels can vary in older men due to decreased numbers and function of Leydig cells, impaired sensitivity of the hypothalamus–pituitary gonadal axis to feedback inhibition, and/or decreased LH pulse amplitude despite normal pulse frequency, and LH pulse amplitude may potentially be related to reductions in neuronal cell secretion of gonadotrophic releasing hormone.[6, 7]

The T trials demonstrated that TTh confers significant and clinically meaningful health benefits in older men with low T and this treatment is safe and effective, irrespective of aetiology.[8]

In addition, the T trials provided compelling evidence that T therapy confers significant benefits in the growing population of men with obesity and/or type 2 diabetes. In fact, T4DM found a 40% reduction in men progressing to type 2 diabetes when hypogonadism was combined with lifestyle change *versus* lifestyle alone. The study also found that intensive lifestyle intervention over 2 years, despite moderate weight loss, did not improve symptoms or free testosterone levels. These findings cast doubt on the reliance of lifestyle advice alone as our only strategy for dealing with the increasing prevalence of type 2 diabetes throughout the world.[9]

BSSM published a guideline on adult testosterone deficiency in January 2018 clarifying the situation that testosterone deficiency (TD) is a condition that can significantly impact on quality of life, causing a wide range of symptoms, including erectile dysfunction, reduced libido, and less specific symptoms like fatigue and sleep disturbance. Testosterone therapy for TD is effective at reducing the symptoms, and may be associated with lower mortality, while the guideline reinforced the importance of a thorough assessment of other risk factors before initiating testosterone therapy. The BSSM also provided a practical guide on the assessment and management of testosterone deficiency in adult men to help clinicians manage the clinical situation.[10]

The many chapters in this book explore the various clinical options in relation to testosterone and other therapies to deal with the multiple problems associated with insulin resistance, type 2 diabetes and cardio-metabolic disease.

References

1. U.S. Food & Drug Administration. FDA Drug Safety Communication: FDA cautions about using testosterone products for low testosterone due to aging; requires labelling change to inform of possible increased risk of heart attack and stroke with use; 2018 [Internet]. Available from: www.fda.gov/Drugs/DrugSafety/ucm436259.htm
2. Nguyen CP, Hirsch MS, Moeny D, *et al.* Testosterone and "age-related hypogonadism"—FDA concerns. N Engl J Med 2015;373:689-91.
3. Rao PK, Boulet SL, Mehta A, *et al.* Trends in testosterone replacement therapy use from 2003 to 2013 among reproductive-age men in the United States. J Urol 2017;197:1121-6.

4. Traish AM. Age-Related Testosterone Deficiency Merits Treatment. Androgens: Clinical Research and Therapeutics 2021;2:46-55.
5. Khera M, Broderick GA, Carson CC, *et al.* Adult-onset hypogonadism. Mayo Clin Proc 2016;91:908-26.
6. Vermeulen A, Kaufman JM. Ageing of the hypothalamic-pituitary testicular axis in men. Horm Res 1995;43:25-8.
7. Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 2005;26:833-76.
8. Snyder PJ, Bhasin S, Cunningham GR, *et al.* Testosterone trials investigators effects of testosterone treatment in older men. N Engl J Med 2016;374:611-24.
9. Wittert G, Bracken K, Robledo KP, *et al.* Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021;9:32-45.
10. Hackett G, Kirby M, Edwards D, *et al.* The British Society for Sexual Medicine guidelines on adult testosterone deficiency with statements for UK practice. J Sex Med 2017;14:1504-23.

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Introduction: the politics of testosterone

David R. Edwards

There's more to testosterone than just sex

I suspect that if testosterone wasn't associated with sexual function then there would be no need to write this chapter. In addition the smouldering media debate and medical discourse which have flared up from time to time concerning the use of testosterone therapy (TTh) would probably have died out.

Testosterone is a word used loosely in everyday life by people who may not know much about it. One hears comments like 'male aggression fuelled by testosterone'. A quick Internet search produced comments by the Urban Dictionary: "During the war grandpa was a testosterone-fuelled madman killing lots of enemies".[1] Often, the only things that the general public know about testosterone are the negative aspects. When Abraham Morgentaler was interviewed by Abdulmaged Traish, about testosterone and negativity he stated: That perspective is made even worse by ads that we see on TV or in the media that promote supplements that are, I suppose, allegedly intended to boost testosterone, to make men more 'manly'.[2]

When checking the definition of manly, it is not meaning male aggression implying that testosterone treatment will turn men into rampaging bull elephants, or start committing sexual abuse or violence. Manliness is a term of approval, when a man feels good about himself, feels strong both physically and mentally, earns respect from his partner and demonstrates traits approved by society. When I see the partners of patients with low testosterone levels they often say "He has lost his oomph", or "I want him to be strong and support me again". One of my patients with both clinical symptoms and several laboratory tests demonstrating low testosterone described himself as a "Weak, feeble creature".

One of the factors that has fuelled the fire of T criticism is its misuse among young men, especially those involved with bodybuilding and sport. These athletes and so-called "sportsmen" cheat by using anabolic androgenic steroids (AAS) in order to gain an advantage over their competitors by adding muscle bulk to make them physically stronger. Medically qualified clinicians increase

the sleaze, undercover and improper use of testosterone by issuing prescriptions to these so called "Olympians". The use of (AAS) has been an international political football with nations being accused of cheating, excluded from competing, or medals retracted. The Countries alleged of doping their athletes bounce back with denials and claims of improper testing or false results. Of worrying concern is the continuing increase in AAS for body image and cosmetic reasons in the UK.[3] Sometimes the medical profession are complicit in prescribing T illegally to such patients and to men that have normal physiological T levels and without signs or symptoms of TD.

As I write this chapter, another Political/Sporting/Media/Testosterone storm is brewing, as the first transgender athlete is set to compete at an Olympics. Three key imperatives, in order to compete in sport, are inclusion, fairness and safety. The International Olympic Committee requires a trans woman to achieve testosterone levels below 10nmol/L for a year to be eligible for women's competition. Critics say that factors such as muscle mass, muscle strength and bone density should also be taken into consideration. Inclusion therefore comes at the expense of fairness to both trans and biological females. Trying to keep all parties happy seems to me to be an insolvable problem at present.

A quick "straw poll" of ten random adults "in the street" as to: "What is the first word that comes into your mind when I mention the word testosterone?" produced the following result. Six said "sex" and four answered "aggression". The public are generally unaware that there is a medical condition called Testosterone Deficiency (TD) which can affect not only the general health but also the quality of life of the affected person.[4]

Many Health Care Professionals (HCPs) seem reticent to proactively search for TD in at risk groups such as men with Type 2 Diabetes (T2D), where the incidence of TD is 40%.[5] However when asked, most will check for thyroid function as part of the annual check-up, even though the pick-up rate of finding thyroid disease is much lower.

The media and testosterone

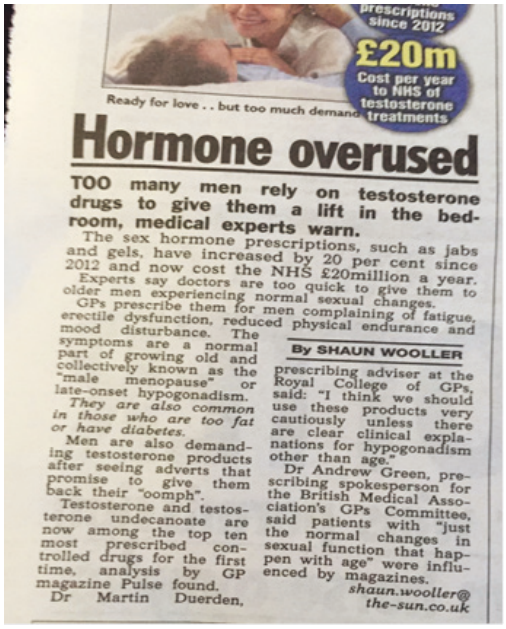
The media continue to have a love hate relationship with testosterone, extoling its value one week and denigrating it the next. Figures 1.1 and 1.2 are typical and illustrate headlines at either end of the press demographic spectrum. They love to use the expression 'male menopause' as it reads well in print. The term 'male menopause' is inappropriate as men do not have 'menses' (periods) and only very rarely (following surgery or trauma for example) does TD occur suddenly 'pause'.

It may not be helpful if men read headlines such as: "My energy is back: how testosterone replacement therapy is changing men's lives", in national newspapers.[6] Because of the link to sex and their embarrassment there could be a tendency for men to be tempted into Quackery seeking help from 'fringe' private clinics or online

Figure 1.1. Peta Bee, The Times 15th June 2021.


Men, have you got brain fog? It may be low testosterone

Figure 1.2. Shaun Wooller, The Sun 24th September 2016.


prescriptions since 2012

£20m

Cost per year to NHS of testosterone treatments

Ready for love . . but too much demand

Hormone overused

TOO many men rely on testosterone drugs to give them a lift in the bedroom, medical experts warn.

The sex hormone prescriptions, such as jabs and gels, have increased by 20 per cent since 2012 and now cost the NHS £20million a year.

Experts say doctors are too quick to give them to older men experiencing normal sexual changes.

GPs prescribe them for men complaining of fatigue, erectile dysfunction, reduced physical endurance and mood disturbance. The symptoms are a normal part of growing old and collectively known as the "male menopause" or late-onset hypogonadism.

They are also common in those who are too fat or have diabetes.

Men are also demanding testosterone products after seeing adverts that promise to give them back their "oomph".

Testosterone and testosterone undecanoate are now among the top ten most prescribed controlled drugs for the first time, analysis by GP magazine Pulse found.

Dr Martin Duerden, prescribing adviser at the Royal College of GPs, said: "I think we should use these products very cautiously unless there are clear clinical explanations for hypogonadism other than age."

Dr Andrew Green, prescribing spokesperson for the British Medical Association's GPs Committee, said patients with "just the normal changes in sexual function that happen with age" were influenced by magazines.

By SHAUN WOOLLER

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sources of testosterone. These men often fall through the net of not being properly investigated and monitored.

For those men that had gone to a bona fide National Health service (NHS) clinician they were then made to feel guilty by another headline; "Increasing demand for testosterone on NHS costing tax payers £20 million".[7] The article implied that "obesity, stress or diabetes, which can cause low testosterone, taking effect in an aging body" could be reversed "By tackling these conditions appropriately" and therefore shouldn't have been given testosterone. From my clinical experience although patients with TD, obesity and Type 2 Diabetes (T2D) are aware of these issues, and are on the correct management and medication, they find it practically impossible achieve targets for weight or HbA1c. However, if they are prescribed TTh they can gradually reach these targets. Haider *et al.*[8] achieved remission in over a third of their hypogonadal T2D participants treated with TTh. This contrasted with no remissions of T2D or reductions in HBA1c or glucose in the control group. Furthermore there were fewer deaths, myocardial infarctions, strokes and diabetic complications in the TTh group vs the controls.

Why didn't that appear as a front page headline? I suspect because of the image that testosterone has built up over the years.

Testosterone deficiency: a new pseudo-condition?

Pharma companies are sometimes accused of "disease mongering" in having created a new "pseudo-condition" of Testosterone Deficiency (TD) in order to promote their medication to an unsuspecting public and trying to 'hoodwink' the medical

profession into prescribing it. These Pharma sceptics consider the interest in testosterone to be a recent affair based on the drug industry's rush to find treatments for "lifestyle problems". I would suggest that nothing could be further from the truth as writings have been discovered going back many centuries, linking the testes with sexual function. Likewise, aphrodisiacs such as pills, pastes and potions for the penis, have formed many a tale or recipe across many cultures and centuries.

Sushrata of India 140 B.C. wrote:

"By eating the testes of a he-goat with (an adequate quantity of) salt and powdered long-pepper (Pippali), fried in clarified butter prepared from churning milk (and not from curd), a man is enabled to visit a hundred women."

Sushruta Samhita, volume 4: Cikitsasthana by Kaviraj Kunja Lal Bhishagratna | 1911

Leonardo da Vinci,[9] in the sixteenth century, wrote this on the side of one of his anatomical diagrams "Are not the testicles the cause of ardour?".

In 1767 John Hunter undertook the first documented testicular transplantation by grafting the testicles from a cock into the abdomen of a hen. Arnold Berthold, in 1849, concluded that a substance produced in the testes affected behavioural and sexual characteristics via the blood stream. An enthusiastic 'transplanter', in the 1920s, was Serge Voronoff, who used slices of primate testis and grafted them to the testicular capsule in humans. Apparently, he operated on 300 men and claimed that hormonal secretion lasted 1-2 years reducing over time due to graft fibrosis.

The pace was speeding up to find the active substance. In 1927, Lemuel Clyde McGee demonstrated a biologically active substance from bull testicles. The first 15g of androgen was isolated from 15,000 -25,000litres of policeman's urine. A Dutch group including Karoly David, Elizabeth Dingemanse, Janos Freud and Ernst Laqueur used several tons of bull testicles to isolate the chief secretion product from the testes and the main androgen in the blood which they called testosterone in 1935. The word testosterone comes from 'testo' (testes), 'ster' (sterol), and 'one' (ketone). Meanwhile the race to publish the chemical synthesis of testosterone was achieved by three groups led by Adolf Butenandt, Ernst Lacueur and Leopold Ruzicka in the same year.

Both the discovery of the biochemical substance and its chemical synthesis meant that modern endocrinology blossomed and the 'therapeutic door' was opened for sexual hormones with perhaps overenthusiastic prescribing of such treatments and patients encouraged by the word and 'promise' of 'rejuvenation'. The Journal of the American Medical Association (JAMA) hinted at this possibility in 1939 when their editorial commented:

"Recently many reports have appeared in medical journals claiming that a climacteric occurs in middle aged men. Brochures circulated by pharmaceutical

manufacturers depict the woeful course of aging man. None too subtly these brochures recommend that male hormone substance, like a veritable elixir of youth, may prevent or compensate for the otherwise inevitable decline".[10]

There is a misconception whereby T is just labelled as a sex hormone. Whilst it does have an impact on sexual drive and function, like many hormones it has an effect on many parts of the body. These numerous attributes will be expanded on in the following chapters.

The Internet: friend or foe?

Our patients for one reason or another are turning to the internet for information about medical conditions. Whilst social media can be a powerful tool for educating patients and HCPs, it can also be festering source of disinformation. The term 'disinformation' refers to 'false information deliberately and often covertly spread in order to influence public opinion or obscure the truth'.[11] Men experiencing sexual problems may resort to the internet before seeking help from a clinician because of embarrassment.[12] Warren *et al.*[13] assessed the patient utilisation and reliability of You Tube videos (YTVs) concerning male hypogonadism and TTh. They found that most of these YTVs were unreliable but that there were some reliable ones. The YTVs featuring a physician were more reliable and less biased but received fewer viewings than unreliable ones. They also noted that many patients (over 38 million views) were using YTVs as an educational resource for male hypogonadism and TTh. Unfortunately, these findings were consistent with previous studies evaluating YTVs and sexual health information. Many nations and international groups have looked at policing social media platforms.[14] Having established that lawsuits cannot effectively stop Internet misinformation, Sableman looked at whether social media companies could be encouraged to kerb such issues. However he concluded: *"In short, self-policing by social media companies is unlikely to keep political misinformation off of their pages"*.[15] If that is the state of affairs for political Internet misdemeanours what hope do the medical profession have in preventing unreliable YTVs?

Past myths and future management of testosterone

Two myths surrounding testosterone have caused concern among Physicians. One going back over 80 years was practically etched in stone, concerning the link between testosterone and prostate cancer. The second in 2013-14 was the allegation that testosterone therapy caused increased cardiovascular risk including heart attack, stroke and death. Both rocked the medical profession to its very foundation, caused a media and legal frenzy, but deprived suitable patients from treatment with testosterone. These topics are discussed more fully in the book together with references. However, the weight of evidence suggests that testosterone therapy does not increase cardiovascular or prostate cancer risk.

Testosterone has pleiotropic properties in producing many effects in many parts of the body. This, until the last few years has been one of the problems in dissipating research evidence among the medical profession. Each medical speciality kept in its own 'silo' whether it is teaching students, on hospital wards, societies, journals or conferences. So communication, sharing experience, research and case studies were fragmented. Hopefully, more integrated journals, conferences and teaching communications will bring together a mélange of HCPs who will be able to have intelligent debate and deliberation so that a consensus of reliable information can be cascaded down to clinicians, the media and patients alike.

Critics of testosterone replacement argue that it is a natural aging process – so why medicalise it? However, research has shown that 75% of men, in old age, maintain normal testosterone levels.[16] Looking at the patient holistically demonstrates that dentists do not generally decline to treat patients when their teeth start to crumble and fall out. When their hearing, eyesight and joints begin to fail do we as clinicians refuse to help them? Concerning hormone replacement, general practitioners actively and routinely check for thyroid function in at risk groups and replace and monitor hormone replacement where necessary. We often prescribe insulin for diabetics and in women offer hormone replacement therapy when indicated.

Surely we owe it to patients, their partner and families to operate within guidelines[17] to actively look for, investigate, treat to within physiological levels and monitor those with testosterone deficiency? This needs to be within the framework of a state health provider (such as the NHS in the UK) or a properly registered bona fide private clinic. There is a need for education of patients, clinicians and the media in demonstrating that there is more to testosterone than just sex.

References

1. Urban Dictionary; 2016 [Internet]. Available from: https://www.urbandictionary.com/define.php?term=testosterone-fueled [accessed 2021, June 15].
2. Traish A, Morgentaler A. Interview with Dr. Abraham Morgentaler. Androgens: Clinical Research and Therapeutics 2020;1:3-7. Available from: https://www.liebertpub.com/doi/10.1089/andro.2020.29000.int [accessed 2021, June 15].
3. Schartau P. Britain's anabolic steroid epidemic. Trends in Urology and Men's Health 2020;11:4:27-30.
4. Edwards D and David J The Journal Of Sex Med 2016;13:5 Suppl 2.
5. Kapoor D, Aldred H, Clark S, *et al.* 2007 Clinical and biochemical assessment of hypogonadism in men with type 2 diabetes: correlations with bioavailable testosterone and visceral adiposity. Diabetes Care 2007;30:911-7.
6. Hill A. The Guardian. 9th Sept 2019.
7. Warley W. The Independent. 26th Sept 2016.
8. Haider KS, Haider A, Saad F. Remission of type 2 diabetes following long-term treatment with injectable testosterone undecanoate in patients with hypogonadism and

type 2 diabetes: 11-year data from a real-world registry study. Diabetes Obes Metab 2020;22:2055-68.
9. Keele KD. Leonardo da Vinci's elements of the science of man. New York Academic Pres; 1983.
10. August A, Werner AA. The Male Climacteric. JAMA 1939;112:1441-3.
11. Library of Congress. COLLECTION Legal Reports (Publications of the Law Library of Congress). Available from: https://www.loc.gov/law/help/social-media-disinformation/compsum.php
12. Gul M, Diri MA. YouTube as a source of information about premature ejaculation treatment. The J Sex Med 2019;16:1734-40.
13. Warren CJ, Wisener J, Ward B. YouTube as a Patient Education Resource for Male Hypogonadism and Testosterone Therapy. Sex Med 2021;9:100324.
14. Library of Congress. COLLECTION Legal Reports (Publications of the Law Library of Congress). Available from: https://www.loc.gov/law/help/social-media-disinformation/compsum.php
15. Sableman M. Muting Misinformation: What's the role of social media companies? Thompson Coburn LLP; 2020 [Internet]. Available from: https://www.thompsoncoburn.com/insights/blogs/internet-law-twists-turns/post/2020-08-17/muting-misinformation-what-s-the-role-of-social-media-companies
16. Tajar A, Forti G, O'Neill TW, *et al.* Characteristics of secondary, primary, and compensated hypogonadism in aging men: evidence from the European Male Ageing Study. J Clin Endocrinol Metab 2010;95:1810-8.
17. Hackett G, Kirby M, Edwards D, *et al.* British Society for Sexual Medicine Guidelines on Adult Testosterone Deficiency, With Statements for UK Practice. Jour Sex Med 2017;14:1504-23.

2

Diagnosis and terminology in hypogonadism

Patricia E.S. Schartau

Introduction

Hypogonadism (testosterone deficiency) is a clinical and biochemical syndrome that stems from a reduced production of testosterone and sperm cells by the testis. It can adversely affect multiple bodily systems and is associated with a marked decrease in quality of life. Hypogonadism is primarily classified according to it being a congenital or acquired disorder; the anatomical level of dysfunction: at testicular (primary hypogonadism), hypothalamic- pituitary (secondary hypogonadism) or combined levels and recognises affected men with idiopathic, metabolic or iatrogenic conditions resulting in testosterone deficiency. Treatment decisions should include lifestyle measures and a holistic review of the patient.

This chapter aims at:

1. Shedding some light on definitions and terminology in patients with testosterone deficiency (hypogonadal patients).
2. Outlining its epidemiology.
3. Exploring the underlying pathophysiology.
4. Discussing issues surrounding diagnosis.
5. Drawing a conclusion.

Definitions and terminology

For the purpose of this chapter, we will refer to the term 'hypogonadism'. However, other nomenclature used in the literature include male hypogonadism, men with testosterone deficiency (TD) and testosterone deficiency syndrome (TDS).

Regarding the decreasing plasma androgen levels in ageing men, in the past, 'andropause', 'male menopause' and 'male climacteric' have been used. However, these terms are generally regarded as inaccurate, since the precipitous changes that occur in women are lacking in men. Unlike menopause, the decrease in testicular function in men is gradual and symptoms can be more non-specific. Indeed, many older men (up to 80%) continue to have free androgen levels in the low normal

range throughout their lifetime. Terms such as "late onset hypogonadism" (LOH), partial androgen decline in aging male (PADAM) and androgen decline in aging male (ADAM) have gained more acceptance.

Hypogonadism is a clinical and biochemical syndrome, associated with reduced testosterone levels and a range of symptoms and signs spanning across body systems which can greatly affect quality of life and potentially fertility.[1] It remains underdiagnosed and undertreated.[2]

Epidemiology

In ageing men who are relatively healthy, there is a small decrease in testosterone, which by itself only accounts for small numbers of hypogonadism diagnoses.[3] For 40-79 years, the incidence of hypogonadism has been reported as 11.7 and 12.3 cases per 1000 people per year respectively[4, 5] and symptomatic incidence between 1.2-5.7%.[6, 7] Regarding prevalence, estimates vary widely: One study[7] found that symptomatic prevalence in American men <70 years of age was 3.1-7%, and increased to 18.4% in men 70 years or older. The Massachusetts Male Ageing study (MMAS) found that 6-12% of men aged 40-69 years had symptomatic hypogonadism.[8]

A high prevalence of hypogonadism can be found in patients with obesity and acquired chronic conditions, such as dysglycaemia (Type 2 diabetes mellitus T2DM; up to 40%),[9] metabolic syndrome, insulin resistance, cardiovascular disease, some cancers, COPD and renal disease.[7] Regarding congenital conditions, Klinefelter syndrome, a trisomy with a 47 XXY karyotype, is the most common cause but less than half of affected patients are formally diagnosed with hypogonadism in their lifetime.

Pathophysiology

The testosterone circulation

In healthy men, most circulating testosterone (98%) is bound, either to sex-hormone binding globulin (SHBG; 60%) or to lower affinity, high-capacity binding proteins (predominantly albumin; 38%), with approximately 2% being free of any binding.[10] Physiological affects are only exhibited by bioavailable testosterone, comprising the free testosterone (2%) and the low affinity bound one (38%). Simplified:

Total testosterone = Free testosterone (2%) + Bound testosterone (98%)
Bound testosterone = SHBG bound (60%) + albumin (and other low affinity proteins; 38%)
Bioavailable testosterone= Free (2%) + albumin-bound (38%)

Testosterone circulation can be altered by changing SHBG levels (Table 2.I for underlying causes), which can distort correct estimation of androgen status. Thus, if any of the causes are present, SHBG should be measured and free and/or bioavailable testosterone calculated (free calculator).[11] This topic will be further discussed in Chapter 4.

Table 2.I. Causes of modified circulating SHBG levels. Adopted from the European Association of Urologists.[12]

SHBG increase	• Drugs: oestrogens, thyroid hormone, anticonvulsants • Hyperthyroidism • Ageing • AIDS/HIV • Smoking • Hepatic disease
SHBG decrease	• Drugs: growth hormone (GH), anabolic androgenic steroids, testosterone, glucocorticoids • Obesity • Hypothyroidism • Cushing's disease • Acromegaly • Insulin resistance (metabolic syndrome, T2DM) • Non-alcoholic fatty liver disease (NAFL) • Nephrotic syndrome

Classification of causes for hypogonadism

Broadly speaking, hypogonadism can be classified according to the anatomical location of the defect causing the inadequate testosterone production: Primary hypogonadism (testicular dysfunction; hypergonadotrophic hypogonadism), secondary hypogonadism (hypothalamic-pituitary axis failure; hypogonadotrophic hypogonadism) or mixed (combination of primary and secondary hypogonadism).[13]

Measuring luteinizing hormone (LH) can provide information about a dysfunction on testicular and/or hypothalamic-pituitary level, and contribute towards therapeutic decision making.

More recently, a new classification system was put forward which differentiates between organic and functional hypogonadism.[9] Organic hypogonadism refers to conditions resulting in low testosterone where there is a proven dysfunction of the hypothalamic- pituitary-testicular (HPT) axis suppression rather than a functional gonadal axis suppression secondary to ill health (functional hypogonadism).[14] Regarding the latter, addressing management of co-morbidities and lifestyle is part of the therapeutic goal,[9] but frequently not sufficient as sole treatment.

Subclinical (compensated) hypogonadism

This is a less studied type of hypogonadism, characterized by normal testosterone levels combined with elevated LH[15] for which the European Male Ageing study showed a prevalence of 10%. Whilst testosterone levels remain normal, there is the possibility that they declined from upper to lower normal. The high LH may reflect an adjustment of the HTT feedback loop in order to compensate.[16] Moreover, subclinical hypogonadism shares some of the adverse health outcomes with overt hypogonadism, hence early identification and interventions such as lifestyle modifications,

Table 2.II shows the classification of male hypogonadism adopted from the European Association of Urologists.[12]

PRIMARY HYPOGONADISM (hypergonadotropic hypogonadism)	
Congenital or developmental disorders	
Common causes	**Uncommon causes**
Klinefelter syndrome	Rare chromosomal abnormalities XX male syndrome 47 XYY syndrome 48 XXYY syndrome 21 Trisomy (Down syndrome) Noonan syndrome Autosomal translocations1 Defects of testosterone biosynthesis CAH (testicular adrenal rest tumours) Disorders of sex development (gonadal dysgenesis) LHR gene mutations Myotonic dystrophy (including type I and II) Uncorrected cryptorchidism (including INSL3 and LGR8 mutations) Bilateral congenital anorchia Sickle cell disease Adreno-leukodystrophy
Acquired disorders	
Drug-induced	**Localised problems**
Chemotherapy agents Alkylating agents Methotrexate Testosterone synthesis inhibitors Ketoconazole Aminoglutethimide Mitotane Metyrapon	Bilateral surgical castration or trauma Testicular irradiation Orchitis (including mumps orchitis) Autoimmune testicular failure Testicular Torsion Alcohol/Cirrhosis Environmental Toxins
Systemic diseases/conditions with hypothalamus/pituitary impact	
Chronic systemic diseases* Chronic organ failure* Glucocorticoid excess (Cushing syndrome)* Aging* HIV	Malignancies Lymphoma Testis cancer Spinal cord injury Vasculitis Infiltrative diseases (amyloidosis; leukaemia)

SECONDARY HYPOGONADISM (hypogonadotropic hypogonadism)	
Congenital or developmental disorders	
Common causes	**Uncommon causes**
Haemochromatosis*	Combined hormone pituitary deficiency Idiopathic hypogonadotropic hypogonadism (IHH) with variants: Normosmic IHH Kallmann syndrome Isolated LH β gene mutations Prader-Willi Syndrome
Acquired disorders	
Drug-induced	**Localised problems**
Oestrogens Testosterone or androgenic anabolic steroids Progestogens (including cyproterone acetate) Hyperprolactinaemia-induced drugs Opiates GnRH agonist or antagonist Glucocorticoids	Traumatic brain injury Pituitary neoplasm (micro/macro-adenomas) Hypothalamus tumours Pituitary stalk diseases Iatrogenic Surgical hypophisectomy Pituitary or cranial irradiation Inflammatory and infectious diseases Lymphocytic hypophysitis Pituitary infections Granulomatous lesions Sarcoidosis Wegener's granulomatosis Other granulomatosis Encephalitis Langerhans' histiocytosis Hyperprolactinaemia as a consequence of localised problems (hypothalamus-pituitary mass)
Systemic diseases/conditions impacting the hypothalamus/pituitary	
Chronic systemic diseases* Metabolic diseases HIV infection Chronic organ failure Chronic Inflammatory Arthritis Glucocorticoid excess (Cushing syndrome)* Eating disorders* Endurance exercise Acute and critical illness Ageing*	Spinal cord injury Transfusion-related iron overload (β-thalassemia)

ANDROGEN RESISTANCE/DECREASED TESTOSTERONE BIOACTIVITY	
Congenital or developmental disorders	
Aromatase deficiency Kennedy diseases (spinal and bulbar muscular atrophy) and other extensions of CAG repeats Partial or complete androgen insensitivity 5α reductase type II (5αR) deficiency	
Acquired disorders	
Drug-induced	**Localised problems**
Drug-induced AR blockage Steroidal antiandrogen Cyproterone acetate Spironolactone Non-steroidal antiandrogen Flutamide Bicalutamide Nilutamide Drug-induced 5α reductase (5αR) activity blockade Finasteride Dutasteride Drug-induced ER blockade Clomiphene Tamoxifen Raloxifene Drug-induced aromatase activity blockade Letrozole Anastrazole Exemestane Increased SHBG	Coeliac disease

* Conditions acting and central and peripheral levels resulting in either primary and secondary hypogonadism.
1 Different autosomal translocations can cause rare cases of hypogonadism and infertility.

are key.[17] However, clinical experience tells us that lifestyle advice alone is often not enough if testosterone is low,[15] partly because motivation and energy to change is poor in the presence of a low testosterone.[18] However, motivation can increase via, for example, an improvement in mood, with help of testosterone replacement therapy.[19]

Late-onset hypogonadism (LOH)

Late onset hypogonadism is defined as "a clinical and biochemical syndrome associated with advancing age and characterised by typical symptoms and a deficiency in serum testosterone levels". Those symptoms are mainly of sexual nature, such as erectile dysfunction, reduced spontaneous erections and reduced libido. LOH may significantly reduce quality of life and adversely affects the function of multiple

organ systems.[20] As highlighted in Section 1, the terms 'andropause' or 'male menopause' are sometimes still being used: However, these are misleading terms. Whilst we know that testosterone production declines with ageing,[21] unlike menopause, testosterone deficiency is not an inevitable result of aging. Indeed, up to 80% of men maintain levels in the normal range throughout life. Moreover, the decrease in testosterone is gradual in contrast to the 'menopause' where there is a steep and relatively sudden decrease in some hormone levels. Returning to the more recent classification of organic *versus* functional hypogonadism, the main causes of functional hypogonadism are obesity, chronic disease co-morbidities (*e.g.* Diabetes Mellitus) and ageing (but mostly the former two). Regarding the 'older' classification, age-related HPT axis changes manifest predominantly as primary and subclinical hypogonadism, and LOH relating to co-morbidity (*e.g.* obesity) often presents as secondary or mixed hypogonadism.[22] However, the question remains of how much LOH contributes towards co-morbidities and vice versa.[23]

Diagnosis

The diagnosis of hypogonadism requires decreased serum concentrations of testosterone combined with one or more clinical symptoms.[21] Clinical signs may also be present.

Symptoms

One way to classify these symptoms is into sexual and non-sexual.[20] The former includes decreased libido, erectile dysfunction, decreased spontaneous (morning erections) and delayed ejaculation. Loss of libido, erectile dysfunction and decreased spontaneous erections have been found to be the most predictive symptoms for low testosterone measurements).[7, 24]

Non-sexual symptoms include depression or dysthymia, decreased energy levels and/or fatigue, impaired concentration, reduced body mass and strength, hot flashes, gynaecomastia, breast discomfort, shrinking testes and, infertility. Signs include anaemia, osteopenia and osteoporosis, increased body fat (abdominal-visceral) and BMI.[14] Different studies have established different testosterone levels at which specific symptoms occur.[12] In reality, most patients present with erectile dysfunction and/or reduced libido, due to its impact on quality of life, and a desire to be treated.[23]

Screening questionnaires

Screening questionnaires on male hypogonadism are available, but have low specificity (although high sensitivity), which limits their utility in clinical practice.[24]

The ADAM questionnaire (Androgen Deficiency in Aging Male)[25] is a widely used and accepted tool, which has been translated into different languages.[26] Its sensitivity has been estimated as up to 97%,[27] with specificity ranging from 24-60%.[26, 28] The AMS (Aging Males' Symptoms Scale) was developed in 1999 in order to assess

symptoms and their changes over time, in particular with testosterone replacement therapy treatment.[29] Sensitivity was estimated as 83% with a specificity of 39%.[25] The MMAS (Massachusetts Male Aging Study questionnaire) was developed in 2000 as a screening tool.[29] When initially administered in a clinical setting, the MMAS had a sensitivity of 76% and a specificity of 49%.[26] A study[30] that subsequently compared the ADAM, AMS and MMAS tools found the MMAS to have a sensitivity of 60%, with a specificity of 59%. The NERI (New England Research Institutes) questionnaire to screen for hypogonadism was developed in 2009.[31] A subsequent study found the tool to have strong psychometric properties, including acceptable discriminant, construct and content validity, as well as good internal consistency and test-retest reliability.[32] Screening for hypogonadism in younger men is less studied, and there is not as much known about the use of these screening tools in this population.[32]

Completing one of these questionnaires would only take a patient a few minutes. This could be done at home or in the waiting room and integrate with the clinical system in order to screen for whether the answers warrant further exploration of symptoms via a more detailed history, examination and laboratory testing. Consensus is that the questionnaires on their own should not be used for systematic screening of hypogonadism in men.[33]

History taking

The history should explore any symptoms (and signs that patients may have noticed) outlined above and provide an understanding how the symptoms have impacted on the patient's life.

Moreover, the history should cover relevant surgical interventions during lifetime, such as for hypospadias and cryptorchidism, as well as explore any known congenital defects. Any prescribed or over the counter medications should be elicited as some of these can potentially interfere with the HPG axis, as well as co-morbidities (*e.g.* Malabsorption, Hypothyroidism, Haemochromatosis, Obstructive Sleep Apnoea, Diabetes Mellitus). It is also worth enquiring about any acute illness, which can cause the development of functional hypogonadism in which case testosterone laboratory testing should be deferred. For example, taking recent data from SARS-CoV-2 infections, a study[34] concluded that COVID-19 might deteriorate serum testosterone level in SARS-CoV-2 infected male patients. Whilst this is beyond the scope of this chapter, a large number of recent papers[35, 36] have discussed the effects of an acute Covid infection on testosterone levels and vice versa: for example, Hackett *et al.* concluded that "furthermore, the virus is associated with a severe primary hypogonadism occurring in addition to the functional secondary hypogonadism associated with comorbid conditions in patients at high risk of infection" (see Chapter 18).[36]

Physical examination

Weight, height, body mass index (BMI) and waist circumference should be recorded as testosterone deficiency is associated with increased body fat (particularly

abdominal), BMI and reduced height and muscle strength.[24] Amount and distribution of body hair should be assessed; presence and degree of breast enlargement and acanthosis nigricans (associated with insulin resistance); abnormalities in the scrotum and penis (size, appearance). As part of good practice, it has been suggested to conduct a digital rectal exam (DRE) in patients with suspected hypogonadism particularly with view of potentially initiating testosterone replacement therapy, TRT).[12]

Any patient presenting with consistent and multiple symptoms (especially erectile dysfunction and/or loss of sexual desire) and signs of hypogonadism should be further investigated, as well as the following groups of patients: men with T2DM, BMI >30 kg/m^2 or waist circumference >102 cm (40.2 inches) and men on long-term opiates, antipsychotic or anticonvulsant medication (see Chapter 20).[37]

Laboratory tests

Serum total testosterone (TT) is the most widely accepted initial parameter to establish a potential diagnosis of hypogonadism. As highlighted before, the diagnosis of hypogonadism is a clinical and biochemical one. Fasting levels is the gold standard and should be taken between 7 A.M. –11 A.M. or for shift workers within three hours of waking up. However, diurnal variation may be masked in older men: it was demonstrated in a cross-sectional study[38] of 3600 men with a mean age of 60.3 years who were undergoing prostate cancer screening, that serum testosterone concentrations remained the same between 6A.M. and 2P.M., with a decrease (13%) between 2 P.M. – 6 P.M. Laboratory tests should not be conducted during acute or subacute illness.[39] Additional blood test investigations may include prostate-specific antigen (PSA), haematocrit, markers of cardiovascular risk (glycaemic control, lipids) and any other tests relevant to exclude alternative diagnoses (*e.g.* thyroid function tests).

Any testosterone level of 12 nmol/L or less should be confirmed on at least two separate occasions preferably at least 4 weeks apart.[37, 39] Measuring serum luteinising hormone (LH) will help to differentiate between primary and secondary hypogonadism whereas a serum prolactin can be added in when there is a suspicion of secondary hypogonadism (low LH) or the testosterone is below 5.2 nmol/L (150 ng/dL). For values between 8-12 nmol/L, it is recommended to add in SHBG in order to calculate free testosterone.[40, 11] Additional tests, such as cortisol and/or oestradiol, are not indicated unless symptoms and signs suggest another underlying endocrine disorder.[41]

The gold standard for measurement of total testosterone is considered to be liquid chromatography tandem mass spectrometry (LC-MS).[39] However, this method is not widely available. The most used methods instead are immunometric assays (IA) and radioimmunoassay (RIA). Whilst very specific, there is a range of antibodies being used and hence results can vary between laboratories. Despite issues with calibration and reliability, most studies demonstrate a good correlation between these methods and the gold standard.[42]

Further issues as well as controversy around interpretation of results will be covered in Chapter 3.

Conclusions

In this chapter, we have explained some of the terminology around hypogonadism, outlined relevant epidemiological issues and explored the underlying physiology. Moreover, we have discussed the different components required to make a diagnosis, as hypogonadism remains a clinical and biochemical syndrome. We have been able to briefly touch on some of the controversies surrounding the diagnosis-particularly around laboratory testing and interpretation of results. Treatment threshold and treatment options (which has its own controversies) is beyond the scope of this chapter. However, subsequent chapters will focus on specific aspects (*e.g.* testosterone reference ranges) and explore these in more depth than has been possible in the current chapter.

One of the primary messages of this chapter is that hypogonadism remains underdiagnosed and mostly undertreated. Hopefully, this chapter contributes towards raising awareness and sheds some light on this condition, which – if unrecognised – has the potential to dramatically impact adversely on mental and physical health.

References

1. Behre HM. Clinical Use of FSH in Male Infertility. Front Endocrinol (Lausanne) 2019;10:322.
2. Trinick TR, Feneley MR, Welford H, *et al.* International web survey shows high prevalence of symptomatic testosterone deficiency in men. Ageing Male 2011;14:10-5.
3. Nieschlag E, HermannM, Behre M. Andrology: male reproductive health and dysfunction. Third edition. Berlin: Springer Berlin Heidelberg; 2010.
4. Khera M, Adaikan G, Buvat J, *et al.* Diagnosis and Treatment of Testosterone Deficiency: Recommendations from the Fourth International Consultation for Sexual Medicine (ICSM 2015). J Sex Med 2016;13:1787-804.
5. Hall SA, Esche GR, Araujo AB, *et al.* Correlates of low testosterone and symptomatic androgen deficiency in a population-based sample. J Clin Endocrinol Metab 2008;93:3870-7.
6. Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 2005;26:833-76.
7. Wu FC, Tajar A, Pye SR, *et al.* Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. J Clin Endocrinol Metab 2008;93:2737-45.
8. Cabral RD, Busin L, Rosito TE, *et al.* Performance of Massachusetts Male Ageing Study (MMAS) and androgen deficiency in the ageing male (ADAM) questionnaire in the prediction of free testosterone in patients aged 40 years or older treated in an outpatient regimen. Aging Male 2014;17:147-54.

9. Grossmann M, Matsumoto AM. A Perspective on Middle-Aged and Older Men With Functional Hypogonadism: Focus on Holistic Management. J Clin Endocrinol Metab 2017;102:1067-75.

10. Büchter D, Behre HM, Kliesch S, *et al.* Pulsatile GnRH or human chorionic gonadotropin/human menopausal gonadotropin as effective treatment for men with hypogonadotropic hypogonadism: a review of 42 cases. Eur J Endocrinol 1998;139:298-303.

11. PCTAG. Free and Bioavailable Testosterone Calculator. Available from: http://www.pctag.uk/testosterone-calculator/

12. Dohle GR, Arver S, Bettocchi C, *et al.* Male Hypogonadism. European Association of Urology Guidelines 2021. Available from: https://uroweb.org/guideline/male-hypogonadism/#7

13. Guyatt GH, Oxman AD, Kunz R, *et al.* Going from evidence to recommendations. BMJ 2008;10;336:1049-51.

14. Morgentaler A, Zitzmann M, Traish AM, *et al.* Fundamental Concepts Regarding Testosterone Deficiency and Treatment: International Expert Consensus Resolutions. Mayo Clin Proc 2016;91:881-96.

15. Tajar A, Forti G, O'Neill TW, *et al.* Characteristics of secondary, primary, and compensated hypogonadism in ageing men: evidence from the European Male Ageing Study. J Clin Endocrinol Metab. 2010;95:1810-8.

16. Liu PY, Pincus SM, Takahashi PY, *et al.* Ageing attenuates both the regularity and joint synchrony of LH and testosterone secretion in normal men: analyses via a model of graded GnRH receptor blockade. Am J Physiol Endocrinol Metab 2006;290:E34-E41.

17. Corona G, Maseroli E, Rastrelli G, *et al.* Characteristics of compensated hypogonadism in patients with sexual dysfunction. The J Sex Med 2014;11:1823-34.

18. Wittert G, Bracken K, Robledo KP, *et al.* Testosterone treatment to prevent and revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double -blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021;9:32-45.

19. Snyder PJ, Bhasin S, Cunningham GR, *et al.* Lessons From the Testosterone Trials. Endocr Rev 2018;39:369-86.

20. Jones TH. Late onset hypogonadism. BMJ 2009;338:b352.

21. The European Male Ageing Study (EMAS). Prevalence, Incidence and Geographical Distribution of Symptoms of Ageing in Men, and Their Endocrine, Genetic and Psychosocial Correlates. Introduction. Available from: http://www.emas.man.ac.uk/Main.asp

22. Kaufman JM, Lapauw B, Mahmoud A, *et al.* Aging and the male reproductive system. Endocr Rev 2019;40:906-72.

23. Nieschlag E. Late-onset hypogonadism: a concept comes of age. Andrology 2019;8:1506-11.

24. Lunenfeld B, Mskhalaya G, Zitzmann M, *et al.* Recommendations on the diagnosis, treatment and monitoring of hypogonadism in men. Aging Male 2015;18:5-15.

25. Morley JE, Charlton E, Patrick P, *et al.* Validation of a screening questionnaire for androgen deficiency in aging males. Metabolism 2000;49:1239-42.

26. Rabah DM, Arafa MA. Validation of an Arabic ADAM questionnaire for androgen deficiency screening in the Arab community. Aging Male 2009;12:95-9.

27. Morley JE, Perry HM 3rd, Kevorkian RT, *et al.* Comparison of screening questionnaires for the diagnosis of hypogonadism. Maturitas 2006;53:424-9.

28. Martínez-Jabaloyas JM, Queipo-Zaragozá A, Rodríguez-Navarro R, *et al.* Relationship between the Saint Louis University ADAM questionnaire and sexual hormonal levels in a male outpatient population over 50 years of age. Eur Urol 2007;52:1760-7.

29. Heinemann LA, Saad F, Zimmermann T, *et al.* The Aging Males' Symptoms (AMS) scale: update and compilation of international versions. Health Qual Life Outcomes 2003;1:15.

30. Smith KW, Feldman HA, McKinlay JB. Construction and field validation of a self-administered screener for testosterone deficiency (hypogonadism) in ageing men. Clin Endocrinol 2000;53:703-11.

31. Rosen RC, Araujo AB, Connor MK, *et al.* The NERI hypogonadism screener: psychometric validation in male patients and controls. Clin Endocrinol (Oxf) 2011;74:248-56.

32. Bernie AM, Scovell JM, Ramasamy R. Comparison of questionnaires used for screening and symptom identification in hypogonadal men. The Ageing Male 2014;17:195-8.

33. Giltay EJ, Tishova YA, Mskhalaya GJ, *et al.* Effects of testosterone supplementation on depressive symptoms and sexual dysfunction in hypogonadal men with the metabolic syndrome. J Sex Med 2010;7:2572.

34. Çayan S, Uğuz M, Saylam B, *et al.* Effects of serum total testosterone and its relationship with other laboratory parameters in the prognosis of Coronavirus disease 2019 (Covid-19) in SARS- CoV-19 infected male patients: a cohort study. Aging Male 2020;23:1493-503.

35. Auerbach JM, Khera M. Testosterone's role in Covid-19. J Sex Med 2021;18:843-8.

36. Hackett G, Kriby M. Testosterone deficiency in men infected with Covid-19. Trends in Urology and Men's Health 2020;11:7-10.

37. British Society of Sexual Medicine (BSSM, 2018): A practical guide on the assessment and management of testosterone deficiency in adult men. Available from: http://www.bssm.org.uk/wp-content/uploads/2018/02/BSSM-Practical-Guide-High-Res-single-pp-view-final.pdf

38. Crawford ED, Barqawi AB, O'Donnell C, *et al.* The association of time and day and serum testosterone concentration in a large scale screening population. BJUI Int 2007;100:509-13.

39. Trost LW, Mulhall JP. Challenges in testosterone measurement, data interpretation, and methodological appraisal of intervention trials. J Sex Med 2016;13:1029-46.

40. Bhasin S, Brito JP, Cunningham GR. Testosterone therapy in men with androgen deficiency syndrome: an Endocrine Society Clinical Practice guideline. J Clin Endocrinol Metabol 2010;95:2536-59.

41. Tsametris C, Isidori AM. Testosterone replacement therapy: For whom, when and how? Metabolism 2018;86.69-78.

42. Nieschlag E. Lat-onset hypogonadism: A concept comes of age. Andrology 2019;8:1506-11.

3

Issues regarding testosterone measurement and use of reference ranges in men

Mark Livingston, Rachel Marrington

Introduction

Serum testosterone (T) assays play an important role in the clinical evaluation of a number of common endocrine disorders in both males and females.[1] The role of the clinical laboratory is to provide tests that, together with clinical judgement, aid in the diagnosis and management of disease. When requesting hormone levels, it is essential that the clinician understands the clinical implications of the laboratory results generated, which must be judged to be appropriate or not, taking into consideration the clinical picture of the individual patient. Where male T levels are concerned, confusion appears to reign in both clinical and laboratory domains; we categorise the reasons for requesting T levels into the following areas, including: 1) diagnosis of primary hypogonadism (HG); 2) diagnosis of secondary HG: pituitary/hypothalamic disease; 3) late onset HG (also known as testosterone deficiency (TD), adult onset HG, and functional HG); and 4) improving patient fertility.

This chapter will focus on the factors that affect the measurement of circulating total T levels, including the T assays as applicable for males; however, it must be remembered that in many cases the same assay system is used on female specimens where the T concentration is an order of magnitude lower. This is important when it comes to the design of the assay and possible limitations.

In males, the measurement of T is mainly to assess gonadal status or to monitor testosterone replacement therapy (TTh). T circulates in both protein-bound and non-protein-bound (free) forms. In men, approximately 50% is loosely bound to albumin, 44% bound to sex hormone binding globulin (SHBG), 4% is bound to other proteins and 2% is free and non-protein bound.[2]

There are many factors which affect the T result which can be broadly classified into either physiological or analytical factors. The physiological factors within a male that play an important role include: age, fasting, nutritional status, weight,

acute and chronic illness (negative acute phase reactant), ethnicity, concentration of relevant binding proteins, and the time of day and season of year that the specimen has been collected.[2, 3]

Total T assays originated using radioimmunoassay techniques (RIA) which required organic extraction and chromatographic separation prior to analysis. These have long since been developed further so that T can be measured routinely on automated platforms using nonradioactive methods.[4] Although a T result can now be produced more rapidly and at a lower cost, this can sometimes be at the price of the accuracy and precision of the result. External Quality Assurance (EQA) data from the United Kingdom National EQA Service (UK NEQAS) for Steroid Hormones Scheme has repeatedly shown variation both within and between methods. This is important not only in terms of individual patient care, but also when data is combined over many years, from a number of centres, using different analytical equipment/methodologies as part of research studies which could then go on to influence future guidelines.

Factors affecting the measurement of circulating total testosterone levels

The circulating concentration of total T measured in human serum is dependent on a number of analytical and physiological factors; these factors can have significant effects on T levels measure and include the following (analytical measurement is dealt with separately).

Age

Male T levels decline with age gradually decreasing with each decade after the age of 30-40 years (markedly after 60 years of age), leading to late onset HG (also known as adult-onset TD and functional TD) in 6-12% of men.[5, 6] This decrease in total T may be exaggerated by SHBG levels rising with age.[2] Declining T and other anabolic hormones in men may influence aging-related deteriorations in body function (*e.g.* frailty, obesity, osteopenia, cognitive decline, and erectile dysfunction.[7]

Taking the mean T at age 40 years as 100%, the expected relative levels are given in Table 3.I. In older males, these levels can be kept in mind as a guide to when the T level is borderline low. If age-related ranges are not quoted, this has the potential to increase the numbers of apparently abnormal low results in older men, with an impact on further investigation and treatment. Male total T reference ranges and guidance on action limits are covered in a later section.

Circadian rhythm (time of day)

Substantial evidence exists to support the circadian variation in testosterone release, which occurs with peak total T levels in adults seen early in the morning

around 09:00 h, whilst the lowest values (up to 60% lower) are found in the evening.[8] Brambilla *et al.*,[8] in an elegant study, showed that in men aged 30-40 years old, T levels were 20–25% lower at 16:00 h than at 08:00 h. The difference observed declined with age, with only a 10% difference by 70 years of age. Importantly, 17 out of 66 (25.8%) men with at least one of three measurements <10.4 nmol/L after midday had normal total T levels at all three visits before midday. Brambilla and colleagues[8] recommended restricting T measurements to morning hours in both young and older men, and this circadian rhythm in circulating T level was also shown by Plymate *et al.*;[9] however, the rhythm in elderly men has been shown to be considerably blunted and shifted in time compared to younger men,[10] providing evidence for age-related changes in the circadian rhythms of luteinizing hormone (LH) pulse frequency and T secretion, suggesting the LH releasing hormone pulse generator loses its circadian rhythmicity with normal male aging.

Table 3.I. Mean testosterone levels for each decade of life after the age of 40 years. Taking the mean testosterone at age 40 years as 100%, the expected relative levels are given. Taken from Livingston *et al.*[6]

Age (y)	Percentage (%)	Lower limit normal (adjusted) nmol/L
40	100	9.4
50	93	8.7
60	85	8.0
70	79	7.4
80	72	6.8
90	67	6.3

Thus, two blood samples for T assay should be drawn between 07:00 h and 11:00 h (preferably 09:00 h), due to this diurnal variation (as T levels are usually at their highest at that time), which is now recommended in guidance for T testing to diagnose HG.[11] This gives a baseline level of T that can then be used to determine whether the patient is hypogonadal or not. Sampling at varying times is not recommended as it could complicate the diagnosis of HG which is defined by T thresholds and symptoms.

A survey in the UK NEQAS for Steroid Hormones Scheme in 2021 showed approximately 50% of laboratories (94 respondents) gave advice on the time of day that specimens should be collected for the measurement of T in males. Variation was observed in the advice that was given for the time of day of sample collection, and there were differences on whether the advice was for all patients or just those who had had an initial low T.

Seasonal variation

Evidence for seasonal fluctuations of serum total T levels is limited and contradictory; most evidence stems from cross-sectional rather than longitudinal studies. Many of the studies are limited by their sample sizes, environmental conditions

(with wide-ranging temperature and day–night patterns), as well as varying sampling protocols, and a lack of consideration for potential confounders (*e.g.* age, nutrition, physical activity and exercise, light and weather patterns).[12] Moreover, most of the studies have come from populations living in Europe or North America, so it is unclear whether seasonal effects on T levels are restricted to populations living at the higher latitudes.

Early studies on seasonal influences reported peaks in T levels in the summer–early autumn with troughs in the winter–early spring.[12] Santi *et al.*,[13] in an observational, retrospective study of 7,491 men (mean age 47.5 years) in Modena, Italy [Latitude: 44.6471° N, Longitude: 10.9252° E], showed a seasonal fluctuation of T levels higher in the summer with a direct correlation to daylight duration and environmental temperatures. When their cohort was stratified by age, they found seasonal changes in men aged between 35 and 57 years, but no seasonal effect was evident for men aged <35 years or >57 years; this differing pattern by age subgroup may account for some of the discrepancies seen between the different studies. In a much smaller cohort (27 healthy male volunteers)[14] in Copenhagen, Denmark [Latitude: 55.6761° N, Longitude: 12.5683° E], during a 17-month period, a seasonal variation was observed in LH and T levels in men with peak levels observed during June–July, with minimum levels present during winter–early spring. The authors suggested that air temperature, rather than light exposure, was a possible climatic variable explaining the seasonal variation.

Monthly variations in mean serum total T levels were reported across the months in 8,367 middle-aged men in Seoul, South Korea[15] [Latitude: 37.5665° N, Longitude: 126.9780]; however, in contrast to the studies already mentioned, a nadir was observed in May (15.3 nmol/L) and a peak in January (20.8 nmol/L), even after adjusting for confounders including age and body mass index (BMI), corresponding to a nearly 25% difference. T levels were inversely related both to the mean outdoor temperature and daylight duration.

In a cross-sectional study involving 1,548 men in Tromsø, north Norway (a population exposed to a wide seasonal variation in daylight and temperature) [Latitude: 69.6492° N, Longitude: 18.9553° E], Svartberg *et al.*[16] found a bimodal seasonal variation of total T with a small peak in February, nadir in June and more prominent peak in October–November; these patterns persisted when adjusted for age and waist-to-hip ratio. The lowest levels of T occurred in months with the longest hours of daylight and highest temperatures; however, samples analysed were collected at varying random times between 08:00 and 16:00 h.

Comparing the latitude and longitude for the different study locations, there was no clear cut relationship; however, the two studies[13, 14] with similar results, in terms of T peaks in summer, were the closest for both latitude and longitude. The Norwegian study[16] was the most northerly at the highest latitude. The greatest difference between all the study locations was for the South Korean study, particularly in terms of its longitude; whether the percentage of sunshine hours/daylight hours

is important here or there is an effect of ethnicity on the differences seen between seasonal patterns of T secretion remains to be determined.

The differences in the patterns of T levels described during the various months of the year, as well as at differing geographies, have the potential to impact on the number of men being diagnosed with late-onset HG and thus the consequent morbidity and mortality associated with this condition.[11] However, as reliable and reproducible data for seasonal variation is limited, and the mechanism(s) are unknown, large longitudinal studies (in different environments/geographies using standardised protocols), with all participants having serial T levels measured, are required for the definitive answer. If seasonal differences are multifactorial in aetiology, this will be even harder to determine. Consequently, these inconsistencies in this evidence preclude its incorporation into existing clinical standards.

Longitudinal studies may shed light on the association between serum T and the seasons, as paired analysis of T levels (between seasons) can be carried out with subgroup analyses (groups stratified by age and other presenting phenotypes may show differing patterns). It is also worth studying the effects of melatonin (the association between serum T and melatonin is not clear-cut at present), vitamin D (again not clear-cut, but probable association between low vitamin D levels and the metabolic syndrome), and sunlight hours/ambient temperature just prior to phlebotomy, on the intra-patient variation in serum T.

Fasting status

On the basis that T levels exhibit significant diurnal and day-to-day variations, and may be suppressed by food intake or glucose, Bhasin *et al.*[17] recommended that clinicians should measure T concentrations on two separate mornings when the patient is fasting; their conclusion was that a fall in circulating T levels in the non-fasted state may give a falsely low serum T and as a result overestimate the number of men with suspected HG; however, this premise is based on a small number of studies, such as Lehtihet *et al.*[18] who reported a reduction in T levels of 30% at 60-120 minutes after a standard 550 kcal meal compared with samples taken in a fasting state. Reductions in serum total T and free testosterone (FT) after a mixed meal were also reported,[19] but, importantly, post-prandial decreases were less pronounced in men aged >40 years and/or with obesity. This is relevant as the majority of men having T levels checked are aged >40 years, so any effect appears less pronounced in the population being tested.

Tremellen *et al.*[20] reported that oral ingestion of high fat, fast food mixed meal produced a 25% fall in serum T within 1 hour of eating, with levels remaining suppressed below the fasting baseline for ≤4 hours; however, intravenous fat (intralipid) had no impact on T levels, suggesting the mechanism may operate through the intestinal tract by an indirectly mediated response. A decrease in circulating T and FT after a fat containing meal was also described by Meikle *et al.*,[21] but with no change after a mixed carbohydrate and protein meal; how this relates to current

dietary habits in the UK and elsewhere in the 21st Century is hard to say, but most people would likely have a mixed carbohydrate +/- protein breakfast on a working day, which is often when the blood sample for T measurement would be taken. Short-term fasting, as a metabolic stressor, may exert an inhibitory impact on the stress-responsive control of both the pulsatile and diurnal regulation of the male hypothalamic-pituitary-gonadal axis; thus, introducing a fast into the middle of an individual's normal routine, out of keeping with their usual circadian rhythm, may actually have an 'abnormal' effect on a number of their hormonal variables including T levels.

In other studies, T was reduced after a 75 g glucose load with a mean drop at the nadir of 3.5 nmol/L, but levels appeared reactive returning towards baseline even with 120 minutes;[22] similarly, Caronia *et al.*[23] also reported a 25% reduction in T after an oral glucose load.

In contrast to these studies and recommendations, only 8.5% of clinical laboratories in a recent UK-wide survey[24] made any reference to service users of the need for a fasting sample for T measurement; moreover, UK NEQAS (in data collected in June 2021) found that only 14% of clinical laboratories reported having this advice available for all users. However, in a retrospective laboratory-based study by our research group,[25] using real world data, no significant difference was found between paired non-fasting *versus* fasting samples [group 1] in the patients studied (nor between paired non-fasting samples [group 2] and paired fasting samples [group 3]), even when the analyses were adjusted for age and time between samples (Table 3.II); our view was that the analytical and within-subject biological variation components in relation to T measurement seemed to account for much of the difference seen. Furthermore, only 27.2% of samples for T requests were actually collected in the fasting state over the 11-year study period; there was also no significant difference between fasting *versus* non-fasting samples in men with T levels ≤10.4 nmol/L and in men with T levels ≤12.0 nmol/L (as indicative thresholds of potential late-onset HG),[6] nor between fasting and non-fasting T in subgroups stratified into those aged ≤50 and >50 years. This has significant implications for patients, in terms of convenience, as a fasting sample does not appear to be necessary for the measurement of serum T based on this data.

Weight and Body Mass Index (BMI)

Serum total T levels are reported to be lower in obese men compared with age-matched non-obese men, a decrease in SHBG concentration being a major contributing factor.[2, 26] The significant effect of increasing BMI and waist size on lowering circulating T levels was clearly shown in the European Male Ageing Study (EMAS; 2,599 men aged 40-79 years, 7% with type 2 diabetes),[5] with obesity (BMI >30 kg/m^2) postulated to impair hypothalamic/pituitary function. Low serum T levels have also been associated with Type 2 diabetes, dyslipidaemia, and the metabolic syndrome.[6] In 2017, the American Association of Clinical Endocrinologists

Table 3.II. Details of total testosterone (T) levels measured in matched samples.

	Median total testosterone (nmol/L) (IQR)					Number of patients	
	Number of patients	**Fasting sample**	**Non fasting sample**	**Change in values (fasting-non-fasting)**	**p (sign-rank test)**	**Increase in values (non-fasting)**	**Decrease in values (non-fasting)**
Group 1 (fasting sample *vs.* non -fasting sample	69	10.8 (8.9/14.1)	11.1 (9.3/13.6)	-0.2 (-1.5/1.7)	0.89	31	38
	Number of patients	Sample 1	Sample 2	Change values (Sample 1-Sample 2)	P (sign-rank test)	Increase in values (Sample 2)	Decrease in values (Sample 2)
Group 2 (two non fasting samples)	126	9.70 (8.1/13.2)	9.45 (7.8/12.9)	0.2 (-1.0/1.4)	0.33	65	59
Group 3 (two fasting samples)	18	14.0 (1.9/16.8)	14.8 (9.9/17.1)	0.4 (-1.4/2.1)	0.53	10	8

Group 1. Median of mean (IQR) T =10.7 (9.35/13.1) nmol/L.
Group 2. Median of mean (IQR) T =9.7 (8.3/13.1) nmol/L, P=0.026 (rank-sum, compared to Group 1).
Group 3. Median of mean (IQR) T =14.8 (10.9 / 17.2) nmol/L, p=0.056 (rank-sum, compared to Group 1).
Group 1. Median of mean T**% difference** between samples (IQR): -0.2 (-1.5/1.7) nmol/L.
Group 2. Median of mean T**% difference** between samples (IQR): 0.2 (-1.0/1.4) nmol/L, P=0.66 (rank-sum, compared to Group 1).
Group 3. Median of mean TT**% difference** between samples (IQR): 0.4 (-1.4 / 2.1) nmol/L, P=0.45 (rank-sum, compared to Group 1).
Group 1. Median Age (IQR) at initial sample: 49 (38/56) years.
Group 2. Median Age (IQR) at initial sample: 51.5 (42/60) years, P=0.26 (rank-sum, compared to Group 1).
Group 3. Median Age (IQR) at initial sample: 51.5 (40 / 59) years, P=0.48 (rank-sum, compared to Group 1).

and the American College of Endocrinology recommended T measurement in all men with type 2 diabetes, BMI greater than 30 kg/m^2 or waist circumference more than 102 cm.[6]

Acute and chronic illness

The interaction of systemic illness and androgen deficiency is not fully understood, but HG associated with acute and chronic illness is been increasingly recognized, whether resulting from the illness itself, its treatment, or compromised nutritional status.[27] Men who are critically ill uniformly develop a temporary hypogonadotropic gonadal insufficiency (at the hypothalamic/pituitary secondary level), regardless of their specific illness (*e.g.* general surgery, burns, acute myocardial infarction, respiratory failure, mechanically ventilated patients, anorexia nervosa), which is manifested by low total T levels not due to reduced sex hormone-binding capacity.[28] How to interpret low/low–normal serum T in systemic illnesses is not straight forward and should be differentiated into overt clinical HG and functional HG.[29] Further studies are needed to delineate the mechanisms by which male HG is related to differing systemic illnesses, and to determine the effects of T replacement in treating HG-associated reductions in quality of life and poorer health outcomes.

Other comorbid conditions not already mentioned that are associated with low circulating T levels include cardiovascular disease, heart failure, osteoporosis, depression, vitamin D deficiency, and Covid-19 (see Chapter 18).[6]

Analytical methodologies for the measurement of total testosterone levels (including common interferences) and the effect of binding proteins

Testosterone analysis

Routine testing laboratories use either immunoassay or mass spectrometry as their methodologies for determination of T levels. Some laboratories may have both techniques and the choice of which is used is usually based on assay availability within the laboratory and patient demographics. Often laboratories will be measuring T in a female matrix by mass spectrometry due to the lower T concentrations and the bigger impact of binding proteins on the T. This may influence the choice of method for male specimens; however, data from the UK NEQAS for Steroid Hormones scheme shows that at least 80% of participants are using an immunoassay for analysis of T in males.

In brief, an immunoassay is a testing procedure that uses an antibody to bind to a specific structure of a molecule. In this case antibodies to T are used to bind T and subsequent reactions allow the quantitative determination of the amount or concentration of T present in the sample. These are sometimes termed 'direct-assays' as there is no extraction or isolation of the T from any binding protein. This in turn means that there could be inaccuracies, especially at low T concentrations, if there

are any anomalies in the concentration of the binding protein present. Mass spectrometry is a more specific and sensitive technique as, theoretically, if used appropriately will only allow detection of a molecule such as T whereas immunoassays do have the potential of interference from molecules that are similar in structure to that being tested. Mass spectrometry has until now been a more laborious and expensive option; however, advances in this area are leading to more automation and random access for analysis of samples.

Immunoassays

In the UK, there are five main diagnostic manufacturers with T assays on their platforms — Abbott Diagnostics (Architect and Alinity), Beckman, Ortho Diagnostics, Roche Diagnostics and Siemens Healthineers (Advia Centaur, Atellica, Dimension and Immulite). All these T assays use similar assay architecture with subtle differences in the technology dependent on how the individual manufacturer handles immunoassays. All assays require endogenous T in the sample to be released from binding proteins and the endogenous T then competes with a 'labelled' T analog for binding sites on the T antibody. The antibody part of the reaction is usually prepared from animal sources, for example sheep. This means that there is the potential for heterophilic antibody interference in the assay if patients are routinely exposed to animals or animal products and they have developed these antibodies. Patients may also have anti-mouse antibodies if they have received preparations of mouse monoclonal antibody for diagnosis or therapy. This human-anti-mouse-antibody interference (HAMA) can cause falsely elevated or decreased results. Heterophilic antibody and HAMA interferences can affect all immunoassays. Unexpected or clinically discordant results on any analyte measured by immunoassay can be followed up by the laboratory by using a different methodology/manufacturer for analysis. Heterophilic blocking tubes are available which can be used to assess whether there is, or is not, interference, but they do not assist in giving the correct result.

The choice of immunoassay used for T is likely to be limited to that of the manufacturer that provides the routine service within the laboratory. A small sample volume is required for an immunoassay (10-200 µL) and immunoassays are random access which means that they are usually available on automated equipment so not analysed in batches. Turn-around times are specific to individual laboratories.

There are other interferences in immunoassays of which laboratories and clinicians need to be aware, for example haemolysis, icterus, lipaemia, biotin and other more specific substances which are similar in structure in this case to T. These and the effect that they have at different concentrations will be detailed in individual manufacturer kit inserts but it is not always clearly available to end users who may be more aware of other medication of clinical conditions for the patient. Haemolysis, icterus and lipaemia interference are often thought to only impact chemistry based spectrophotometric assays. This is not always the case. It is important that laboratories measure serum indices on all samples, and apply the corresponding cut off

values, even when only a test is requested that would be analysed on an immunoassay analyser.

A patient's sample is sometimes analysed for SHBG and albumin, at the same time as the T. This allows a calculated FT to be determined.

Mass spectrometry

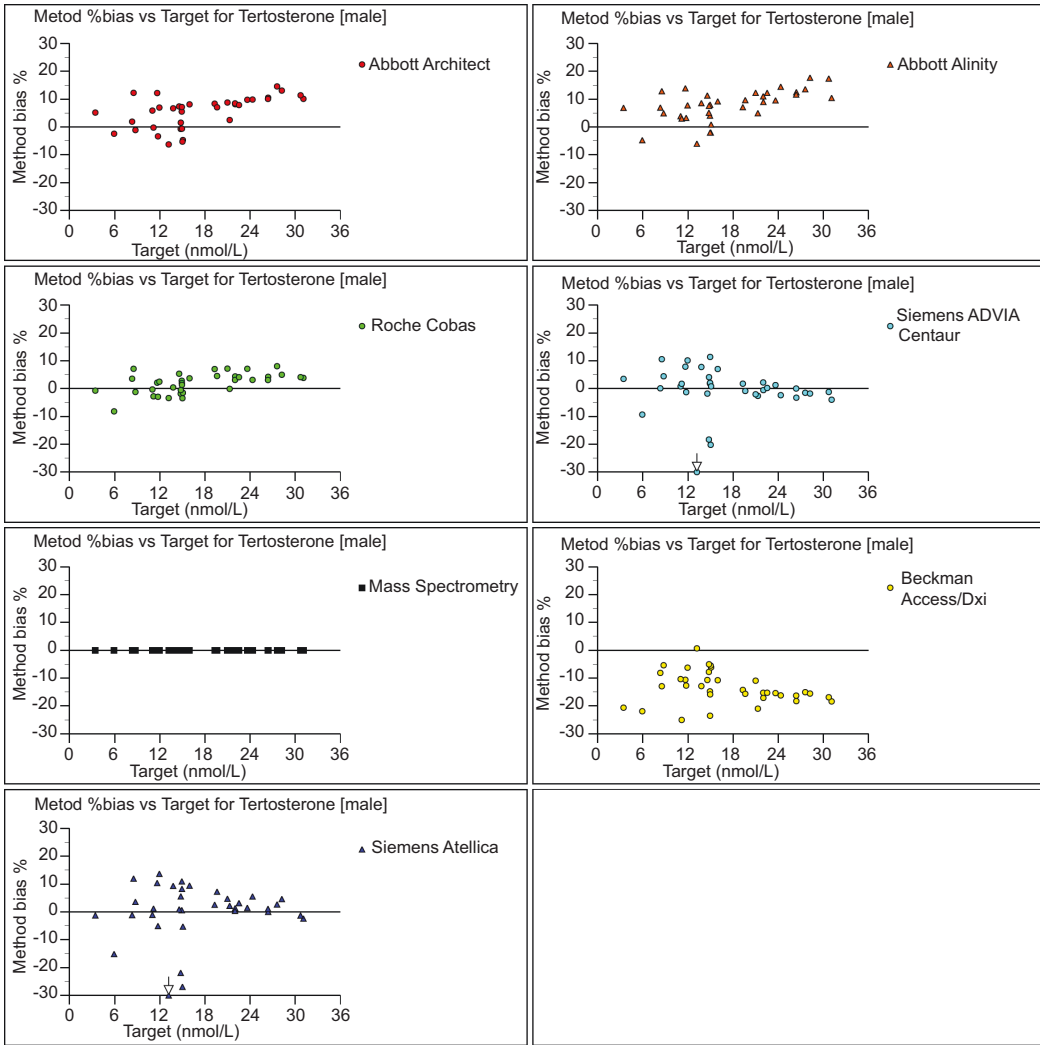
Mass spectrometry methods for T were primarily developed to improve the accuracy of T at low concentrations as found in samples from female patients. An extraction procedure is usually required, which removes binding proteins etc. from the sample. Liquid chromatography is then used to separate T from other components in the sample. The mass spectrometer quantifies the 'amount' of T and fragment(s) present based on their molecular weight. This can be converted to T concentration by the use of deuterated internal standards and appropriate calibrators. Due to the high specificity of mass spectrometry for specific mass to charge ratios, which are unique for each analyte, there is a minimal impact of cross reactivity from other substances.

Though mass spectrometry as a technique is analytically superior to immunoassay, for T, variation is still observed between mass spectrometry users which could be due to the differences in assay protocols used. Some laboratories have developed and optimised their own assays for T whilst others use commercially available kits which standardise columns, reagents and plates. In general, the between-laboratory agreement for mass spectrometry users is similar to that for immunoassay. From UK NEQAS for Steroid Hormones EQA data in 2021 the between-laboratory imprecision for all assays is between 5-10% across the concentration range 0.5-35 nmol/L.

In 2007, the Endocrine Society issued a Position Statement relating to the utility, limitations and pitfalls in measuring T.[30] The main conclusion was that when standardisation was in place, normative values for total T and FT could be established. They recommended that laboratory proficiency testing (PT/EQA) should be based on the ability to measure T accurately and precisely not only agreement with other users of a method. The UK NEQAS for Steroid Hormones EQA Scheme distributes single serum donations from males, multiple serum donations from males pooled together and male serum with known amounts of exogenous T added. The mass spectrometry mean is used as the target value. Figure 3.1 shows the method bias of the major manufacturers against the mass spectrometry target value. It is clear to see that there are concerning differences in bias, across the concentration range, between the different manufacturers. It will be difficult to fully harmonise the measurement of total T and harmonise associated male T reference ranges whilst these differences in T assays exist.

Also in 2007, the Centers for Disease Control and Prevention (CDC) began a project to standardise and harmonise hormone measurements to ensure accurate and comparable results across assays, across laboratories, over time. Reference method analysis for T now exists, but sample volume, cost and time for analysis

Figure 3.1. Method bias of testosterone against the mass spectrometry method mean (based on average of at least 30 individual results) for the major manufacturers of testosterone immunoassay during the period 2020–2021 (Data from the UK NEQAS for Steroid Hormones EQA Scheme 2021).



prohibits the use to specific areas such as manufacturer assay validation and verification of target values for PT/EQA providers. Recent data from the T assay standardisation programme[31] supports the findings routinely observed by UK NEQAS with the conclusion that there has been an improvement in the accuracy and precision of T assays but there is still variability at low concentrations, which is a key area in the investigation of HG. Assay manufacturers should be encouraged to use

international standard reference materials to better standardise the measurement of total T in their assays to reduce the analytical variation of results.

Assay performance

Assay bias can and does change over time. Bias can also be dependent upon the concentration of the analyte. This is not unique to T assays. Changes in bias can be due to 1) planned assay changes made by the manufacturer or 2) insidious assay drift which is often caused by lot-to-lot variation of the reagent. Figure 3.2 shows the T assay bias over the five year period 2016 to 2021 for the major manufacturers and methods. Box and whisker plots (the median is a bar, the box is the 25th to 75th centiles and the whiskers 5th and 95th centiles) of bias data for each individual method is shown with time on the x axis and bias (individual method relative to mass spectrometry mean) on the y axis. Figure 3.2 shows not only the overall bias of individual methods at specific time points, but also the spread in results within that method. Data is from at least 200 laboratories in total, but the individual method data ranges from 5 to 80 laboratories dependent on the method. The overall bias for mass spectrometry is 0 due to it being used as the 'target value'. There are a range of biases for the other manufacturers and variation is seen even within a manufacturer.

The bias of an individual assay is an issue when universal reference ranges and/or cut off values are used for the diagnosis and management of HG. It is advised that laboratories make users aware of the assay systems that have been used to generate results and it is important that there is good communication between laboratories and service users on assay changes both at the high level (change in assay) but also any assay drift.

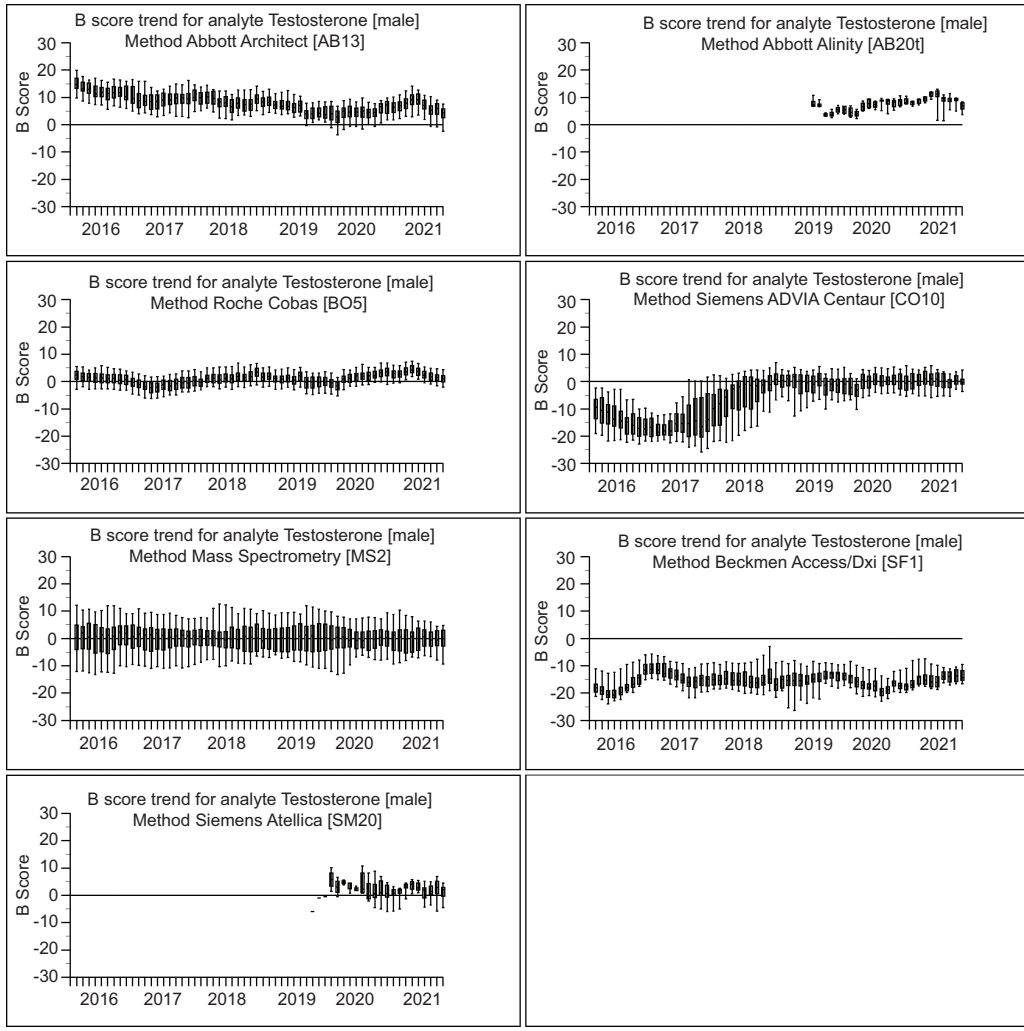
Changes in overall bias and variation in methods not only affects the management of individual patients but can also affect data held within clinical trials etc. Clinical trials often take place over a number of years and various assay systems could be used during this time. Though it may be difficult to restrict the assay systems that are used, knowledge of the variation in assay bias allows an appropriate uncertainty in the overall data to be calculated.

Variance in methodology and practices within Laboratory Networks pose a clinical risk if patient samples could be analysed across a range of laboratories. Differences in results may be analytical rather than physiological.

Calculated free testosterone

The free hormone theory states that only unbound T is active and able to bind to androgen receptors in target tissues in the body.[32] The assessment of FT is sometimes used in cases where the total T is near to the lower limit of normal or in patients with conditions that affect their SHBG levels. FT is most commonly calculated using equations such as the Vermeulen equation but analysis by equilibrium dialysis is often seen as the gold standard. The important point to remember about

Figure 3.2. Seismograph plots showing the bias and spread of bias (using standard box and whisker plots) for the different manufacturers, against the mass spectrometry mean as the target value, during the time period 2016-2021.



the calculation of FT is that the assay performance of the three assays T, SHBG and albumin all impact on the final calculation.

SHBG assays are immunoassays, like T, therefore prone to similar analytical variability, and indeed different manufacturers do have different biases and give different results on the same specimen for SHBG. Albumin assays are mainly chemical colorimetric. Colorimetric/spectrophotometric assays are known to be more heavily influenced by pre-analytical factors such as haemolysis.

The measurement of FT and its associated problems are discussed in another chapter in this volume and will not be further described here (see Chapter 4).

The clinical importance of using appropriate reference ranges/action limits for male testosterone levels and optimising the methodology for its measurement

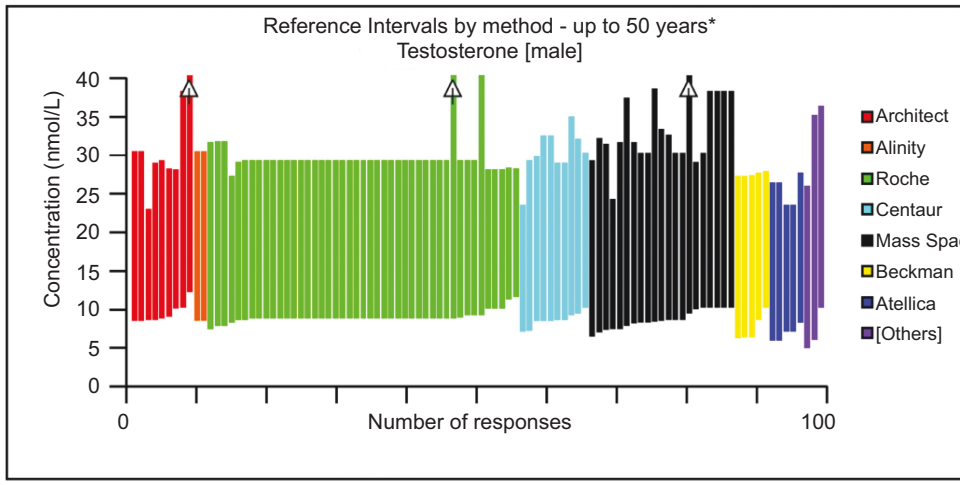
In a national survey of UK-wide NHS clinical laboratories in 2018,[24] most laboratories (84.4%) reported using immunoassays to measure male total T levels. A significant variation in reference intervals quoted by these laboratories for total T was evident; these varied significantly between the laboratories and different methods used, and even amongst users of the same method (as seen in Figure 3.1 and Figure 3.2). The varying lower limits seen have potential significant consequences for men with all types of HG (as defined in the introduction) with regards to initiation of TTh; a varying upper limit could cause adjustment to treatment, such as lowering of TTh dose (gels) or increasing the time between administrations (injectable), or even discontinuation. These data are supported by that reported in the UK NEQAS for Steroid Hormones Scheme (Figure 3.3). Here reference range data is shown by method and split into two age groups (<50 years and >50 years).

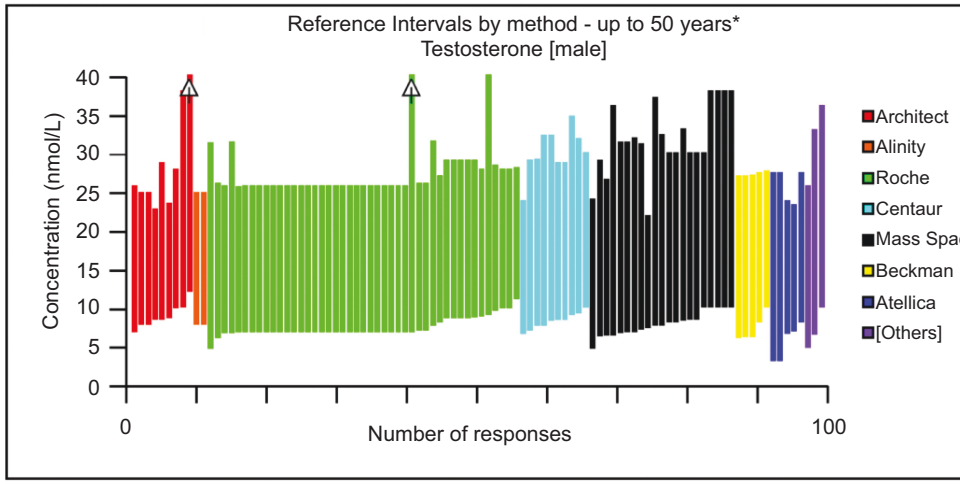
Reference intervals based on population distributions are often misinterpreted as the "normal" range and can lead to confusion in the absence of any treatment guidelines; interestingly, we have previously questioned whether reference intervals should now be replaced by action limits in male HG (with reference to the guidelines) to overcome this clinical issue,[33] particularly as treatment ambiguity often arises when T levels are borderline and, unfortunately, a borderline range is not acknowledged by a majority of laboratories.

Great disparity was also revealed in the actions taken by the laboratories for total T outside variable triggering levels. A calculated FT or bioavailable testosterone (BT) would be important here, in view of the free hormone hypothesis, especially when total T levels are between 8.0 and 12.0 nmol/L (230-346 ng/dL),[34-36] but again was often not provided/available. Studies have demonstrated associations between FT/BT levels and clinical conditions. Higher BT levels have been associated with a lower risk of the metabolic syndrome and of cardiovascular mortality.[37] These may also be more appropriate measures than total T; for example, their decrease with male ageing is steeper and associated with features of HG, whilst a decrease in total T can be exaggerated by SHBG levels rising with age (recently shown to be associated with symptoms of HG and mortality).[38, 39]

Any move away from reference ranges to action limits should be based on current evidence of benefit derived from the management of the pathology, analogous to diabetes and dyslipidaemia, for example, where we do see laboratory

Figure 3.3. Adult male total testosterone reference ranges by manufacturer method reported in the UK NEQAS for Steroid Hormones Scheme (2021) for those aged ≤50 years and aged >50 years (N.=100 different laboratories).





report comments including evidence-based action limits for glycated haemoglobin A1c and low-density lipoprotein cholesterol, respectively. Late onset HG is defined as a combination of low serum total T levels and associated symptoms (*e.g.* reduced bone mineral density (BMD), muscular strength and cognition, increased fatigue and sexual dysfunction).[6] The prevalence is estimated at 6-12% (and up to 40% in men with type 2 diabetes) and men with HG present to varying specialties including urology, general practice, diabetes and endocrinology. Longitudinal studies have demonstrated both HG and erectile dysfunction (an

associated symptom) to be independently associated with increased mortality, so the diagnosis is important clinically. A combination of symptoms associated with HG and total T <8.0 nmol/L (<230.5 ng/dL) has been shown to be significantly associated with increased total mortality and mortality related to cardiovascular disease (CVD).[40, 41] In the Testosterone Trial[42] total cohort, significant benefits were shown in sexual function, depression, mood, physical performance, vitality, quality-of-life, anaemia and BMD following TTh. Further, improvement in symptoms and reduced mortality rates have been shown with TTh in men aged >40 years with type 2 diabetes (where the prevalence is greater than in the general population) with a TT ≤8.7 nmol/L (≤250.7 ng/dL).[43] Two longitudinal studies in men with low T levels and type 2 diabetes confirmed this finding using a cut-off of 10.4 nmol/L (299.7 ng/dL),[44] and using T and calculated FT cut-offs of 12 nmol/L (345.8 ng/dL) and 0.25 nmol/L (7.2 ng/dL), respectively.[45, 46]

After a review of the available evidence, guidelines for the diagnosis and treatment of HG in men were published by the British Society for Sexual Medicine (BSSM)[11] suggesting that those T levels <8 nmol/L (<230.5 ng/dL) or calculated FT <0.180 nmol/L (<5.2 ng/dL) usually need TTh, while those with T in the range 8-12 nmol/L (230.5-345.8 ng/dL) may require TTh dependent on symptoms present associated with HG. Similarly, other national and international guidelines now recommend screening T levels in men with type 2 diabetes, the metabolic syndrome, and obesity, which will lead to an increased detection of candidates for treatment.[47-49] A number of clinical guidelines have been published now addressing the diagnosis and treatment of male HG, however, due to the quantity published (we counted eight published in recent years) this may actually contribute to the level of uncertainty amongst clinicians.[50]

Laboratory practice should support the prevailing clinical guidance. Thus, the diagnosis and management of HG in men (a condition with a high prevalence) is reliant on the analytical methodology to be both accurate and precise for the laboratory measurement of T concentrations; this will have a direct impact on treatment decisions for the initiation and monitoring of TTh. However, in a national audit of UK Biochemistry laboratories,[24] the responses showed considerable variation in practice in the measurement and reporting of male T levels. This is not surprising as there has been, for a number of years, awareness about this issue. The national laboratory survey indicated the need for harmonisation of laboratory function in this area. One option may be for the laboratory to assume a central role in the guidance provided for the diagnosis and treatment of HG with standardised pre-analytical protocols, information of test assays and post-analytical advisory comments. In doing so, clear interpretation of results could be given ensuring that the relevant treatment guidelines are accessible and/or referenced in the laboratory report. Nonetheless, a lack of assay standardisation remains across the existing T methods/platforms.

Conclusions

This chapter highlights the factors that introduce variation into the measurement of serum total T levels in men (and women) and need to be borne in mind when measuring this analyte. It reveals the inconsistencies between clinical laboratories and reinforces the requirement for ongoing action regarding the analytical standardisation of T assays and the harmonisation of pre- and post-analytical laboratory function (of reference ranges and advice provided to clinicians by the laboratory), particularly in relation to providing evidence-based action thresholds. Existing gaps between clinical associations and laboratory medicine associations can be addressed with joint ownership of testing recommendations. If this can be achieved, the situation could change for the better, in a relatively short period of time, for patients requiring measurement of their T levels.

References

1. Matsumoto AM, Bremner WJ. Serum testosterone assays--accuracy matters. J Clin Endocrinol Metab 2004;89:520-4.
2. Diver MJ. Analytical and physiological factors affecting the interpretation of serum testosterone concentration in men. Ann Clin Biochem 2006;43:3-12.
3. Trost LW, Mulhall JP. Challenges in Testosterone Measurement, Data Interpretation, and Methodological Appraisal of Interventional Trials. J Sex Med 2016;13:1029-46.
4. Wang C, Catlin DH, Demers LM, *et al.* Measurement of total serum testosterone in adult men: comparison of current laboratory methods *versus* liquid chromatography-tandem mass spectrometry. J Clin Endocrinol Metab 2004;89:534-43.
5. Wu FC, Tajar A, Pye SR, *et al.* Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. J Clin Endocrinol Metab 2008;93:2737-45.
6. Livingston M, Kalansooriya A, Hartland AJ, *et al.* Serum testosterone levels in male hypogonadism: Why and when to check-A review. Int J Clin Pract 2017;71:e12995.
7. Wu FC, Tajar A, Beynon JM, *et al.* Identification of late- onset hypogonadism in middle-aged and elderly men. N Engl J Med 2010;363:123-35.
8. Brambilla DJ, Matsumoto AM, Araujo AB, *et al.* The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. J Clin Endocrinol Metab 2009;94:907-13.
9. Plymate SR, Tenover JS, Bremner WJ. Circadian variation in testosterone, sex hormone-binding globulin, and calculated non-sex hormone-binding globulin bound testosterone in healthy young and elderly men. J Androl 1989;10:366-71.
10. Bremner WJ, Vitiello MV, Prinz PN. Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. J Clin Endocrinol Metab 1983;56:1278-81.
11. Hackett G, Kirby M, Edwards D, *et al.* British Society for Sexual Medicine guidelines on adult testosterone deficiency, with statements for UK practice. J Sex Med 2017;14:1504-23.
12. Smith RP, Coward RM, Kovac JR, *et al.* The evidence for seasonal variations of testosterone in men. Maturitas 2013;74:208-12.

13. Santi D, Spaggiari G, Granata ARM, *et al.* Seasonal Changes of Serum Gonadotropins and Testosterone in Men Revealed by a Large Data Set of Real-World Observations Over Nine Years. Front Endocrinol (Lausanne) 2020;10:914.

14. Andersson AM, Carlsen E, Petersen JH, *et al.* Variation in levels of serum inhibin B, testosterone, estradiol, luteinizing hormone, follicle-stimulating hormone, and sex hormone-binding globulin in monthly samples from healthy men during a 17-month period: possible effects of seasons. J Clin Endocrinol Metab 2003;88:932-7.

15. Lee JH, Lee SW. Monthly variations in serum testosterone levels: Results from testosterone screening of 8,367 middle-aged men. J Urol 2021;205:1438.

16. Svartberg J, Jorde R, Sundsfjord J, *et al.* Seasonal variation of testosterone and waist to hip ratio in men: the Tromsø study. J Clin Endocrinol Metab 2003;88:3099-104.

17. Bhasin S, Brito JP, Cunningham GR, *et al.* Testosterone Therapy in Men with Hypogonadism: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018;103:1715-44.

18. Lehtihet M, Arver S, Bartuseviciene I, *et al.* Serum testosterone decrease after a mixed meal in healthy men independent of SHBG and gonadotrophin levels. Andrologia 2012;44:405-10.

19. Van de Velde F, Reyns T, Toye K, *et al.* The effects of age and obesity on postprandial dynamics of serum testosterone levels in men. Clin Endocrinol (Oxf) 2020;92:214-21

20. Tremellen K, Hill A, Pearce K. Mechanistic insights into the aetiology of post-prandial decline in testosterone in reproductive-aged men. Andrologia 2019;51:e13418.

21. Meikle AW, Stringham JD, Woodward MG, *et al.* Effects of a fat-containing meal on sex hormones in men. Metabolism 1990;39:943-46.

22. Gagliano-Jucá T, Li Z, Pencina KM, *et al.* Oral glucose load and mixed meal feeding lowers testosterone levels in healthy eugonadal men. Endocrine 2019;63:149-56.

23. Caronia LM, Dwyer AA, Hayden D, *et al.* Abrupt decrease in serum testosterone levels after an oral glucose load in men: implications for screening for hypogonadism. Clin Endocrinol (Oxf) 2013;78:291-96.

24. Livingston M, Downie P, Hackett G, *et al.* An audit of the measurement and reporting of male testosterone levels in UK clinical biochemistry laboratories. Int J Clin Pract 2020;74:e13607.

25. Livingston M, Hackett G, Ramachandran, *et al.* Is a Fasting Testosterone Level Really Necessary for the Determination of Androgen Status in Men Clin Chim Acta 2021;521:64-9.

26. Travison TG, Vesper HW, Orwoll E, *et al.* Harmonized Reference Ranges for Circulating Testosterone Levels in Men of Four Cohort Studies in the United States and Europe. J Clin Endocrinol Metab 2017;102:1161-73.

27. Kalyani RR, Gavini S, Dobs AS. Male hypogonadism in systemic disease. Endocrinol Metab Clin North Am 2007;36:333-48.

28. Turner HE, Wass JAH. Gonadal function in men with chronic illness. Clin Endocrinol 1997;47:379-403.

29. Rochira V. Hypogonadism in Systemic Diseases. In: Simoni M, Huhtaniemi I, editors. Endocrinology of the Testis and Male Reproduction. Cham, Switzerland: Springer International Publishing; 2017.

30. Rosner W, Auchus RJ, Azziz R, *et al.* Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. J Clin Endocrinol Metab 2007;92:405-13.

31. Cao ZT, Botelho JC, Rej R, *et al.* Impact of testosterone assay standardization efforts assessed via accuracy-based proficiency testing. Clin Biochem 2019;68:37-43.

32. Keevil BG, Adaway J. Assessment of free testosterone concentration. J Steroid Biochem Mol Biol 2019;190:207-11.

33. Ramachandran S, Konig CS, Hackett G, *et al.* Managing clinical heterogeneity: an argument for benefit based action limits. J Med Diag Therapy 2018;1:034701.

34. Antonio L, Wu FCW, O'Neill TW, *et al.* Low free testosterone is associated with hypogonadal signs and symptoms in men with normal total testosterone. J Clin Endocrinol Metab 2016;101:2647-57.

35. Nanjee MN, Wheeler MJ. Plasma free testosterone—is an index sufficient? Ann Clin Biochem 1985;22:387-90.

36. Morgentaler A, Zitzmann M, Traish AM, *et al.* Fundamental concepts regarding testosterone deficiency and treatment: international expert consensus resolutions. Mayo Clin Proc 2016; 91:881-96.

37. Lin J-W, Lee J-K, Wu C-K, *et al.* Metabolic syndrome, testosterone, and cardiovascular mortality in men. J Sex Med 2011;8:2350-60.

38. Morley JE, Perry HM. Androgen treatment of male ypogonadism in older males. J Steroid Biochem Mol Biol 2003;85:367-73.

39. Van den Beld AW, de Jong FH, Grobbee DE, *et al.* Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. J Clin Endocrinol Metab 2000;85:3276-82.

40. Pye SR, Huhtaniemi IT, Finn JD, *et al.* Late-onset hypogonadism and mortality in aging men. J Clin Endocrinol Metab 2014;99:1357-66.

41. Dong JY, Zhang YH, Qin YQ. Erectile dysfunction and risk of cardiovascular disease meta-analysis of prospective cohort studies. J Am Coll Cardiol 2011;58:1378-85.

42. Snyder PJ, Bhasin S, Cunningham GR, *et al.* Effects of testosteronetreatment in older men. N Engl J Med 2016;374:611-24.

43. Shores MM, Smith NL, Forsberg CW, *et al.* Testosterone treatment and mortality in men with low testosterone levels. J Clin Endocrinol Metab 2012;97:2050-8.

44. Muraleedaran V, Marsh H, Kapoor D, *et al.* Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. Eur J Endocrinol 2013;169:725-33.

45. Hackett G, Heald AH, Sinclair A, *et al.* Serum testosterone, testosterone replacement therapy and all-cause mortality in men with type 2 diabetes: retrospective consideration of the impact of PDE5 inhibitors and statins. Int J Clin Pract 2016;70:244-53.

46. Hackett G, Jones PW, Strange RC, *et al.* Statin, testosterone and phosphodiesterase 5-inhibitor treatments and age related mortality in diabetes. World J Diabetes 2017;8:104-11.

47. Seftel AD, Kathrins M, Niederberger C. Critical Update of the 2010 Endocrine Society Clinical Practice Guidelines for Male Hypogonadism: a systematic analysis. Mayo Clin Proc 2015;90:1104-15.

48. Handelsman Y, Bloomgarden ZT, Grunberger G, *et al.* American association of clinical endocrinologists and american college of endocrinology - clinical practice guidelines for developing a diabetes mellitus comprehensive care plan - 2015. Endocr Pract 2015;21:1-87.
49. Mulhall JP, Trost LW, Brannigan RE, *et al.* Evaluation and management of testosterone deficiency: AUA guideline. J Urol 2018;200:423-32.
50. Salter CA, Mulhall JP. Guideline of guidelines: testosterone therapy for testosterone deficiency. BJU Int 2019;124:722-9.

4

Adult-onset testosterone deficiency: changes in SHBG and the role of calculated free testosterone

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Testosterone has multiple roles in male physiology. These include the development of the reproductive system, preservation of bone/muscle health, impact on both cognitive and sexual function as well as effects on the drivers of the metabolic syndrome including central obesity and insulin resistance.[1] Adult-onset testosterone deficiency (TD), a condition characterised by low testosterone levels and associated symptoms, has a high prevalence (around 40%) in men with type 2 diabetes (T2DM).[1] Importantly adult-onset TD has been associated with increased mortality, and in men with T2DM, longitudinal studies have shown that testosterone therapy (TTh) abolishes this increased mortality.[2, 3] Thus, accurate diagnosis of this highly prevalent condition and appropriate treatment is essential. As the symptoms of adult-onset TD are varied, patients may be referred to various clinical specialties (endocrinologists, urologists, general practitioners etc.) indicating the need for robust guidelines.

The use of low serum testosterone (total testosterone and in some cases free testosterone) in diagnosing adult-onset TD, varies amongst guidelines issued by the various societies listed below.

British Society for Sexual Medicine – 2017:[1] total testosterone ≤12 nmol/L (346 ng/dL), free testosterone ≤0.225 nmol/L (6.5 ng/dL), with a recommendation that free testosterone should be checked if total testosterone lies between 8 (231 ng/dL) -12 nmol/L (346 ng/dL).

Endocrine Society – 2018:[4] total testosterone <10.4 nmol/L (300ng/dL) or free testosterone <0.20 nmol/L (5.8 ng/dL).

International Society for Sexual Medicine – 2015:[5] total testosterone <12.0 nmol/L (346 ng/dL).

American Urological Association – 2018:[6] total testosterone <10.4 nmol/L (300 ng/dL).

International Society for the Study of the Aging Male – 2015:[7] total testosterone <12.1 nmol/L.

Use of free or bioavailable testosterone level is recommended when total testosterone levels do not relate to the presenting symptoms. Though the guideline does not propose a free testosterone threshold for the management of adult-onset TD, it acknowledged that some evidence supports use of the following free testosterone levels; 0.225 nmo/L (65 pg/mL), 0.243 nmol/L (70 pg/mL) and 0.347 nmol/L (100 pg/mL) as lower thresholds based on clinical experience.

Clearly, the above guidelines vary in the serum total testosterone thresholds used in diagnosing adult-onset TD. Further, the role of free testosterone is unclear. We will discuss clinical use of these variables together with sex hormone binding globulin (SHBG), a major determinant of the free hormone concentration.

Free hormone hypothesis and SHBG

Lipophilic hormones are mainly bound to specific and non-specific proteins for transport in plasma. The free (unbound) fraction is determined by the concentration of carrier proteins, particularly SHBG, and the respective binding affinities. The free hormone hypothesis is based on the premise that only the unbound fraction is physiologically functional after passively crossing into the target cell.[8] Mutations in the gene coding for SHBG can alter hormone affinity and binding leading to changes in the total free hormone concentration, but do not appear to be associated with significant pathology. These findings have been suggested as support for the free hormone hypothesis.[8] By contrast, Handlesman in 2017, argued strongly against the free hormone hypothesis, refuting three of the core assumptions; only unbound hormone is internalised, protein-bound testosterone is inert and, the mode of action of free testosterone on all tissues is via similar mechanism(s).[9] Thus, in addition to the free hormone concentration it can be argued that intra-cellular free hormone is dependent on blood flow, rate of cellular hormone uptake and intracellular metabolism of the hormone, with all these factors possibly having a bearing on clinical phenotype. The validity of the free hormone hypothesis is further questioned by the demonstration that cellular uptake of hormone bound to SHBG may occur via the endocyte receptor, megalin.[10] In addition, there are suggestions from *in vitro* research that the internalized SHBG could modify the effects of testosterone on prostate specific antigen and androgen receptor mRNA expression.[11]

Testosterone is mainly bound to SHBG (44%) with high affinity ($K_a \sim 10^9$ L/mol), as well as the more abundant albumin (50%) with lower affinity (K_a; $\sim 3X10^4$ L/mol). Only about 2% of testosterone is found in the unbound state.[8] The term bioavailable testosterone is used when in addition to the free testosterone, the hormone fraction bound weakly to albumin is also taken into account. SHBG, a homodimeric

glycoprotein (90 kDa) is largely synthesised in the liver with each monomer having a single steroid binding site, thus permitting transport of two steroid molecules/dimer.[8, 12] SHBG expression is increased by liver disease, nephrotic syndrome, oestrogens, thyroid hormones, adiponectin and PPAR-gamma agonists and decreased by proinflammatory cytokines.[8] Cortisol binding globulin (K_a 5.3X10^6 L/mol) and orosomucoid (K_a 3.0X10^5 L/mol) bind the steroid weakly and are usually not taken into clinical consideration. [8, 12] We have previously shown that in men with T2DM, SHBG levels were positively associated with age and negatively with glycaemic control, body mass index, lipids (triglycerides, high density lipoprotein cholesterol) and statin treatment.[13] Interestingly, variations in the gene encoding SHBG have been proposed to contribute to the development of T2DM.[14] Furthermore, we demonstrated that in men with T2DM and adult-onset TD, TTh (testosterone undecanoate) reduced SHBG levels, suggesting a more complex regulation of SHBG levels than a basic feedback on synthesis by plasma testosterone concentrations.[15]

The symptoms and characteristics of adult-onset TD are nonspecific and varied and not always associated with low testosterone levels, prompting interest in the relationship between SHBG and clinical phenotype.[12, 13] While SHBG may not have a direct effect on clinical outcomes we might expect higher serum SHBG levels to be associated with greater morbidity/mortality as free/bioavailable testosterone levels would presumably be lower. An insight into the biological role of SHBG may be gained by investigating individuals who have no peptide; Vos *et al.* described an extremely rare missense mutation that encoded a defective SHBG variant that accumulated in cells.[16] A brother and sister, homozygotes for this mutant allele, had no detectable serum SHBG. In the brother, serum total testosterone levels were low but free levels appeared adequate. Gonadal development and sperm production and function appeared normal though he had symptoms of hypoandrogenism. These findings suggest SHBG is required for some of testosterone's anabolic activities but is not essential for male reproductive development.[16]

It is noteworthy that a physiological role for SHBG is not indicated by the finding that serum concentrations vary with marked inter- and intra-individual variation.[17] For example, Krakowsky *et al.* found SHBG ranging between 6-109 nmol/L,[18] while we found in 857 men with T2DM, concentrations ranging between 7.9-185.5 nmol/L.[13] Further, SHBG levels increase with age during adulthood.[13, 18]

It is possible that the free hormone hypothesis is overly simplistic regarding the passive role of SHBG and therefore, mechanism(s) of action of testosterone. Indeed, presently available knowledge is insufficient to properly understand the role of free and bioavailable testosterone in determining clinical phenotypes. Nonetheless, there is data indicating a key role for free testosterone. For example, the European Male Aging Study showed men with normal total but low free testosterone (using the algorithm of Vermeulen *et al.*[19]) had higher luteinising hormone (LH) levels, and more sexual and physical symptoms associated with adult-onset TD than men with normal total and free testosterone, or men with low total and

normal free testosterone levels.[20] Thus, it is probably right at present, to adopt a pragmatic approach and use free and bioavailable testosterone as useful adjuncts to total testosterone as suggested by some of the previously cited guidelines. Another more difficult and perhaps cumbersome option, not mentioned by guidelines is to use serum total testosterone and SHBG independently (as we have demonstrated SHBG to be associated with mortality, independent of calculated free testosterone) to help with the diagnosis and treatment of adult-onset TD, although more research is required in this area.

Use of free testosterone in clinical practice

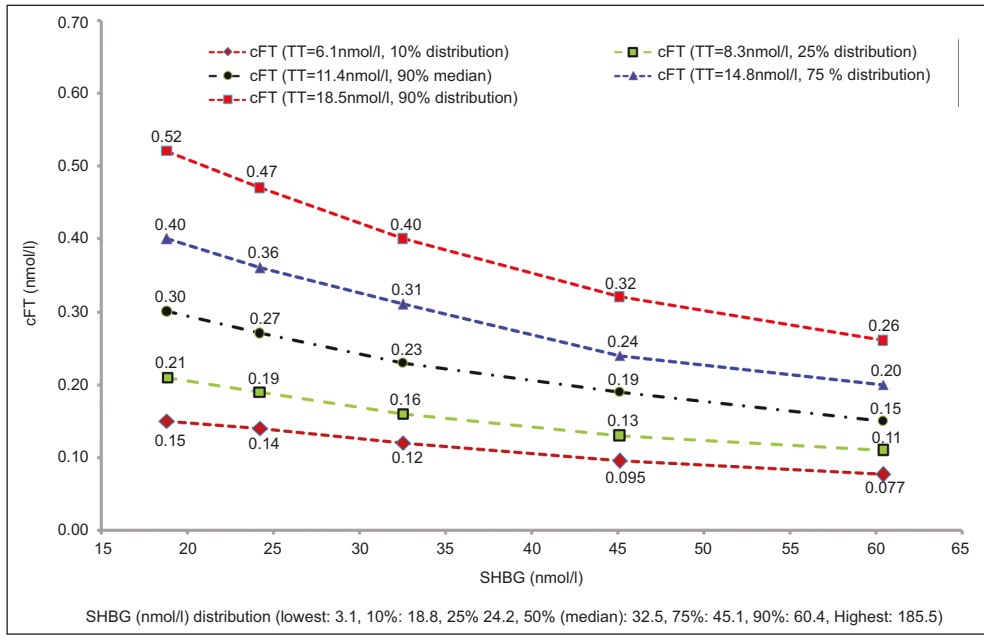
The direct measurement of free and bioavailable testosterone is not without issue. The reference method of estimating free testosterone, equilibrium dialysis, lacks standardisation.[21, 22] Non-SHBG bound testosterone can be measured by precipitating the bound fraction with ammonium sulphate.[22] Both methods are laborious and time consuming, and hence, not available in routine healthcare laboratories.[22] Free testosterone can be relatively rapidly measured by immunoassay using an analogue ligand, but the values obtained vary when compared with equilibrium dialysis.[19, 21, 22] A recent questionnaire-based audit involving scientists from 96 laboratories in the United Kingdom (UK)/Republic of Ireland showed 50 of 58 laboratories offering calculated free testosterone as part of a cascade protocol after initially measuring total testosterone.[23] None of the laboratories offered direct measurement of free or bioavailable testosterone. The audit included data from UK National External Quality Assurance Service that showed only 32 (14.7%) of 218 laboratories enrolling into the scheme (December 2018) used tandem mass spectrometry.[23]

Free testosterone concentration is calculated using the serum levels of albumin, SHBG and total testosterone in one of a variety of derived equations.[21, 24] Some equations assume mass-action binding between testosterone, albumin and SHBG and require values for the dissociation constants of the albumin-testosterone and SHBG-testosterone complexes in addition to concentrations of albumin, SHBG and total testosterone.[19] Importantly while SHBG is a homodimer with each monomer capable of binding testosterone,[8,12] some calculations assume that one molecule of the dimer binds a single molecule of testosterone.[19, 25, 26] A further issue is the wide variation in published values used for testosterone binding affinities to SHBG. These are determined *in vitro* under conditions that make it difficult to replicate the physiological *milieu.* A more recently reported model described by Zakharov *et al.* proposes testosterone binds to two allosteric sites with distinct affinities rather than two sites with the same affinity.[27] These considerations mean that free testosterone determinations can at best only be approximations of the true levels of the hormone particularly given the complex milieu of serum. Further, in addition to the above fundamental issues, the inadequate quality of both testosterone and SHBG laboratory measurements *via* immunoassays have been highlighted, adding to the difficulty of

obtaining only approximations of the actual level of free hormone. In recognition of this the Endocrine Society states that in the event of equilibrium dialysis not being available for measuring free testosterone, clinicians should use a formula that accurately estimate free testosterone concentrations using total testosterone, SHBG, and albumin concentrations.[4] Emphasis is also placed upon accurate measurements of total testosterone, SHBG, and albumin. Figure 4.1 and Figure 4.2 show the change in calculated free testosterone and calculated bioavailable testosterone in relation to SHBG and total testosterone levels, using the algorithm by Vermeulen *et al.*[19] A near doubling of both calculated free testosterone and bioavailable testosterone is seen with the SHBG at 10% of the distribution, compared to SHBG at 90% of the distribution. This near doubling was observed at all total testosterone levels.

However, while determination and the clinical use of free/bioavailable testosterone levels remain unsettled, it is important to recognise the limitations of total testosterone levels resulting from both inter-individual variation in SHBG levels and other determinants of androgen sensitivity including the functionality of androgen receptors and possible actions of other hormones. This paradigm is supported by

Figure 4.1. Calculated free testosterone levels at various SHBG and total Testosterone levels (10^{th}-90^{th} percentile in men with T2DM) in the BLAST screened cohort. cFT was calculated using the algorithm by Vermeulen *et al.*[19] with albumin concentrations maintained at the default 43 g/L.



the European Male Aging Study showing men with normal total but low free testosterone levels (using the algorithm by Vermeulen *et al.*[19]), had higher LH levels and more sexual and physical symptoms than men with both normal total and free testosterone levels or men with low total and normal free testosterone levels.[20] Thus, low free testosterone (even with a normal total testosterone) appeared to be associated with TD, while low total testosterone with normal free testosterone was not. Further, studies have shown significant associations between levels of free/bioavailable testosterone and clinical endpoints. For example, higher bioavailable testosterone levels were associated with a lower risk of the Metabolic Syndrome and of cardiovascular mortality.[28] Thus, in clinical practice, calculated free testosterone may be useful in the assessment of men with symptoms suggestive of adult-onset TD and a borderline total testosterone of 8-11 nmol/L but less helpful in men with a total testosterone level <8 nmol/L.[1]

Use of total testosterone and SHBG as independent factors

In view of the problem of using free testosterone (measured or calculated), another option, especially in a research setting, is to consider total testosterone and SHBG (in the event of it being independently associated with clinical outcomes) as independent risk factors.

Rastrelli *et al.* showed that higher SHBG concentrations were significantly associated with features of TD; this association being independent of total testosterone levels.[29] Based on the above finding, they suggested that TD could be associated with both, lower testosterone production and decreased biological activity.[29] Higher SHBG, independently of total testosterone, is associated with either subjective or objective androgen deficiency features. This indicates that besides a hypogonadism due to an impaired testosterone production, a hypogonadism due to a lower biological activity of testosterone does exist.

Tint *et al.* demonstrated that SHBG levels (which increased with age) were positively correlated with all-cause mortality after adjusting the analysis for age.[30] We initially confirmed these finding using data from individuals not on TTh in the BLAST screened cohort, by studying the association between SHBG, age, total testosterone and other factors associated with SHBG and mortality in men with T2DM.[13] Age, SHBG and total testosterone were significantly associated with all-cause mortality. When calculated free testosterone (using the algorithm by Vermeulen *et al.*[19]) was substituted for total testosterone, it was significantly associated with mortality. Interestingly age and SHBG remained significantly associated with mortality.[13]

We then stratified total testosterone and SHBG and studied associations with all-cause mortality. Age, SHBG (median=35nmol/L) and total testosterone levels

(12nmol/L was chosen as a threshold for adult-onset TD) were significantly and independently associated with all-cause mortality. The median SHBG values were slightly different from the values shown in Figure 4.1 and Figure 4.2, which were baseline values in the total cohort, that included men who were subsequently commenced on TTh. Mortality and age increased with SHBG quartiles. Importantly the association of SHBG with mortality appeared age-dependent; the combination of median age >66 years/median SHBG >35 nmol/L was significantly associated (Hazard ratio (HR): 9.44, 95% CI: 3.24, 27.53, P<0.001) with mortality (25.22%) compared to age ≤ 66 years/SHBG ≤35 nmol/L (mortality: 3.63%).[13] We also showed that in men >66 years, SHBG ≤35 nmol/L (cf. SHBG >35 nmol/L) was associated with lower mortality (HR: 0.35, 95% CI: 0.16, 0.76, P=0.007). In men aged ≤66 years, SHBG stratified by the median of 35 nmol/L was not associated with mortality, hence, the association between SHBG and all-cause mortality only appears in older men with T2DM. In contrast, men with total testosterone <12 nmol/L were significantly associated with increased mortality in both age categories. Thus, while the association of SHBG with mortality in T2DM men is mediated by age, a total testosterone <12 nmol/L was not. Figure 4.3A (men ≤66 years) and Figure 4.3B (men

Figure 4.2. Bioavailable testosterone levels at various SHBG and total Testosterone (both analytes at 10th-90th percentile in men with T2DM) in the BLAST screened cohort. Bioavailable testosterone was calculated using the algorithm by Vermeulen *et al.*[19] with albumin concentrations maintained at the default 43 g/L.

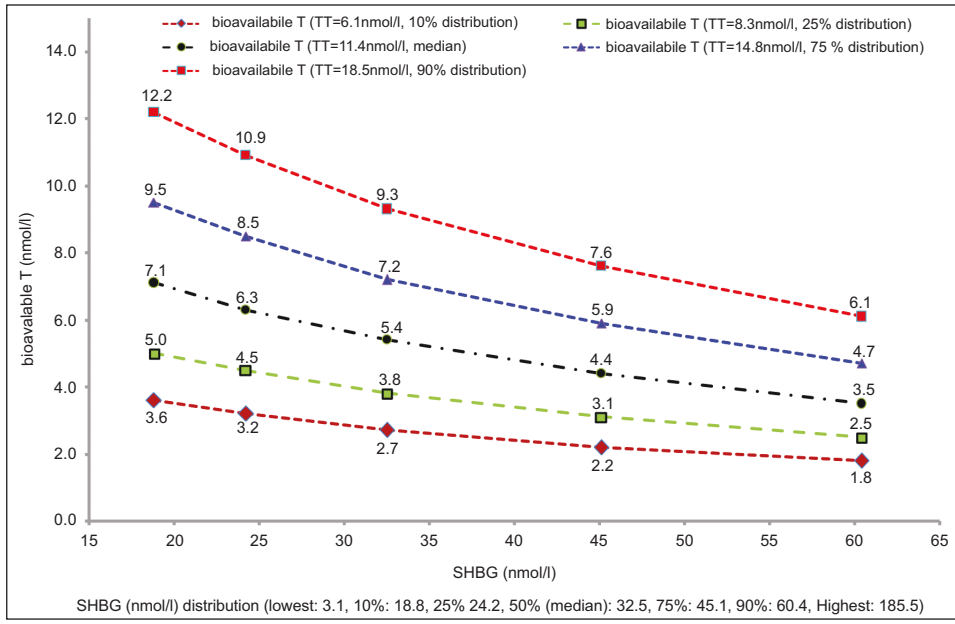
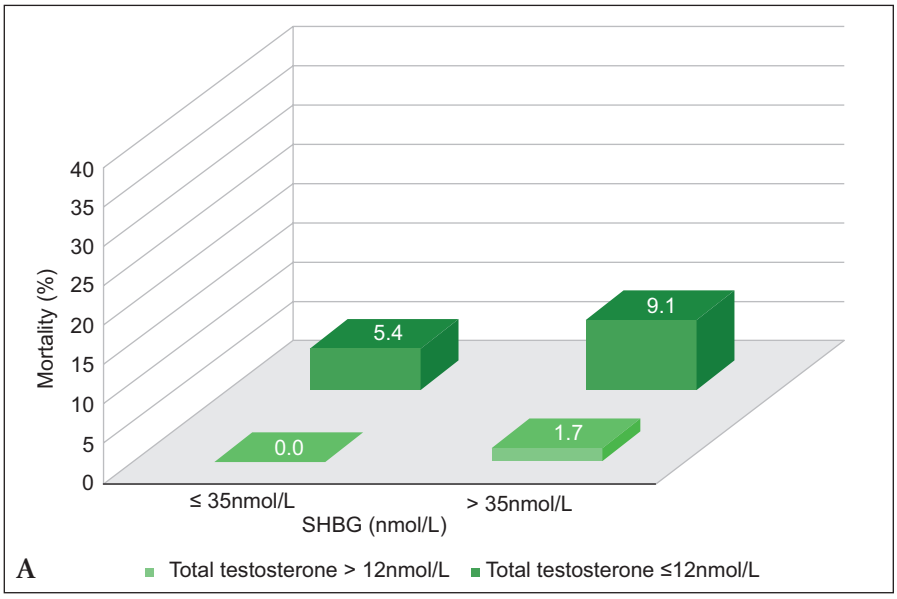
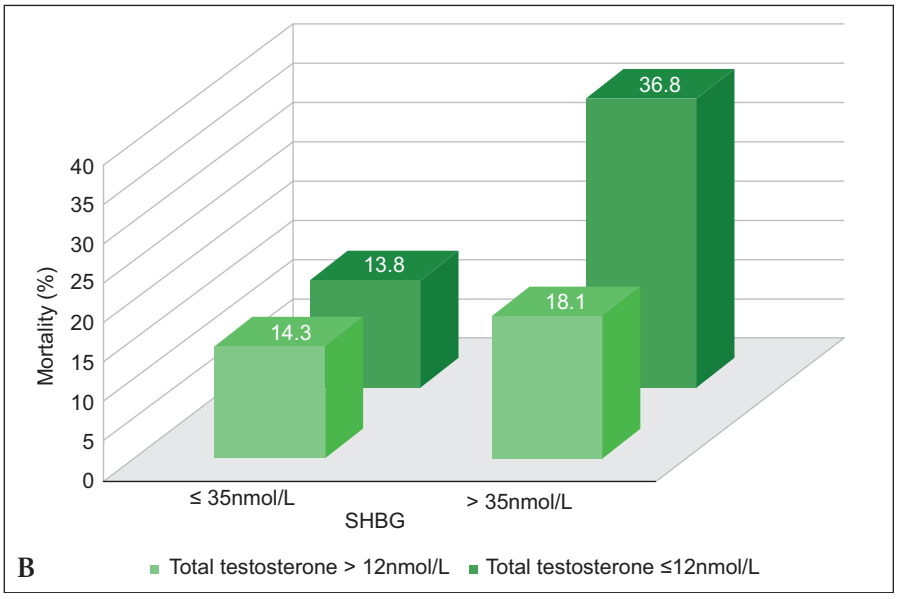


Figure 4.3. Association between total testosterone, SHBG (stratified by median values) and mortality during follow-up in men with T2DM from the BLAST cohort study, not on TTh. Separate bar charts are shown in men ≤66 years (median) (Figure 4.3A, N.=180) and > (Figure 4.3B, N.=182). Figure 4.3A (age ≤66 years, median follow-up =4.0 years). Figure 4.3B. (age >66 years, median follow-up =3.7 years).





>66 years) show all-cause mortality in men with T2DM stratified by SHBG and total testosterone. In men >66 years, the combination of total testosterone ≤12 nmol/L and SHBG >35 mmol/L increased mortality risk greatly. Cox regression analysis in the same cohort did not show any significant difference in survival between the other 3 groups.[13] This together with data shown in Figures 4.3A and 4.3B suggest that the risk associated with total testosterone, SHBG and calculated free testosterone (as it did not negate the association between SHBG and mortality) is complex. The role of SHBG is clearly not straight forward. We do not suggest that SHBG is directly associated with mortality given the wide distribution. It could be that the algorithm used to calculate free testosterone does not accurately estimate the free hormone. Figures 4.3A and 4.3B also suggest that the risk of mortality does not correlate in a linear pattern with both total testosterone and SHBG. Thus, with more studies using stratified SHBG groups as opposed to a continuous variable, risk thresholds may be adopted.

Conclusions

In this chapter we initially described the differing recommendations from various guidelines regarding the use of total testosterone and free testosterone in the diagnosis of adult-onset TD. Understanding the role of free testosterone is essential if the free hormone hypothesis is to be accepted, and we discuss the merits of this perhaps flawed theory. We accept that the free/bioavailable form of testosterone is active. However, questions remain whether testosterone bound to SHBG is active; the negative correlation between SHBG and clinical symptoms/outcome[13, 29, 30] suggest that even if the testosterone bound to SHBG was active, it would be lower than that seen with the free/bioavailable testosterone. Less than ideal performance of immunoassays used commonly to measure total testosterone and SHBG hamper calculation of free testosterone. There appear to be fundamental issues in the algorithms commonly utilised in the calculation of the free hormone. Even direct measurement of both free and bioavailable testosterone has methodological issues in addition to impracticality. Thus, given the above uncertainties, is stopping use of free/bioavailable testosterone in clinical practice until further clarity exists, a reasonable option? Although this point can be debated, our view is that this should not be the case. It is though important that clinicians are aware of the issues surrounding the use of free/bioavailable testosterone in their clinical decision making. Clinical practice is often pragmatic and subjective, making use of the available evidence, even if imperfect. Adult-onset TD is interesting in that it combines clinical presentation with laboratory testosterone measurements. This is appropriate as testosterone alone would be inadequate as other factors such as testosterone receptor sensitivity would have an impact on TD.[31] Despite all the issues with the free hormone hypothesis and methodology, free testosterone levels appear to correlate with clinical symptoms of adult-onset TD.[20] Thus, use of free/bioavailable

testosterone as an adjunct in the clinical management of adult-onset TD is perhaps merited. This is in view of free testosterone levels correlating with clinical symptoms of adult-onset TD. Using free testosterone in an ordinal scale (as opposed to continuous) with adopted thresholds mirroring some of the guidelines[1, 4] may therefore overcome some of the problems. However, GH and SR, as practising consultants find free testosterone values useful (as an adjunct to total testosterone) in making TTh treatment decisions in men with symptoms of adult-onset TD who have otherwise normal total testosterone/low free testosterone. The definition of adult-onset TD, with both clinical and biochemical components permits such a flexible approach. Indeed, this flexibility is compatible with the guidelines of the British Society for Sexual Medicine in 2017[1] and the Endocrine Society in 2018 (see Chapter 11).[4] Despite the pragmatic approach that we would advocate when using free testosterone at this time, it is clear that further clinical/mechanistic research and method development are required.

References

1. Hackett G, Kirby M, Edwards D, *et al.* British Society for Sexual Medicine guidelines on adult testosterone deficiency, with statements for UK practice. J Sex Med 2017;14:1504-23.
2. Muraleedharan V, Marsh H, Kapoor D, *et al.* Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. Eur J Endocrinol 2013;169:725-33.
3. Hackett G, Heald AH, Sinclair A, *et al.* Serum testosterone, testosterone replacement therapy and all-cause mortality in men with type 2 diabetes: retrospective consideration of the impact of PDE5Inhibitors and statins. Int J Clin Pract 2016;70:244-53.
4. Bhasin S, Brito JP, Cunningham GR, *et al.* Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018;103:1715-44.
5. Dean JD, McMahon CG, Guay AT, *et al.* The International Society for Sexual Medicine's Process of Care for the Assessment and Management of Testosterone Deficiency in Adult Men. J Sex Med 2015;12:1660-86.
6. Mulhall JP, Trost LW, Brannigan RE, *et al.* Evaluation and management of testosterone deficiency: AUA guideline. J Urol 2018;200:423-32.
7. Lunenfeld B, Mskhalaya G, Zitzmann M, *et al.* Recommendations on the diagnosis, treatment and monitoring of hypogonadism in men. Aging male 2015;18:5-15.
8. Bikle DD. The Free Hormone Hypothesis: When, Why, and How to Measure the Free Hormone Levels to Assess Vitamin D, Thyroid, Sex Hormone, and Cortisol Status. JBMR Plus 2020;5:e10418.
9. Handelsman DJ. Free Testosterone: Pumping up the Tires or Ending the Free Ride? Endocr Rev 2017;38:297-301.
10. Hammes A, Andreassen TK, Spoelgen R, *et al.* Role of endocytosis in cellular uptake of sex steroids. Cell 2005;122:751-62.

11. Li H, Pham T, McWhinney BC, *et al.* Sex Hormone Binding Globulin Modifies Testosterone Action and Metabolism in Prostate Cancer Cells. Int J Endocrinol 2016;2016:6437585.

12. Ramachandran S, Hackett GI, Strange RC. Sex Hormone Binding Globulin: A Review of its Interactions with Testosterone and Age, and its Impact on Mortality in Men with Type 2 Diabetes. Sex Med Rev 2019;7:669-78.

13. Ramachandran S, Strange RC, Fryer AA, *et al.* The association of sex hormone-binding globulin with mortality is mediated by age and testosterone in men with type 2 diabetes. Andrology 2018;6:846-53.

14. Ding EL, Song Y, Manson JE, *et al.* Sex hormone-binding globulin and risk of type 2 diabetes in women and men. N Engl J Med 2009;361:1152-63.

15. Ramachandran S, Hackett GI, Strange RC. Testosterone replacement therapy: Pretreatment sex hormone-binding globulin levels and age may identify clinical subgroups. Andrology 2020;8:1222-32.

16. Vos MJ, Mijnhout GS, Rondeel JM, *et al.* Sex hormone binding globulin deficiency due to a homozygous missense mutation. J Clin Endocrin Metab 2014;99:E1798-E1802.

17. Hammond GL. Diverse roles for sex hormone-binding globulin in reproduction. Biol Reprod 2011;85:431-41.

18. Krakowsky Y, Conners W, Morgentaler A. Serum Concentrations of Sex Hormone-binding Globulin Vary Widely in Younger and Older Men: Clinical Data from a Men's Health Practice. Eur Urol Focus 2019;5:273-79.

19. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666-72.

20. Wu FC, Tajar A, Beynon JM, *et al.* Identification of late-onset hypogonadism in middle-aged and elderly men. N Engl J Med 2010;363:123-35.

21. Ly LP, Sartorius G, Hull L, *et al.* Accuracy of calculated free testosterone formulae in men. Clin Endocrinol (Oxf) 2010;73:382-8.

22. Surampudi PN, Wang C, Swerdloff R. Hypogonadism in the aging male diagnosis, potential benefits, and risks of testosterone replacement therapy. Int J Endocrinol 2012;2012:625434.

23. Livingston M, Downie P, Hackett G, *et al.* An Audit of the Measurement and Reporting of Male Testosterone Levels in UK Clinical Biochemistry Laboratories. Int J Clin Pract 2020;74:e13607.

24. Goldman AL, Bhasin S, Wu FCW, *et al.* Reappraisal of Testosterone's Binding in Circulation: Physiological and Clinical Implications. Endocr Rev 2017;38:302-24.

25. Mazer NA. A novel spreadsheet method for calculating the free serum concentrations of testosterone, dihydrotestosterone, estradiol, estrone and cortisol: with illustrative examples from male and female populations. Steroids 2009;74:512-9.

26. Södergard R, Bäckström T, Shanbhag V, *et al.* Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem 1982;16:801-10.

27. Zakharov MN, Bhasin S, Travison TG, *et al.* A multi-step, dynamic allosteric model of testosterone's binding to sex hormone binding globulin. Mol Cell Endocrinol 2015;399:190-200.

28. Lin JW, Lee JK, Wu CK, *et al.* Metabolic syndrome, testosterone, and cardiovascular mortality in men. J Sex Med 2011;8:2350-60.

29. Rastrelli G, Corona G, Cipriani S, *et al.* Sex hormone-binding globulin is associated with androgen deficiency features independently of total testosterone. Clin Endocrinol 2018;88:556-64.

30. Tint AN, Hoermann R, Wong H, *et al.* Association of sex hormone-binding globulin and free testosterone with mortality in men with type 2 diabetes mellitus. Eur J Endocrinol 2016;174:59-68.

31. Zitzmann M, Nieschlag E. Androgen receptor gene CAG repeat length and body mass index modulate the safety of long-term intramuscular testosterone undecanoate therapy in hypogonadal men. J Clin Endocrinol Metab 2007;92:3844-53.

5

Adult-onset testosterone deficiency, incident diabetes and mortality

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This chapter will initially focus on the association between adult-onset testosterone deficiency (TD) and type 2 diabetes (T2DM). This will be followed by a description of studies demonstrating increased mortality in this condition of high prevalence. We then move on to the role of testosterone therapy (TTh) in modifying the association between adult-onset TD and T2DM. Finally, we describe studies, including one by our own research group that shows the effects of TTh on the risk of mortality in men with adult-onset TD and T2DM. We hope that this chapter will encourage clinicians to actively screen for adult-onset TD and associated conditions (*e.g*, metabolic syndrome (MetS), T2DM, obesity etc.), and consider TTh in altering the pathogenesis and risk of mortality. Primary hypogonadism and pituitary disease leading to secondary hypogonadism (classical hypogonadism) will not be covered as there is little controversy regarding TTh in these circumstances.

Adult-onset testosterone deficiency

Testosterone is a pleiotropic hormone, with effects on muscle, adipose tissue, cardiovascular system, liver and brain, in addition to its better-known roles in male sexual development and reproduction. TD is defined by the presence of symptoms and signs of androgen deficiency, together with low serum testosterone.[1]

The term functional or adult-onset TD (also referred to as late-onset TD or hypogonadism) has been introduced over the last 20 years in order to describe symptomatic androgen deficiency in males (usually middle aged or older) in the absence of any intrinsic disorder of the hypothalamic-pituitary-gonadal axis.[2] The European Male Aging Study studied 3369 middle aged and older men for adult-onset TD from 8 European countries over a median follow-up of 4.4 years.[3] TD was defined herein as having 3 or more sexual dysfunction symptoms in the presence of a total

testosterone (TT) level <11.1 nmol/L and free testosterone (FT) level <0.22 nmol/L and was evident in 5.1% of men aged between 70 and 79 years compared to just 0.4% of men aged between 40 and 49 years.[3]

Adult-onset TD, insulin resistance, sex hormone binding globulin, MetS and T2DM

About 40% of men with T2DM may have adult-onset TD.[4] There is accumulating evidence for an association between low testosterone in men and prevalence of T2DM, with much of this suggesting that low testosterone predicts insulin resistance, MetS and T2DM development.[5, 6] Central obesity is considered to be a driver of insulin resistance and MetS, both of which predispose to T2DM. However, it must be borne in mind that the MetS is not one pathological entity, but a cluster of conditions defined by committees to aid clinical practice (ensuring that physicians address all the associated classifying pathologies when presented with one of them).[7] There is also a strong argument that adult-onset TD should be included within the MetS classification for men.[7]

The association between low testosterone and T2DM is not straightforward, with some research showing that sex hormone binding globulin (SHBG) may perhaps play an important independent role in this association. Low SHBG appears to predict MetS/T2DM with some studies suggesting this association to be independent of serum testosterone.[8] In fact, there is evidence that both low SHBG and low testosterone can predict the incidence of the MetS and T2DM independently of each other.[5, 6]

It has also been seen that low testosterone levels have been associated with hepatic steatosis.[9] Low testosterone and hepatic steatosis are both associated with MetS and T2DM. The hepatic synthesis of SHBG may be suppressed by intrahepatic fat.[10] Thus, it is possible that the relationship between SHBG and MetS/T2DM is influenced by worsening hepatic fat impairing SHBG production. SHBG production is also inhibited by testosterone, with SHBG levels falling during TTh.[11] It must be stated that serum SHBG and testosterone levels in men not on TTh, are positively correlated. The authors believe that clarity on the above independent associations with MetS/T2DM will only be achieved when the mechanisms of the changes in both SHBG and testosterone levels as well as the direction of the relationship are fully understood.

Increased insulin resistance has been found to be associated with lower serum testosterone.[12] A longitudinal study by Ottarsdottir *et al* found low endogenous testosterone was predictive of insulin resistance after 9.7 years follow-up.[13] Furthermore, high baseline homeostasis model assessment index of insulin resistance (HOMA-IR) did not predict low testosterone at follow up, hinting at low testosterone levels being potentially causal of insulin resistance.[13] More evidence of the direction of the association between low testosterone and insulin resistance is provided by intervention

trials. Rubinow *et al.* randomised healthy male volunteers to acute androgen deprivation, using gonadotrophin releasing hormone (GnRH) agonist, or to placebo.[14] The men in the deprivation arm developed greater insulin resistance.[14] Similarly, men with T2DM who underwent androgen deprivation therapy (for prostate cancer) have been found to have worsening glycaemic control.[15] Additionally a trial in non-diabetic men with prostate cancer showed increase in fasting insulin levels 3 months post therapeutic castration, even though fasting glucose remained similar.[16]

The roles of visceral adiposity (central obesity being a surrogate) and reduced muscle mass in adult-onset TD – both well-known effects– may explain the increase in insulin resistance.[17] However, increase in adiposity appears to lead to a reduction in testosterone, thus the association is perhaps bidirectional.[17] Interestingly inappropriately normal, or sometimes marginally low luteinizing hormone (LH) is evident in adult-onset TD whereas a suppressed/very low or high LH level would suggest pituitary pathology or primary testicular failure respectively. The hypogonadal-obesity hypothesis was an early proposed theory relating to a testosterone- oestradiol (E2) shunt due to adipose tissue aromatase increasing conversion of testosterone to E2.[18] The resulting low T:E2 ratio was thought to lead to inhibition of GnRH and LH secretion. However, men with TD have been found to have low E2 levels[19] hence an update of this hypothesis (hypogonadal-obesity-adipocytokine hypothesis) proposes increased levels of adipocyte inflammatory cytokines (*e.g.* TNFα, IL6), leptin and E2, which inhibit GnRH release and LH.[17] In contrast, a longitudinal study by Kupelian *et al.* found that low baseline testosterone in non-overweight patients predicted MetS and T2DM 15 years later.[6]

The above section has demonstrated the associations between adult-onset TD and MetS, as well as the drivers of MetS such as central obesity and insulin resistance, low SHBG and T2DM. Causation and directionality has not been demonstrated. Complete clarity is absent regarding the role of E2, and LH levels may be dependent on both testosterone and E2. We will now examine the effects of TTh on some of these associations.

Effect of TTh in adult-onset TD

There have been many studies investigating the impact of giving hypogonadal men testosterone replacement therapy (TTh), on the risk and progression of MetS/T2DM. In the recently published T4DM randomised control trial (RCT) which investigated the effect of TTh (compared with placebo) as an adjunct to lifestyle advice in men with impaired glucose tolerance or newly diagnosed T2DM, there were significant intra (cf. baseline levels) and inter (cf. placebo) decreases in glucose (fasting and 2 hours post oral glucose load) in the TTh group.[20] The fall in HbA1c was not significantly different to the controls. The investigators suggested that the HbA1c levels may have been affected by an increase in erythrocyte lifespan (which may be a

mechanism of the rise in haematocrit seen with TTh) as an explanation of these results (see Chapter 8).[20] There were other factors that may have contributed to the non-significant HbA1c decrease; the mean baseline HbA1c of 5.7% may not have been sufficiently high to demonstrate benefit, and the inclusion criteria was for men with Total testosterone ≤14.0 nmol/L irrespective of associated symptoms (thus, not all the men had true adult-onset TD). Interestingly, favourable changes in waist circumference, total muscle mass and total fat mass following TTh were evident.[20] Thus improvements in the glucose levels seen in the T4DM RCT are in accordance with the findings of the Moscow study, a 30-week RCT of 184 men with adult-onset TD, MetS and/or T2DM, where TTh was shown to decrease insulin resistance measured by HOMA-IR.[21] However, a RCT by Gianatti *et al* studied the effects of TTh (testosterone undecanoate (TU)) in 88 hypogonadal men with T2DM for 40 weeks and showed improvement in fat mass, lean mass and lipid parameters, but not in HbA1c levels or insulin resistance indicated by HOMA-IR.[22]

However, there have been studies which have shown improvement in HbA1c associated with TTh. Yassin *et al.* in 2019 examined data on 316 men with prediabetes (HbA1c between 5.7-6.4%) and adult-onset TD from 2 urology registries, followed up over 8 years.[23] TU was prescribed to 229 men of the cohort whilst the remaining 87 men opted out of TTh. HbA1c decreased significantly (mean ±SD: 0.39±0.1%) in the men on TU compared with an increase (mean ±SD: 0.63±0.03%) in the untreated men. Whilst 90% of the men on TU achieved normal glycaemic control (HbA1c <5.7%), 40.2% of the untreated men progressed to T2DM (HbA1c ≥6.5%).[23]

Groti *et al.* in 2018 carried out a 52-week RCT in obese men with T2DM not requiring insulin and similarly found a significant fall in HbA1c in men on TTh (10 weekly TU) compared with placebo.[24] Cai *et al.* performed a systematic review in 2014 of 5 randomised control trials (including 351 participants with a mean follow-up time of 6.5 months) and showed that TTh significantly reduced fasting plasma glucose levels, fasting serum insulin levels, HbA1c and triglyceride levels.[25]

Traish *et al.* analysed data from an observational, prospective, cumulative registry study including 656 men (age: 60.7±7.2 years) with total testosterone <12.1 nmol/L and symptoms of TD (360 men were prescribed TU whilst the remaining 296 men opted against TTh).[26] During the median follow up of 7 years, fasting glucose and HbA1c fell significantly compared with baseline values and also compared with controls.[26]

Thus, confusion exists regarding the effect TTh has on insulin resistance and glycaemic control. This may be due to heterogeneity within adult-onset TD.[27] The definition of adult-onset TD is perhaps a pragmatic entity to aid clinicians and may contribute to the confusion. Serum total testosterone is dependent on SHBG levels and the latter is not always taken into account. Although it may be argued that inclusion of FT may negate the need for SHBG to be a factor in the definition of adult-onset TD, the methods used to measure or calculate FT are not straightforward.

Further, the androgen receptor sensitivity can vary,[28] thus in an ideal scenario the testosterone thresholds used in the diagnosis of adult-onset TD should be relative and not absolute concentrations. The symptoms of testosterone deficiency included in the diagnosis are not unique to the condition, hence other factors contributing to the clinical phenotype will exist. All the above issues may contribute to the varying results from the studies. Thus, it is worth reducing the patient heterogeneity by recognising subgroups based on presenting symptoms/signs as well as factors that may contribute to the pathophysiology, *i.e.* using narrower inclusion criteria within studies.

Adult-onset TD and diabetes: risk of mortality

There have been many observational studies evaluating endogenous serum testosterone and mortality. A systematic review and meta-analysis of community-based studies revealed that lower total testosterone levels were associated with a higher incidence of cardiovascular and all-cause mortality.[29] 21 studies were included in the systematic review and 12 of these in the meta-analysis. The pooled relative risk of all-cause mortality comparing the lowest third to the highest third of the endogenous testosterone levels was 1.35 (95% C.I. 1.13, 1.62). Similarly the pooled relative risk for cardiovascular mortality was 1.25 (95% C.I. 0.97, 1.60). However, considerable between- study heterogeneity existed, perhaps due to differences in the specified cohort selection; none of which included a pre-specified diabetic population.[29]

Using prospective data on a cohort of 2599 men from the European Male Aging Study (EMAS), a 5-fold increase in all-cause mortality compared with eugonadal men was observed in those men with severe TD (defined as men with 3 sexual symptoms and TT<8 nmol/L and cFT<220 pmol/L), after adjustments for age, Body Mass Index (BMI), smoking status, chronic poor health, and regional centre (Hazard Ratio [HR] 5.5, 95% CI 2.7,11.4).[30] If men with TT<8 nmol/L were considered, irrespective of their symptoms status, HR of 2.3 (9.5% CI 1.2,4.2) for all-cause mortality was found compared with eugonadal men. Further, the presence of three symptoms of sexual dysfunction resulted in a 3-fold higher risk in all- cause mortality irrespective of testosterone level compared with men who had no sexual symptoms. 7% of the cohort were noted to have T2DM at baseline. The proportion of men with T2DM was greater in the group that died *versus* the group that survived – 14.8 *versus* 6.2% (P<0.001). However, no adjustment for mortality and hypogonadism was made for the subgroup with T2DM.[30]

Shores *et al* carried out a retrospective observational study of 1031 American veterans with adult-onset TD (TT<8.7 nmol/L) over a mean duration of 40.5 months.[31] Numerous co-morbidities existed within the cohort, including diabetes mellitus (38%), coronary heart disease (21%) and sexual dysfunction (36%). TTh was prescribed in 398 men (39%) for a median duration of 16.6 months with intramuscular preparations being the most commonly used formulation. 633 men (61%)

remained untreated. Unadjusted analysis revealed lower all-cause mortality in men on TTh compared to untreated men (10.3% *versus* 20.7%, P<0.0001). This association was confirmed in a Cox regression analysis adjusted for confounding variables: age, study site, BMI, baseline TT, comorbidities, hospitalisation, coronary heart disease and diabetes mellitus (HR for TTh *versus* no treatment: 0.61, 95% CI: 0.42, 0.88). In men without T2DM TTh was not significantly associated with mortality (HR: 0.72, 95% CI: 0.46, 1.13). In contrast TTh was associated with reduced mortality in men with T2DM (HR: 0.44, 95% CI: 0.23, 0.84) hinting that this subgroup was more likely to benefit from TTh.[31]

In a prospective longitudinal study of 550 men with T2DM, 154 (28%) and 142 (25.8%) men were found to have low TT (<10 nmol/L) and low calculated free testosterone (cFT) (<0.19 nmol/L) at baseline, respectively.[32] Association of baseline androgen level with all-cause mortality over a 14-year follow-up was assessed. Mortality was significantly higher (P<0.0001) in the men with low testosterone during the follow-up. 36.1% (143/396) of men with normal baseline testosterone died whereas 55.8% (86/154) of men with hypogonadism died. The age-adjusted HR for mortality of patients with adult-onset TD was 1.54 (95% CI: 1.2, 2.0, P<0.002) with eugonadal men as reference. Further, both low baseline cFT and dihydrotestosterone were associated with increased mortality.[32] However, impact of TTh on mortality was not assessed in this study.

Two other prospective longitudinal studies by Muraleedaran *et al.*[33] and Hackett *et al.*[34] in men with T2DM also showed that low serum testosterone was associated with increased mortality. However, as these studies also assessed the association between TTh and mortality they will be described in the next section.

Adult-onset TD and diabetes: TTh and mortality risk

The above studies have suggested that men with adult-onset TD maybe at greater risk of all-cause mortality, with this association perhaps driven by the subgroup of men with T2DM. Thus, it is of considerable interest if TTh could reverse this association, especially in men with T2DM.

Muraleedharan *et al.* prospectively examined the association between low serum testosterone levels, TTh and mortality exclusively in a cohort with T2DM.[33] 581 diabetic men were followed up over a mean period of 5.8 years. Patients were stratified into two groups according to baseline TT concentration of 10.4 nmol/L. 343 men had TT >10.4 mmol/L (mean TT ± SD: 15.7±4.5 mmol/L) and 238 men had a TT ≤10.4 mmol/L (mean TT±SD: 7.5±2 mmol/L). Mortality was then compared between the two groups. When adjustments were made for age, BMI, HbA1c, SHBG, smoking status, statin, ACE inhibitor or Angiotensin Receptor Blocker use as well as presence of pre-existing cardiovascular disease, mortality rate in the low TT group (17.2%) was significantly higher compared to the normal total testosterone (9%) group. Multi-variate adjusted Cox regression analysis revealed a significant reduction

in survival in those with low TT levels (HR: 2.02, 95% CI: 1.2, 3.4).[33] The mortality analysis, with the men divided into only these two groups, resulted in the group with low TT being comprised of both those who did and did not receive TTh. This could have diluted the death rate in this group as TTh improved mortality, as the authors went on to show. The 238 with low serum TT were further stratified into 2 groups based on whether or not they received TTh. 64 men were treated for a mean ± SD of 41.6±20.7 months. 85.9% of these received testosterone gel, with the remaining 14.1% having TU administered. Mortality was significantly higher in men with low testosterone levels who remained untreated (20.1%), compared with the men with low serum TT receiving TTh (9.38%) (P=0.002), and compared with men with normal serum TT levels (9.12%). After adjusting for co-variates, Cox regression showed survival was lower in the men with low serum TT, not on TTh (HR: 2.3; 95% CI: 1.3, 3.9) than in men with normal TT levels. Interestingly the survival in men with low serum TT on TTh was closely aligned to those men with normal levels.[33]

The association between low serum testosterone, TTh and mortality was studied in the BLAST (Burntwood, Lichfield, Atherstone, Sutton Coldfield, Tamworth) screened cohort (857 men with T2DM who were screened for low TT and/or cFT).[34] This was a prospective longitudinal study over a median (IQR) follow-up of 3.9 (3.2, 4.6) years. The total cohort of 857 men with T2DM were categorised into the following groups: men with normal testosterone (TT >12 nmol/L and FT >0.25 nmol/L, N.=320) and men with low testosterone levels (TT≤12 nmol/L or FT≤0.25 nmol/L, N.=537). Figure 5.1 shows the differences in mortality between these 2 groups in three age categories. In the 60-70 year and 70-80 year categories, there was reduced mortality in the normal TT group compared with the low TT group. The group with low TT was further stratified into men untreated (N.=362) and treated (N.=175) with TTh. A Cox regression analysis showed that normal testosterone (HR: 0.62, 95% CI: 0.41, 0.94) and low testosterone treated with TTh (HR: 0.38, 95% CI: 0.16, 0.90) were independently associated with reduced mortality compared with the reference group (men with low testosterone not provided TTh). Figure 5.2 shows the comparison of mortality in men with low testosterone who were treated *versus* those who were untreated. The difference in mortality, especially in the 2 upper age categories, is even greater here than in the comparison between normal and low TT groups seen in Figure 5.1. The above regression model was adjusted for age, statin and phosphodiesterase-5 inhibitor (PDE5-i) therapy. Interestingly PDE5-i use was associated with greater survival but the association between TTh and reduced mortality was also evident in men with low testosterone levels not prescribed PDE5-i agents.[34] It is beyond the scope of this chapter to delve into possible mechanisms of this association. However, it is important that future research methodology takes into account PDE5-i treatment as a confounder.

Further analysis of the same cohort demonstrated that factors (weight, body mass index, HbA1c, lipids, blood pressure) associated with CVD (either baseline or change during follow-up) did not appear to mediate the above-mentioned association

Figure 5.1. Bar chart demonstrating mortality (%) in the group of men with normal testosterone levels (N.=320) and the men with low testosterone levels not on TTh (N.=362), across three age categories within the BLAST screened cohort.

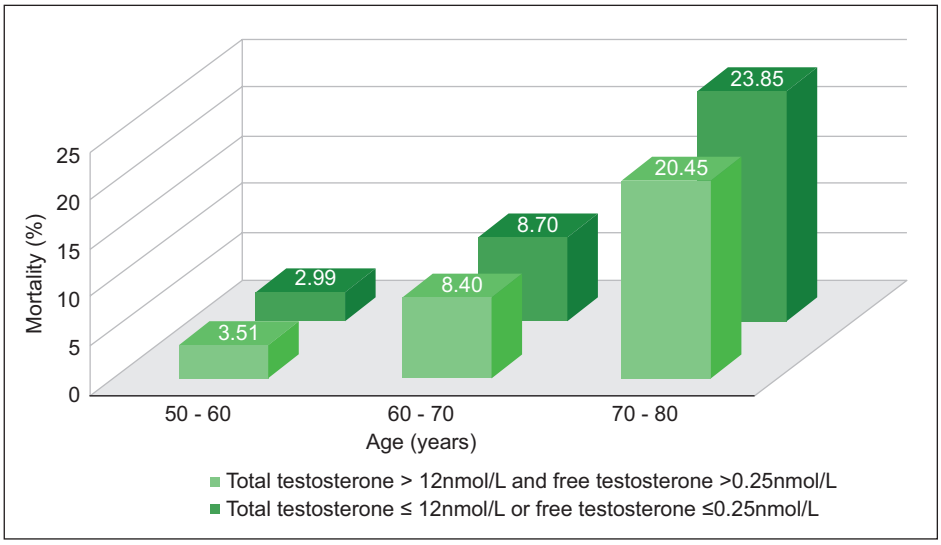
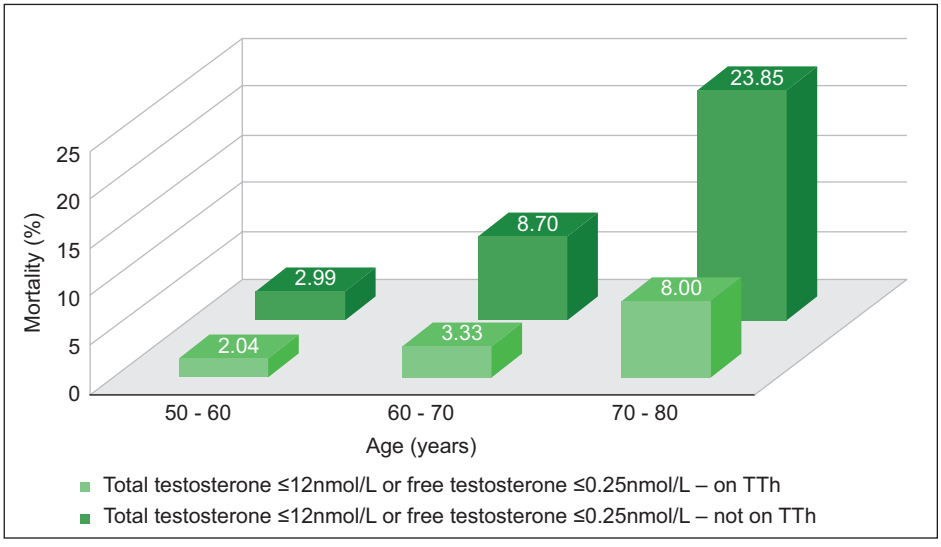


Figure 5.2. Men with low testosterone were stratified into untreated (N.=362) and treated (N.=175) groups. The bar chart below demonstrates mortality (%) across three age categories, in men with low TT who received TTh and who did not receive TTh.



between TTh and survival.[35] Hence, the mechanism(s) of the impact of TTh on mortality in men with low testosterone and T2DM remains to be fully established.

Conclusions

In the above chapter we have demonstrated that adult-onset TD is associated with many of the classifying features of MetS, especially the two drivers; central obesity and insulin resistance. Thus, not surprisingly it is highly prevalent in both MetS and T2DM. Although TTh is seen to reverse some of these associations, one cannot be certain whether causation and bidirectionality of the associations is possible. We have previously speculated that adult-onset TD should perhaps be added to the classification of MetS, and the gathering evidence, in our view, adds weight to this. It would remind physicians to screen for adult-onset TD when a male patient presents with any of the features of MetS or with T2DM. We have described some longitudinal studies suggesting that TTh is associated with increased survival in men with adult-onset TD and T2DM. Currently TTh is recommended in men with adult-onset TD to alleviate the associated symptoms and signs, thereby improve quality of life. The possible reduction in mortality in addition would clearly be a welcome bonus. The TRAVERSE RCT (https://www.clinicaltrials.gov/ct2/show/NCT03518034) which includes 6000 men diagnosed with TD and at high risk of CVD, is due to report in 2022 on the effects of topical TTh on time to major adverse cardiac event as the primary outcome. However, we urgently require a RCT with adequate follow-up, with all-cause mortality as an outcome, initially in men with T2DM.

References

1. Dohle G, Arver S, Bettocchi C, *et al.* EAU guidelines on male hypogonadism [Internet] 2018; Available from: http://uroweb.org/guideline/ male-hypogonadism/ [cited 2021, May 10].
2. Hackett G, Kirby M, Edwards D, *et al.* British Society for Sexual Medicine guidelines on adult testosterone deficiency, with statements for UK practice. J Sex Med 2017;14:1504-23.
3. Wu FC, Tajar A, Beynon JM, *et al.* Identification of late-onset hypogonadism in middle-aged and elderly men. N Engl J Med 2010;363:123-35.
4. Kapoor D, Aldred H, Clark S, *et al.* Clinical and biochemical assessment of hypogonadism in men with type 2 diabetes: correlations with bioavailable testosterone and visceral adiposity. Diabetes Care 2007;30:911-7
5. Haring R, Völzke H, Spielhagen C, *et al.* The role of sex hormone-binding globulin and testosterone in the risk of incident metabolic syndrome. Eur J Prev Cardiol 2013;20:1061-8.
6. Kupelian V, Page ST, Araujo AB, *et al.* Low Sex Hormone-Binding Globulin, Total Testosterone, and Symptomatic Androgen Deficiency Are Associated with Development of the Metabolic Syndrome in Nonobese Men. J Clin Endocrinol Metab 2006;91:843-50.

7. Strange RC, Burdett H, Hackett G, *et al.* The Metabolic Syndrome : A More Useful Prognostic Marker for CVD if Testosterone and Urate were Included? J Endocrinol Diab Res 2016;2:E100107.
8. Bhasin S, Jasjua GK, Pencina M, *et al.* Sex hormone-binding globulin, but not testosterone, is associated prospectively and independently with incident metabolic syndrome in men: The Framingham heart study. Diabetes Care 2011;34:2464-70.
9. Völzke H, Aumann N, Krebs A, *et al.* Hepatic steatosis is associated with low serum testosterone and high serum DHEAS levels in men. Int J Androl 2010;33:45-53.
10. Hua X, Li M, Pan F, *et al.* Non-alcoholic fatty liver disease is an influencing factor for the association of SHBG with metabolic syndrome in diabetes patients. Sci Rep 2017;7:1-7.
11. Ramachandran S, Hackett GI, Strange RC. Testosterone replacement therapy: Pretreatment sex hormone-binding globulin levels and age may identify clinical subgroups. Andrology 2020;8:1222-32.
12. Pitteloud N, Mootha VK, Dwyer AA, *et al.* Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men. Diabetes Care 2005;28:1636-42.
13. Ottarsdottir K, Nilsson AG, Hellgren M, *et al.* The association between serum testosterone and insulin resistance: A longitudinal study. Endocr Connect 2018;7:1491-500.
14. Rubinow KB, Snyder CN, Amory JK, *et al.* Acute testosterone deprivation reduces insulin sensitivity in men. Clin Endocrinol (Oxf) 2012;76:281-8.
15. Haidar A, Yassin A, Saad F, *et al.* Effects of androgen deprivation on glycaemic control and on cardiovascular biochemical risk factors in men with advanced prostate cancer with diabetes. Aging Male 2007;10:189-96.
16. Dockery F, Bulpitt CJ, Agarwal S, *et al.* Testosterone suppression in men with prostate cancer leads to an increase in arterial stiffness and hyperinsulinaemia. Clin Sci 2003;104:195-201.
17. Kelly DM, Jones TH. Testosterone: A metabolic hormone in health and disease. J Endocrinol 2013;217:25-45.
18. Cohen PG. The hypogonadal-obesity cycle: Role of aromatase in modulating the testosterone-estradiol shunt--a major factor in the genesis of morbid obesity. Med Hypotheses 1999;52:49-51.
19. Dhindsa S, Furlanetto R, Vora M, *et al.* Low estradiol concentrations in men with subnormal testosterone concentrations and type 2 diabetes. Diabetes Care 2011;34:1854-9.
20. Wittert G, Bracken K, Robledo KP, *et al.* Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021;9:32-45.
21. Kalinchenko SY, Tishova YA, Mskhalaya GJ, *et al.* Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: The double-blinded placebo-controlled Moscow study. Clin Endocrinol (Oxf) 2010;73:602-12.
22. Gianatti EJ, Dupuis P, Hoermann R, *et al.* Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: A randomized controlled trial. Diabetes Care 2014;37:2098-107.
23. Yassin A, Haider A, Haider KS, *et al.* Testosterone therapy in men with hypogonadism prevents progression from prediabetes to type 2 diabetes: Eight-year data from a registry study. Diabetes Care 2019;42:1104-11.

24. Groti K, Žuran I, Antonič B, *et al.* The impact of testosterone replacement therapy on glycemic control, vascular function, and components of the metabolic syndrome in obese hypogonadal men with type 2 diabetes Aging Male 2018;21:158-69.

25. Cai X, Tian Y, Wu T, *et al.* Metabolic effects of testosterone replacement therapy on hypogonadal men with type 2 diabetes mellitus: A systematic review and meta-analysis of randomized controlled trials. Asian J Androl 2014;16:146-52.

26. Traish AM, Haider A, Haider KS, *et al.* Long-Term Testosterone Therapy Improves Cardiometabolic Function and Reduces Risk of Cardiovascular Disease in Men with Hypogonadism. J Cardiovasc Pharmacol Ther 2017;22:414-33.

27. Ramachandran S, König CS, Hackett G, *et al.* Managing Clinical Heterogeneity: An Argument for Benefit-Based Action Limits. J Eng Sci Med Diagnostics Ther 2018;1:034701.

28. Zitzmann M, Nieschlag E. Androgen receptor gene CAG repeat length and body mass index modulate the safety of long-term intramuscular testosterone undecanoate therapy in hypogonadal men. J Clin Endocrinol Metab 2007;92:3844-53.

29. Araujo AB, Dixon JM, Suarez EA, *et al.* Clinical review: endogenous testosterone and mortality in men: a systematic review and meta-analysis. J Clin Endocrinol Metab 2011;96:3007-19.

30. Pye SR, Huhtaniemi IT, Finn JD, *et al.* Late-onset hypogonadism and mortality in aging men. J Clin Endocrinol Metab 2014;99:1357-66.

31. Shores MM, Smith NL, Forsberg CW, *et al.* Testosterone treatment and mortality in men with low testosterone levels. J Clin Endocrinol Metab 2012;97:2050-8.

32. Malipatil NS, Yadegarfar G, Lunt M, *et al.* Male hypogonadism: 14-year prospective outcome in 550 men with type 2 diabetes Endocrinol Diabetes Metab 2019;2:E00064.

33. Muraleedharan V, Marsh H, Kapoor D, *et al.* Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. Eur J Endocrinol 2013;169:725-33.

34. Hackett G, Heald AH, Sinclair A, *et al.* Serum testosterone, testosterone replacement therapy and all-cause mortality in men with type 2 diabetes: retrospective consideration of the impact of PDE5 inhibitors and statins. Int J Clin Pract 2016;70:244-53.

35. Hackett G, Cole N, Mulay A, *et al.* Long-term testosterone therapy in type 2 diabetes is associated with reduced mortality without improvement in conventional cardiovascular risk factors. BJU Int 2019;123:519-29.

6

Testosterone, obesity and metabolic dysfunction

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Introduction

Obesity is one of the biggest threats to our species, with global incidence having tripled since 1975, global prevalence at >650 million people and just under a quarter of the world's population (1.9 billion people) being overweight.[1, 2] The health implications of obesity stem from its association with multiple non-communicable chronic diseases that include metabolic dysfunction (Type 2 Diabetes Mellitus [T2D], dyslipidaemia, hypertension, Obstructive Sleep Apnoea [OSA] and Non-Alcoholic Fatty Liver Disease [NAFLD]), psychiatric, cardiovascular and respiratory conditions, biomechanical disorders and increased risk of certain malignancies.[3] The pathogeneses of these obesity-related conditions are complex and likely implicate insulin resistance, hyper-inflammatory processes and oxidative stress.[3] In addition to multiple obesity-related co-morbidities, obesity also associates with a reduced life expectancy.[3] Furthermore, there are substantial global health-economic implications of obesity.

Amongst the plethora of obesity-related co-morbidities, an important consideration is the complex effects of weight-gain and obesity on the Hypothalamo-Pituitary Gonadal (HPG) axis that underlies reproductive function. Curiously, although obesity-related compromise of the HPG axis occurs in both sexes, the underlying mechanisms are sex-specific.[4] Whilst it is beyond the scope of this chapter to explore in detail these sex-related differences, here we focus on the impact of weight gain and obesity on HPG axis functioning in men. Following an overview of the vulnerability of the HPG axis to obesity mediated via effects of leptin, we discuss the diagnosis and pathogenesis of Male Obesity-associated Secondary Hypogonadism (MOSH). We explore the association and complex interlinks between MOSH, obesity and obesity-related metabolic dysfunction (including T2D and OSA) and provide an overview of the metabolic benefits of Testosterone Replacement Therapy (TRT) in men with MOSH.

The HPG axis and its vulnerability to obesity

Amongst all physiological functioning, the reproductive system holds a unique status as a facultative process: one that can either be switched on or off. From an evolutionary perspective, this is entirely understandable. In times of stress, famine or immediate danger, a focus on survival predominates. Conversely, reproductive function is only permitted at times of 'environmental optimization' when the process of reproduction and its potential longer-term sequelae of nurturing offspring are not likely to threaten survival prospects. Accordingly, the HPG axis in both men and women has multiple 'built-in brakes' acting through complex neural and endocrine pathways, that can switch off the HPG axis in response to a plethora of diverse signals.

One such signal, leptin, originates from the adipose tissue as an adipokine. Release of leptin from adipocytes (and therefore levels of serum leptin) are commensurate with adiposity.[5] Leptin crosses the blood-brain-barrier, and acts as the main communication signal between the peripheral adipose tissue depots and the brain. Essentially, leptin informs the brain of the energy storage status of the body, analogous to a fuel indicator on a car. Evolutionarily, famine and starvation represent a major threat to species survival. At such times, adipose tissue reserves diminish, serum leptin levels drop, and this results in a potent suppression of the HPG axis in both men and women, with a switch away from reproductive and towards survival strategies.

Remarkably, leptin acts within a relatively narrow range of serum concentration. When serum leptin levels reach this narrow range (euleptinaemia), leptin functions as a hormone of permission, enabling reproductive function to proceed. Interestingly, leptin has an indirect effect on the HPG axis, with no expression of the leptin receptor on the Gonadotrophin Releasing Hormone (GnRH) neurons themselves.[6] Rather, within the hypothalamus, leptin suppresses activity within the agouti-related peptide neurons.[7] These agouti-related peptide neurons supress activity within kisspeptin neurons that in turn regulate functioning within the GnRH neurons.[8] Given that kisspeptin activates GnRH neuron functioning, agouti-related peptide neurons work effectively like a brake on the HPG axis. In the context of euleptinaemia, the suppression of agouti-related peptide neuron activity therefore acts like the release of a brake on the HPG axis, analogous to lifting one's foot off a brake in a car and enabling it to move forward (in this analogy, the car's forward motion represents functionality of the reproductive axis, enabling the car to be 'sex-driven' in the search for a mate!). In states of energy deficiency (including anorexia nervosa, lipodystrophies, starvation, and malnutrition), hypoleptinaemia ensues. In this context, agouti-related peptide neurons become activated, and this results in increased suppression of kisspeptin neurons, and therefore suppression of the HPG axis. In this scenario, hypoleptinaemia acts like putting the foot down onto a car brake and bringing the car to a stop. In addition to regulation of the HPG axis, leptin also has a dual function to centrally regulate appetite control and metabolism.

Hypoleptinaemia-induced activation of agouti-related peptide neurons also acts to enhance appetite: to extend our analogy, to enable the driver to get out of the car once it has stopped, and to have appetitive motivation to search for food!

Following weight-gain and the development of obesity, increased leptin is released from an expanded adipose tissue store. Hyperleptinaemia ensues, which also associates with suppression of the HPG axis. However, the mechanisms implicated are different from those that underlie hypoleptinaemia-associated HPG axis suppression. In the context of obesity (especially associated with metabolic dysfunction and T2D), leptin resistance ensues.[9] In this context, despite the presence of hyperleptinaemia, there is hypothalamic insensitivity to the effects of elevated serum levels of leptin, and therefore a central response akin to that of hypoleptinaemia (with HPG axis suppression and appetite enhancement). Although incompletely understood, leptin resistance likely results from a combination of obesity-related insulin resistance (with associated secondary hyperinsulinaemia), and an inflammatory milieu (including elevated levels of Tumour Necrosis Factor-alpha [TNFα]).[10] In addition, there are also direct negative effects of hyperleptinaemia peripherally, with a diminishment of Luteinizing Hormone (LH)- and human Chorio-Gonadotrophin (hCG)-stimulated testicular androgen production, and reduced responsiveness of testicular Leydig cells to the stimulatory effects of gonadotrophin stimulation.[11]

In addition to leptin, other factors regulate functioning of the HPG axis. This includes clock genes such as the 'Brain and Muscle Aryl hydrocarbon receptor nuclear translocator-Like 1' gene (*BMAL1*) that plays an important role in the regulation of fertility.[12] Weight gain and the development of obesity and metabolic dysfunction associates with dysregulation of the circadian control of the HPG axis. Furthermore, the imbalance in serum estradiol and testosterone levels in MOSH may have further tissue-specific effects on the expression of clock genes.[12] However, the extent to which clock gene regulation mediates the adverse effects of obesity on the HPG axis remains incompletely understood.

The sensitivity of the androgen receptor (AR) also likely mediates some of the effects of weight gain and obesity on the functioning of the HPG axis. In both men and women, the polymorphic 'CAG-repeat' sequence length within the *AR* gene influences the sensitivity of the *AR* to the effects of androgens. In studies on both euglycaemic men[13] and women with Polycystic Ovary Syndrome,[14] the association of serum levels of testosterone with insulin resistance was influenced by the *AR* CAG repeat number. It seems likely, therefore, that the interactions between obesity, HPG axis function and the development of insulin resistance and other metabolic dysfunction are also influenced by the *AR* CAG repeat number.

To summarize this section, in both men and women, the HPG axis is vulnerable to multiple and diverse signals. Through leptin and its effects on activity within the central agouti-related peptide neurons, the peripheral adipose tissue depots communicate with the hypothalamic regulation of both the reproductive axis and appetitive control. Any deviation from euleptinaemia (stemming from both depleted

adipose tissue depot stores and weight-gain with the development of obesity) result in the combination of suppressed reproductive functioning and enhanced appetite.

Diagnosis and pathogenesis of Male Obesity-associated Secondary Hypogonadism (MOSH)

In men who gain weight and become obese, relatively commonly there is the biochemical and clinical development of MOSH (a diagnosis of exclusion). In the diagnostic process, it is important to take a detailed history and physical examination to clarify any clinical features of MOSH (including both reproductive and non-specific features of hypogonadism that include tiredness, lethargy, and physical weakness).[15] In morbidly obese men, multiple factors may contribute towards such clinical features, including, for example, respiratory compromise or the physical effort of walking and moving, or the development of OSA. Therefore, the presence of the non-specific clinical features of hypogonadism should not be assumed to be related to MOSH, and other investigations are often required (such as polysomnography in obese men with an elevated score on a sleep screening test such as 'Epworth'). A detailed drug history is important, including the use of opioid drugs and/or anabolic steroids, each of which can cause secondary hypogonadism. Finally, there should be ascertainment of any other potential insult to the HPG axis, including history of chemotherapy, radiotherapy, repeated head trauma (including from engagement in certain sports like boxing and football), and any testicular insult (such as trauma or mumps in adulthood).

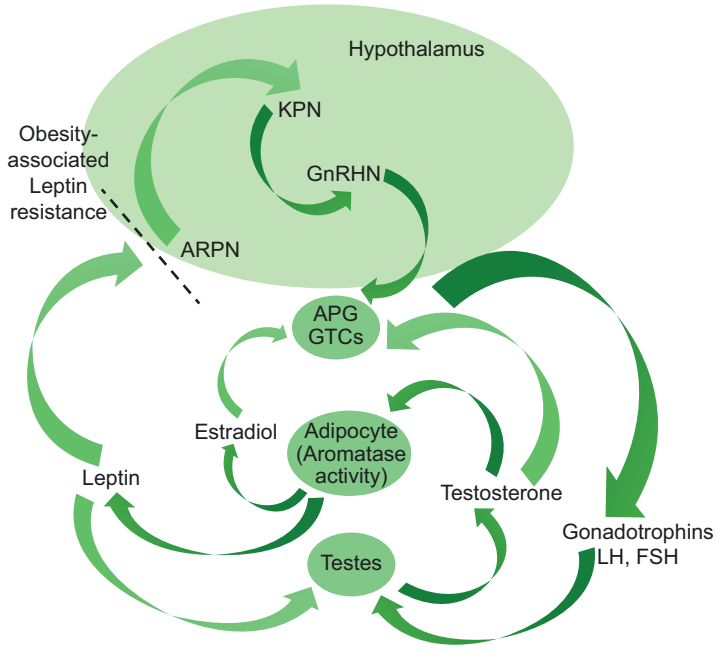
Baseline investigation for MOSH includes a fasting 9am serum testosterone level, coupled with LH and Follicle Stimulating Hormone (FSH). The timing of this test is critical given the diurnal rhythm of testosterone in men.[16] Furthermore, fasting status is essential given the known effects of food ingestion on serum testosterone level. The presence of a suppressed serum testosterone in the context of a normal or elevated serum level of LH and FSH is consistent with secondary hypogonadism.[16] Screening tests should include a serum prolactin, to exclude hyperprolactinaemia as a potential cause. Furthermore, a serum ferritin level is useful to exclude the possibility of haemochromatosis. Biochemical assessment of the rest of the pituitary function is required to ascertain the extent of pituitary dysfunction: in MOSH, pituitary dysfunction is usually restricted to the HPG axis.[16] The biochemical picture of secondary hypogonadism should usually also prompt Magnetic Resonance Imaging (MRI) of the pituitary gland to exclude any pituitary lesion (including non-functioning pituitary tumours), given that the HPG axis is usually affected early in the presence of pituitary tumours.[16] Following a detailed history and examination and thorough biochemical and radiological investigation, the presence of secondary hypogonadism in an obese man in the absence of all other potential causes of secondary hypogonadism can assume the diagnosis of MOSH.[16] Table 6.I provides a summary of the clinical features and investigations for suspected MOSH.

The pathogenesis of MOSH is complex (schematized in Figure 6.1). In addition to the role of obesity-related hyperleptinaemia and leptin resistance outlined above, multiple other factors conspire to further suppress the HPG axis. This includes the conversion of testosterone into estradiol within the adipose tissue through the

Table 6.I. Clinical features and investigations for suspected MOSH.

History	• Tiredness, lethargy, physical weakness, sexual dysfunction, low libido, depression, sleep problems (Epworth score), breast pain, drugs (opioids, anabolic steroids), head trauma, stress, mumps (as an adult), cancer (including radiotherapy and chemotherapy), testicular trauma
Examination	• Elevated BMI and waist circumference • Presence of gynaecomastia
Investigations	• Fasting 9 A.M. serum testosterone, LH, FSH, SHBG, prolactin, • TSH, T3, T4, oestradiol, GH, IGF1, ACTH, cortisol, ferritin, HbA1c • MRI pituitary

Figure 6.1. Overview of the main pathogenic mechanisms that underlie MOSH. APG: Anterior Pituitary Gland; ARPN: Agouti-Related Peptide neurons; FSH: Follicle Stimulating Hormone; GnRHN: Gonadotrophin Releasing Hormone Neurons; GTC: Gonadotroph Cells; KPN: Kisspeptin neurons; LH: Luteinizing Hormone. Solid arrows denote stimulatory effects (or hormonal release); dotted arrows denote suppressive effects.



increased activity of the aromatase enzyme. In this way, adipose tissue related aromatase activity acts like a sump mechanism to diminish serum testosterone, whilst enhancing serum levels of estradiol, that in turn have a suppressive effect on gonadotrophin release from the pituitary gland. Furthermore, obesity, particularly in the context of metabolic dysfunction (such as T2D), often associates with chronic inflammation. Certain inflammatory mediators such as Interleukin-1β (IL-1β), act as potent down-regulators of the HPG axis.[17] Interleukin-6 (IL-6) may also suppress the male HPG axis in men with features of the metabolic syndrome. Finally, free fatty acids may influence directly the production of androgen precursors,[18] that may also influence the clinical manifestation of MOSH.

To summarize this section, MOSH should only be used as a diagnosis following careful clinical assessment through thorough history and examination, biochemical and radiological investigation, given the diverse plethora of potential factors that can suppress the HPG axis. The complex pathogenesis of MOSH centres around obesity and the sequelae of obesity, that include leptin resistance, hyperleptinaemia, enhanced adipose tissue-related aromatase activity and inflammatory sequelae.

Association of MOSH with obesity, T2D and OSA

There is a strong association between MOSH, obesity and obesity-related conditions such as T2D and OSA, with complex interlinks between these clinical entities. Regardless of T2D-status, there is an inverse association between Body Mass Index (BMI) and plasma levels of testosterone in men.[16] In one study on 160 men, Secondary Hypogonadism (SH, defined by a free plasma testosterone level <225 pmol/L) affected >40% of those with a BMI ≥40 $kg.m^{-2}$.[19] In a further study on >1,800 men with a BMI ≥30 $kg.m^{-2}$ and aged ≥45 years, SH affected 40% and 50% of those with euglycaemia and Diabetes Mellitus (DM), respectively.[20] In men with T2D, estimated prevalence of SH ranges between 25-40%, and up to 50% in the context of both T2D and obesity.[16] Furthermore, SH may affect up to a third of young men (aged 18-35 years) with T2D.[21]

The complex interlinks between obesity and T2D with SH in men is influenced by the development of OSA through neuro-endocrine mechanisms.[16] OSA manifests with repetitive collapse of the pharyngeal airway during sleep due to excessive adipose tissue within the neck, with recurrent apnoeic and hypoxic episodes and sleep fragmentation, that in turn can impact negatively on HPG axis functioning.[16, 22] Interestingly, the standard treatment for OSA, Continuous Positive Airways Pressure (CPAP) does not appear to improve plasma levels of free testosterone but may increase plasma levels of Sex Hormone Binding Globulin (SHBG) and total testosterone.[16, 22] OSA associates independently with a reduction in the pulse amplitude of LH,[23] insulin resistance and a higher serum level of leptin than in non-apnoeic control men (independent of BMI and age).[24] As outlined earlier, hyperleptinaemia suppresses the testicular production of testosterone.[11] Therefore, a combination of

diminished LH signals and testicular responsiveness to the steroidogenic effects of LH has a 'double-whammy' effect on HPG axis functioning, with both centrally- and peripherally-mediated effects.

In addition to the effects of OSA on the development of MOSH, it is likely that MOSH itself may adversely influence sleep quality and the development and progression of OSA, resulting in a vicious cycle. There are indirect effects of androgens (via their central aromatase-mediated conversion to oestradiol) on the control of respiration through the serotoninergic system.[16] In the context of MOSH, the depletion of androgens may also decrease the uptake of serotonin in the phrenic and hypoglossal nuclei, and negatively impact on the longer-term control of respiration.[25]

To conclude this section, MOSH does not exist in isolation, but rather by definition always manifests in the context of obesity. In men who gain weight and become obese, the development of MOSH occurs in a substantial proportion (perhaps 40%), that is even greater in the context of T2D. Obesity itself associates with >50 obesity-related conditions including T2D and OSA.[26] It is not surprising, therefore that MOSH, T2D and OSA frequently co-exist epidemiologically. However, there are also pathogenic explanations for the close associations between these clinical entities, with complex bi-directional causal pathways. As such, we cannot simply view MOSH, T2D and OSA as obesity-related conditions that are linked epidemiologically by their common association with obesity. Rather, these obesity-related conditions cluster together through a complex web of pathogenic mechanisms that extends well beyond mere epidemiological association. Analogous to gravitational attraction, this pathogenic web acts like a hidden force that underlies the clustering and strong associations between MOSH, T2D and OSA. The clinical implication of such clustering is that the identification of one of these three entities should prompt active screening for the other two, especially given that each may present asymptomatically.

Metabolic benefits of TRT in MOSH

Having explored the underlying pathogenesis of MOSH and its complex interlinks with metabolic dysfunction (including obesity, T2D and OSA), it is important to consider the benefits of TRT in men with MOSH. Whilst it is beyond the scope of this chapter to provide an exhaustive overview of the various trials that have explored the use of TRT in men with MOSH, reviewed recently,[27] we provide a summary of the main trials reported recently.

In one of the largest and well-phenotyped studies to date, Wittert and colleagues reported on the 'T4DM' study, a placebo-controlled randomized double-blinded trial on >1,000 men aged 50-74 years with a serum testosterone concentration ≤14.0nmol/L, a waist circumference ≥95cm and presence of either impaired glucose tolerance or newly diagnosed T2D. All participants were enrolled into a lifestyle program and were randomly assigned on a 1:1 basis to receive either an intramuscular

testosterone undecanoate (IMTU) injection (1000 mg) or placebo at baseline, at 6-weeks and then at 3-monthly intervals for 2-years.[28] Compared with the placebo group, the men who received IMTU-based TRT had a significant reduction in their risk of developing T2D (relative risk 0.59) at 2-years, that was independent of their baseline serum testosterone concentration.[28] There were additional benefits of TRT that included improved muscle mass, strength and sexual function, and a reduction in fat mass. There was also a note of elevated haematocrit in 22% of the men treated with TRT (see Chapter 8).[28]

Other randomized placebo-controlled trials on the effects of TRT have also shown metabolic benefits. In one study on 220 men with T2D or metabolic syndrome and low serum levels of testosterone, there was a 15% reduction in Homeostasis Model Assessment of Insulin Resistance (HOMA IR) over 12-months for men treated with testosterone gel *versus* placebo, although no changes in glucose metabolism occurred.[29] In the 'Birmingham, Lichfield, Atherstone, Sutton Coldfield and Tamworth' (BLAST) study, hypogonadal men with T2D randomised to IMTU (compared with placebo) had improvements in body composition and glycaemic control (measured with HbA1C).[30] In a prospective observational study, our own group also showed significant metabolic improvements in men with MOSH treated with IMTU (1000 mg), half of whom had a diagnosis of T2D.[31] There were significant improvements in HbA1C (mean reduction of 9 mmol/mol) and pancreatic beta-cell function (assessed through the Homeostasis Model Assessment of β-cell function [HOMA%β]) between pre- and post-IMTU injections.[31] Furthermore, IMTU resulted in a significant reduction in fat mass and increase in lean mass, although no discernible effect on lipid profile and energy expenditure occurred.[31] Table 6.II provides a summary of the metabolic effects of TRT in patients with MOSH.

Despite the positive trials outlined above, it should be noted that there are some inconsistencies within the literature. In one study that compared placebo with IMTU in hypogonadal men with T2D, there was no improvement in either HOMA-IR or glycaemic control in the IMTU group, despite significant improvements in subcutaneous fat mass and lean mass.[32] Similarly, in a separate study on ageing hypogonadal men with T2D controlled on metformin monotherapy, compared with placebo, the application of testosterone gel did not improve glycaemic control or peripheral insulin sensitivity despite an improvement in body composition.[33] In one meta-analysis on 7 studies and >830 participants with T2D and/or metabolic syndrome, there was a beneficial effect of TRT on one measure of insulin resistance, but not on levels of HbA1C.[34] In a further meta-analysis on the metabolic effects of TRT In T2D in 37 studies, TRT

Table 6.II. Summary of metabolic effects of TRT in patients with MOSH.

Fat mass	Reduced
Lean mass	Increased
Body Weight	Unchanged
Fasting plasma glucose	Reduced
Fasting serum insulin/HOMA-IR	Reduced
HbA1c	Reduced

associated with significant reductions in HbA1C, fat mass, triglycerides and fasting plasma glucose level.[35] A further systematic review and meta-analysis from 5 studies also showed improvements in glycometabolic parameters in response to TRT in patients with T2D.[36]

To summarize this section, direct comparisons of data between studies is often problematic due to differences in populations, study design, study duration, type of TRT and selection criteria. As outlined, although many studies on TRT in MOSH show clear metabolic benefits, these apparent metabolic benefits are not consistent across all studies. Accordingly, the management of MOSH with TRT remains contentious, with relatively modest metabolic benefits, some unresolved potential safety issues, and a prevailing view that the management of MOSH should focus on lifestyle management and weight-loss.[27] There is a need for more randomized controlled trials in this field, with prospective designs to address the longer-term metabolic efficacy of TRT in men with MOSH, and to allay any potential safety concerns. Whilst it is incontrovertible that lifestyle improvement (with consequent weight-loss) is beneficial for overall health and wellbeing in men with MOSH,[16] we should also acknowledge the inherent challenges of successful and long-term lifestyle change, and associated weight-loss. Furthermore, it could also be argued that men with MOSH would perhaps be better prepared for the challenges of lifestyle change if they also received TRT, and that if left untreated, their hypogonadal state would likely contribute to ongoing tiredness, lethargy, malaise and physical weakness,[15] that would diminish one's ability to engage with successful lifestyle change. Furthermore, even if glycaemic benefits do not occur, the improved body composition (with reduced fat mass and improved lean mass) shown in response to TRT in most studies on MOSH outlined here, would likely improve overall metabolic health, and optimise successful weight-loss with healthy lifestyle changes. This is coupled with improvements in muscle strength, emotional and cognitive functioning, energy levels, motivation, sexual function, and overall wellbeing that generally manifest from TRT in hypogonadal men, regardless of underlying aetiology.[15] For these reasons, it would seem reasonable to consider the administration of TRT in combination with encouragement of healthy lifestyle changes in men with MOSH, particularly in the context of clinical features of hypogonadism, unless there is a contra-indication, or other clinical reason not to administer TRT. In this way, we can optimize the future metabolic outlook and overall health and wellbeing of men with MOSH.

Conclusions and future directions

Any obesity-related condition suffers from various challenges, given that these conditions never occur in isolation, but rather co-exist with obesity. The high global prevalence of obesity[1, 2] drives increased prevalence of obesity-related conditions. Furthermore, given the numerous co-morbidities of obesity,[26] it is easy to

mis-attribute symptoms erroneously to some other condition or even to the presence of obesity itself. To compound this problem, obesity is a highly stigmatised condition within our society,[37] and inevitably, this stigma spills over to obesity-related conditions. MOSH is no exception. As an obesity-related condition, we should not under-estimate the true prevalence of MOSH. For all the reasons outlined here, in addition to the frequent non-specific nature of symptoms related to MOSH,[15, 16] this important condition appears under-recognised as a clinical entity. Unlike its obesity-related counterpart T2D, with its media exposure, high profile, and financially incentivized screening program within primary care clinical services, MOSH seems like a 'poor relative', with none of these accolades. This is at odds with the known clinical sequelae of male hypogonadism, including adverse effects on bone and cardiovascular health, sexual functioning, emotional regulation, and general overall wellbeing, and the clear benefits of effective management (including the administration of TRT).[15, 16] Coupled with the current controversies within the field alluded to in the last section, simply put, MOSH suffers from multiple perspectives as a clinical entity.

How can we address these challenges? Improving the stigma of obesity and its associated conditions is a good starting point, although this is likely to take decades (possibly even a generational change) to implement. There is also a need for greater awareness generally of certain obesity-related conditions, especially those that are not financially incentivised for screening, like MOSH. Finally, we should not forget that at the centre of all of this is a person suffering from obesity and at least one of its associated co-morbidities, and who may be suffering from the stigma of obesity, the effects of hypogonadism with impaired sexual functioning, diminished strength, emotional regulation, motivation, and general overall wellbeing. Furthermore, close personal relationships and work productivity may have deteriorated. This person is like a stone dropped into a lake, with the ripple effects ultimately felt throughout our society. Let us not forget that person. Like the stone, that person should be kept at the centre of our clinical approach to MOSH.

References

1. Organization WH. Obesity and Overweight: World Health Organization.(2018). 2019.
2. Oduro-Donkor D, Turner MC, Farnaud S, *et al.* Modification of fecal microbiota as a mediator of effective weight loss and metabolic benefits following bariatric surgery. Expert Rev Endocrinol Metab 2020;15:363-73.
3. Fruh SM. Obesity: Risk factors, complications, and strategies for sustainable long-term weight management. J Am Assoc Nurse Pract 2017;29(S1):S3-S14.
4. Dimitriadis GK, Barber TM. Obesity-related metabolic and reproductive dysfunction: variations between the sexes. Expert Rev Endocrinol Metab 2016;11:387-93.
5. Caron A, Lee S, Elmquist JK, *et al.* Leptin and brain-adipose crosstalks. Nat Rev Neurosci 2018;19:153-65.

6. Petrine JCP, Franci CR, Del Bianco-Borges B. Leptin actions through the nitrergic system to modulate the hypothalamic expression of the kiss1 mRNA in the female rat. Brain Res 2020;1728:146574.
7. Xu J, Bartolome CL, Low CS, *et al.* Genetic identification of leptin neural circuits in energy and glucose homeostases. Nature 2018;556:505-9.
8. Donato J Jr, Silva RJ, Sita LV, *et al.* The ventral premammillary nucleus links fasting-induced changes in leptin levels and coordinated luteinizing hormone secretion. J Neurosci 2009;29:5240-50.
9. Pan H, Guo J, Su Z. Advances in understanding the interrelations between leptin resistance and obesity. Physiol Behav 2014;130:157-69.
10. Costanzo PR, Knoblovits P. Male gonadal axis function in patients with type 2 diabetes. Horm Mol Biol Clin Investig 2016;26:129-34.
11. Mammi C, Calanchini M, Antelmi A, *et al.* Androgens and adipose tissue in males: a complex and reciprocal interplay. Int J Endocrinol 2012;2012:789653.
12. Angelousi A, Kassi E, Nasiri-Ansari N, *et al.* Clock genes alterations and endocrine disorders. Eur J Clin Invest 2018;48:e12927.
13. Mohlig M, Arafat AM, Osterhoff MA, *et al.* Androgen receptor CAG repeat length polymorphism modifies the impact of testosterone on insulin sensitivity in men. Eur J Endocrinol 2011;164:1013-8.
14. Mohlig M, Jurgens A, Spranger J, *et al.* The androgen receptor CAG repeat modifies the impact of testosterone on insulin resistance in women with polycystic ovary syndrome. Eur J Endocrinol 2006;155:127-30.
15. Arver S, Lehtihet M. Current guidelines for the diagnosis of testosterone deficiency. Front Horm Res 2009;37:5-20.
16. Saboor Aftab SA, Kumar S, Barber TM. The role of obesity and type 2 diabetes mellitus in the development of male obesity-associated secondary hypogonadism. Clin Endocrinol (Oxf) 2013;78:330-7.
17. Herman AP, Krawczynska A, Bochenek J, *et al.* LPS-induced inflammation potentiates the IL-1beta-mediated reduction of LH secretion from the anterior pituitary explants. Clin Dev Immunol 2013;2013:926937.
18. Mai K, Bobbert T, Kullmann V, *et al.* Free fatty acids increase androgen precursors in vivo. J Clin Endocrinol Metab 2006;91:1501-7.
19. Hofstra J, Loves S, van Wageningen B, *et al.* High prevalence of hypogonadotropic hypogonadism in men referred for obesity treatment. Neth J Med 2008;66:103-9.
20. Dhindsa S, Miller MG, McWhirter CL, *et al.* Testosterone concentrations in diabetic and nondiabetic obese men. Diabetes Care 2010;33:1186-92.
21. Chandel A, Dhindsa S, Topiwala S, *et al.* Testosterone concentration in young patients with diabetes. Diabetes Care 2008;31:2013-7.
22. Hammoud AO, Carrell DT, Gibson M, *et al.* Updates on the relation of weight excess and reproductive function in men: sleep apnea as a new area of interest. Asian J Androl 2012;14:77-81.
23. Luboshitzky R, Lavie L, Shen-Orr Z, *et al.* Altered luteinizing hormone and testosterone secretion in middle-aged obese men with obstructive sleep apnea. Obes Res 2005;13:780-6.
24. Schafer H, Pauleit D, Sudhop T, *et al.* Body fat distribution, serum leptin, and cardiovascular risk factors in men with obstructive sleep apnea. Chest 2002;122:829-39.

25. Behan M, Zabka AG, Thomas CF, *et al.* Sex steroid hormones and the neural control of breathing. Respir Physiol Neurobiol 2003;136:249-63.

26. Pi-Sunyer X. The medical risks of obesity. Postgrad Med 2009;121:21-33.

27. Lapauw B, Kaufman JM. Management of endocrine disease: Rationale and current evidence for testosterone therapy in the management of obesity and its complications. Eur J Endocrinol 2020;183:R167-R83.

28. Wittert G, Bracken K, Robledo KP, *et al.* Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021;9:32-45.

29. Jones TH, Arver S, Behre HM, *et al.* Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). Diabetes Care 2011;34:828-37.

30. Hackett G, Cole N, Bhartia M, *et al.* Testosterone replacement therapy improves metabolic parameters in hypogonadal men with type 2 diabetes but not in men with coexisting depression: the BLAST study. J Sex Med 2014;11:840-56.

31. Dimitriadis GK, Randeva HS, Aftab S, *et al.* Metabolic phenotype of male obesity-related secondary hypogonadism pre-replacement and post-replacement therapy with intra-muscular testosterone undecanoate therapy. Endocrine 2018;60:175-84.

32. Gianatti EJ, Dupuis P, Hoermann R, *et al.* Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: a randomized controlled trial. Diabetes Care 2014;37:2098-107.

33. Magnussen LV, Glintborg D, Hermann P, *et al.* Effect of testosterone on insulin sensitivity, oxidative metabolism and body composition in aging men with type 2 diabetes on metformin monotherapy. Diabetes Obes Metab 2016;18:980-9.

34. Grossmann M, Hoermann R, Wittert G, *et al.* Effects of testosterone treatment on glucose metabolism and symptoms in men with type 2 diabetes and the metabolic syndrome: a systematic review and meta-analysis of randomized controlled clinical trials. Clin Endocrinol (Oxf) 2015;83:344-51.

35. Corona G, Monami M, Rastrelli G, *et al.* Type 2 diabetes mellitus and testosterone: a meta-analysis study. Int J Androl 2011;34:528-40.

36. Cai X, Tian Y, Wu T, *et al.* Metabolic effects of testosterone replacement therapy on hypogonadal men with type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. Asian J Androl 2014;16:146-52.

37. Phelan SM, Burgess DJ, Yeazel MW, *et al.* Impact of weight bias and stigma on quality of care and outcomes for patients with obesity. Obes Rev 2015;16:319-26.

7

Testosterone and anaemia

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Introduction

An association between androgens and erythropoiesis was first reported almost a century ago;[1-3] murine studies demonstrated that the castration of rats decreased their haemoglobin level and the use of testosterone had the opposite effect. Moreover, it is well recognised that males have a higher haemoglobin and red blood cell count than females.[1, 3] Indeed, prior to the use of immunosuppression and erythropoietin treatments, testosterone was routinely used to treat aplastic anaemia, bone marrow conditions and anaemia caused by renal disease.[4]

There is increasing data showing that hypogonadism is a potential mechanism of unexplained anaemia and the benefits of testosterone therapy. This chapter will discuss the contemporary literature regarding testosterone and anaemia including limitations to current data and the need for future work.

Epidemiology

Anaemia has a global prevalence of 1.62 billion and 12.7% (260 million) of affected individuals are men.[5] It has been reported that the prevalence of anaemia increases with age and data from the National Health and Nutrition Survey (NHANES III) showed that within the US population the prevalence of anaemia increased from 1.5% in the age group 17-49 years to 15.7% in the age group 75-84 years and 26.1% in the ages 85 years and above.[6] Anaemia has been observed to be an independent risk factor for both cardiovascular disease death and all-cause mortality.[7] Hamilton *et al.* performed a longitudinal observational study of 788 type 2 diabetic men and observed that serum testosterone was independently associated with anaemia ($P<0.001$) and that there was a significant increase in death of patients with a total serum testosterone concentration below <10 nmol/L ($P=0.006$).[8]

Furthermore, an American study showed that presence of anaemia confers to higher healthcare costs ($14,535 *vs.* $9,451).[9] The presence of anaemia is associated with functional impairment in geriatric patients[10] and thus treatment could improve

clinical outcomes. In approximately 30% of cases of anaemia the cause is unclear,[11] but there is data showing that hypogonadism may be contributory. Ferrucci *et al.* reported that unexplained anaemia patients had a lower total testosterone than non-anaemic patients (332 ng/dL *vs.* 438 ng/dL, P<0.01).[12] Therefore, anaemia is a global and costly healthcare issue and hypogonadism may be causative and therefore a potential target for treatment.

Hypogonadism and anaemia

Observational studies

There is extensive literature[1, 3] showing that men have a higher haemoglobin level than women and this has been attributed to differences in hormone levels.

Ferruci *et al.*[12] studied data from the InCHIANTI study (an epidemiological study of 1,453 persons living in the Tuscany region of Italy) to investigate any association between testosterone and anaemia. The authors observed that total testosterone levels were linearly correlated with haemoglobin and this trend remained the same when adjusting for age, BMI, smoking, creatinine clearance, diabetes mellitus, hypertension, coronary heart disease, congestive heart failure, stroke, chronic obstructive pulmonary disease, and IL-6 and EPO levels. The authors also reported that testosterone levels were linearly correlated with haemoglobin when analysis was limited to those with normal serum iron, vitamin B12 and folate levels. Moreover, the prevalence of anaemia was associated with lower testosterone (P=0.05) and men within the lowest total testosterone level quartile were 13.1 times more likely to be anaemic than those men in the highest total testosterone quartile.

Yeap *et al.* performed a cross sectional analysis of data on 492 men in Western Australia.[13] The authors performed linear regression analysis and observed that a higher total testosterone was associated with a higher haemoglobin level (P=0.024) and this persisted (P=0.003) when excluding confounding factors (age, waist circumference, smoking status, alcohol consumption, eGFR and log ferritin).[13]

Furthermore, androgen deprivation therapy (either in the form of medical or surgical castration) has been observed to result in a significant drop in haemoglobin levels.[14]

Mechanisms of action

The underlying mechanisms of which hypogonadism causes anaemia are still not completely understood. However, there is literature suggesting that erythropoietin, chronic inflammation and hepcidin suppression may be contributory.

Stimulation of erythroid progenitor cells and the role of chronic inflammatory disease

Moriyama *et al.*[15] studied the effects of testosterone on erythroid colony development in normal human bone marrow cultures. The administration of testosterone

and erythropoietin worked together in a synergistic manner to increase erythroid colony formation compared to each hormone alone. The authors postulated that testosterone acted on the haematopoietic stem cell compartment causing stem cell differentiation into the erythrocyte responsive cell compartment thereby stimulating erythropoiesis.

There is data showing that inflammation can suppress erythropoiesis through inhibition of erythropoietin or apoptosis of red cell progenitor cells[16]. Makipour *et al.*[17] postulated that the decline of testosterone seen with age results in an increased level of inflammatory cytokines as androgens are inhibitors of nuclear factor kappa B and this protein is required for the transcription of several inflammatory mediators. This is supported by data from Allen *et al.*[18] showing that bone marrow cultures from uremic patients with inflammatory disease had a significantly inhibited response to erythropoietin and reduced erythroid colony formation compared to controls. Furthermore, treatment of these bone marrow cultures with a combination of antibodies to TNF-α and IFN-γ resulted in an improved response to erythropoietin.

Hepcidin suppression

Coveillo *et al.*[19] observed that testosterone administration significantly increased both haemoglobin and haematocrit in both younger and older men ($P<0.0001$). However, this was not associated with a significant change in erythropoietin or soluble transferrin levels. Hepcidin is a polypeptide produced in the liver that degrades ferroportin (an iron channel) thereby reducing iron absorption and increasing iron sequestration. Consequently, hepcidin inhibits erythropoiesis.[20, 21] Hepcidin increases with inflammation[22] and therefore the use of exogenous testosterone may cause suppression of hepcidin through inhibition of inflammatory cytokines.

Bachman *et al.*[21] performed a randomised controlled trial investigating the effects of exogenous testosterone on haemoglobin, haematocrit and hepcidin levels. The authors observed that testosterone increased haematocrit and haemoglobin and caused a decrease of serum hepcidin. Moreover, the dose of testosterone correlated with the level of hepcidin inhibition ($P<0.0001$).

Guo *et al.*[23] investigated the effects of exogenous testosterone on hepcidin knockout mice and also liver specific androgen receptor knockout mice (testosterone effects hepcidin expression through the androgen receptor). The authors reported that hepcidin knockout mice had higher baseline haematocrit and serum and liver iron levels than wild-type mice and that the administration of testosterone resulted in a significant increase in haematocrit, haemoglobin and serum iron levels in both hepcidin knockout and wild-type mice. However, testosterone use significantly decreased liver iron levels in the wild type mice.

Liver androgen receptor knockout and wildtype mice had similar haematocrit levels at baseline and testosterone treatment was associated with an increase in haematocrit levels in both wild type and liver androgen receptor knockout mice. This

increase with haematocrit levels was most pronounced in the first two weeks after administration of testosterone and plateaued for the rest of the study duration. The authors reported that exogenous testosterone resulted in similar iron indices and haemoglobin levels between wild type and liver androgen receptor knockout mice. However, testosterone administration resulted in decreased hepcidin expression in wild-type mice and liver androgen receptor knockout mice. Collectively, exogenous testosterone administration reduced hepcidin and increased iron availability and erythropoiesis even in hepcidin knockout mice.

Artz *et al.*[24] performed a placebo-controlled study investigating the effects of exogenous testosterone on iron metabolism in 95 hypogonadal men with either unexplained or iron deficiency anaemia. The authors reported that exogenous testosterone use significantly increased haemoglobin ($P<0.001$) and decreased hepcidin ($P=0.004$) and ferritin ($P=0.004$) in the unexplained anaemic cohort but not the iron deficiency group ($P=0.19$). Moreover, testosterone use did not result in a significant increase in erythropoietin in both anaemic cohorts. These results suggest that testosterone stimulates iron mobilisation, and these effects are limited in those with iron deficiency anaemia.

Combined mechanisms

There is data showing that exogenous testosterone may cause both an increase in erythropoietin and suppression of hepcidin. Bachman *et al.*[25] performed a randomised controlled trial investigating the endocrinological and haematological responses to exogenous testosterone in a cohort of 166 men aged 65 years and older. The authors reported that in the intervention group there was an increase in haemoglobin and haematocrit by 1.1 g/dL and 4.4%, respectively. This corresponded with a significant increase in erythropoietin levels and decrease in ferritin and hepcidin levels at 3 months of treatment. However, by 6 months of treatment erythropoietin and hepcidin levels returned to baseline. Interestingly, erythropoietin levels did not become supressed despite elevated haemoglobin and haematocrit with respect to baseline readings suggesting a new set point threshold. These changes were associated with an increase in iron utilisation and soluble transferrin receptor levels in the intervention group. The authors postulated that exogenous testosterone stimulated erythropoiesis by increasing the set threshold for erythropoietin release in relation to haemoglobin levels and iron utilisation.

Testosterone therapy trial data

There are studies investigating the effects of testosterone therapy on haematological indices.

Snyder *et al.*[26] performed a randomised controlled trial containing 108 men receiving either a testosterone patch or a placebo patch for 36 months. The authors reported that haemoglobin and haematocrit levels significantly increased in the

intervention group but not the placebo group (P<0.001). Moreover, 4/7 men who were anaemic at the start of the study in the intervention group developed a normal haemoglobin level. However, 2/5 in the placebo group who were anaemic at baseline also developed a normal haemoglobin during the study. Importantly, three men given exogenous testosterone developed erythrocytosis (defined as a haemoglobin >17.5 g/dL and haematocrit >52%). This highlights the risks associated with testosterone therapy and clinicians should monitor for erythrocytosis.

Roy *et al.*[27] performed a multi-centre randomised controlled trial investigating the use of testosterone gel in anaemic men (defined as a haemoglobin <12.7 g/dL) aged 65 years or older with a testosterone level <275 ng/dL. The authors reported that the use of testosterone increased haemoglobin levels by 1.0 g/dL or more compared to placebo in both anaemia of unknown cause (P=0.002) and also anaemia of known cause (P=0.003). Moreover, exogenous testosterone use significantly increased the percentage of men who transitioned from anaemic to normality compared to placebo in the unexplained anaemic group (58.3% *vs.* 2.2%, P=0.002) and explained anaemic group (52% *vs.* 19%, P=0.003). A significant increase in haemoglobin was observed in the non-anaemic cohort (odds ratio 20.7, P<0.001) and 1.7% of this cohort (6/336 men) developed erythrocytosis (defined as a haemoglobin ≥17.5 g/dL) during treatment but there were no significant differences between treatment arms in major adverse effects. The authors reported that in anaemic men an increase in haemoglobin levels of 1.0 g/dL was associated with a significant improvement in 6-minute walking distance (P<0.001) and the FACIT-Fatigue Scale (P<0.02). This study was criticised because its cut-off values for vitamin B12 and iron were considered too low and thus a proportion of patients may have been wrongly categorised as unexplained anaemia.[28]

Haematological complications of testosterone treatment

The use of testosterone therapy has potential side effects and complications.

Fernandez-Balsells *et al.*[29] performed a systematic review and meta-analysis of 51 studies investigating testosterone therapy and observed that treatment was associated with a significant increase in both haemoglobin and haematocrit compared to placebo or no treatment. However, the use of testosterone treatment was also associated with a higher risk of erythrocytosis than placebo or no treatment (relative risk: 3.15, 95% confidence interval 1.56-6.35) and thus haematological parameters need to be closely monitored with testosterone therapy.

Baillargeon *et al.*[30] performed a case control study of 30,572 men aged 40 years or older using data from a commercial insurance program. The authors observed that exposure to testosterone treatment in the 15 days prior to the event/index date had no association with an increased venous thromboembolism (VTE) risk.[30]

However, Martinez *et al.*[31] examined data from 370 primary care facilities in the U.K. (928,745 men studied) and reported that testosterone therapy was associated with an increased risk of VTE which was most pronounced in the first 6 months of starting treatment and declined thereafter.

However, two meta-analyses[32, 33] have found no statistically significant association between VTE and testosterone therapy and the VTE risk appears to be rare.[33]

Both The Endocrine society and The British Society for Sexual Medicine have recommended monitoring of haematocrit levels with testosterone therapy (at baseline, 3-6 months after starting therapy and annual screening thereafter).[7, 34] Both committees recommend cessation of therapy or dose reduction if the haematocrit levels are >54% (see Chapter 8).[7, 34]

Limitations in our understanding and future work

Further research is required to improve our understanding of the pathophysiological mechanisms underpinning the association between hypogonadism and anaemia and future studies should consider measurement of hepcidin, erythropoietin and iron utilisation markers as these are the most prominent putative mechanisms. Furthermore, studies should consider other factors such as oxidative stress, insulin resistance and inflammation as these may be potential confounding factors.[35] Moreover, clinical studies should adopt lifestyle questionnaires to identify whether the effects of testosterone therapy on haemoglobin levels reflect improvements in quality of life.

Conclusions

There is a clear association between hypogonadism and anaemia and there is extensive data showing that testosterone therapy can increase both haemoglobin and haematocrit levels. However, the mechanisms which govern this relationship are still not definitively established. Given that testosterone can cause erythrocytosis with a subsequent risk of VTE, all patients started on testosterone therapy should have their haematocrit levels monitored and be counselled accordingly.

References

1. Williamson CS. Influence of age and sex on hemoglobin: A spectrophotometric analysis of nine hundred and nineteen cases (preliminary report). JAMA 1915;65:302-7.
2. Steinglass P, Gordon AS, Charipper HA. Effect of Castration and Sex Hormones on Blood of the Rat. Proc Soc Exp Biol Med 1941;48:169-76.
3. Shahani S, Braga-Basaria M, Maggio M, *et al.* Androgens and erythropoiesis: Past and present. J Endocrinol Invest 2009;32:704-16.
4. Ershler WB. Unexplained Anemia in the Elderly. Clin Geriatr Med 2019;35:295-305.

5. WHO. WHO | Global anaemia prevalence and number of individuals affected. Available from: https://www.who.int/vmnis/anaemia/prevalence/summary/anaemia_data_status_t2/en/
6. Guralnik JM, Eisenstaedt RS, Ferrucci L, *et al.* Prevalence of anemia in persons 65 years and older in the United States: Evidence for a high rate of unexplained anemia. Blood 2004;104:2263-8.
7. Hackett G, Kirby M, Edwards D, *et al.* British Society for Sexual Medicine Guidelines on Adult Testosterone Deficiency, With Statements for UK Practice. J Sex Med 2017;14:1504-23.
8. Hamilton EJ, Davis WA, Makepeace A, *et al.* Prevalence and prognosis of a low serum testosterone in men with type 2 diabetes: the Fremantle Diabetes Study Phase II. Clin Endocrinol (Oxf) 2016;85:444-52.
9. Nissenson AR, Wade S, Goodnough T, *et al.* Economic burden of anemia in an insured population. J Manag Care Pharm 2005;11:565-74.
10. Röhrig G, Becker I, Schulz RJ, *et al.* Association between hematologic parameters and functional impairment among geriatric inpatients: Data of a prospective cross-sectional multicenter study ("GeriPrävalenz2013"). Maturitas 2016;90:37-41.
11. Saad F, Röhrig G, Von Haehling S, *et al.* Testosterone Deficiency and Testosterone Treatment in Older Men. Gerontology 2017;63:144-56.
12. Ferrucci L, Maggio M, Bandinelli S, *et al.* Low testosterone levels and the risk of anemia in older men and women. Arch Intern Med 2006;166:1380-8.
13. Yeap BB, Beilin J, Shi Z, *et al.* Serum testosterone levels correlate with haemoglobin in middle-aged and older men. Intern Med J 2009;39:532-8.
14. Grossmann M, Zajac JD. Hematological changes during androgen deprivation therapy. Asian J Androl 2012;14:187-92.
15. Moriyama Y, Fisher JW. Effects of testosterone and erythropoietin on erythroid colony formation in human bone marrow cultures. Blood 1975;45:665-70.
16. Bhatia V, Chaudhuri A, Tomar R, *et al.* Low testosterone and high C-reactive protein concentrations predict low hematocrit in type 2 diabetes. Diabetes Care 2006;29:2289-94.
17. Makipour S, Kanapuru B, Ershler WB. Unexplained Anemia in the Elderly. 2008;45:250-4.
18. Allen DA, Breen C, Yaqoob MM, *et al.* Inhibition of CFU-E Colony Formation in Uremic Patients with Inflammatory Disease: Role of IFN-γ and TNF-α. J Investig Med 1999;47:204-11.
19. Coviello AD, Kaplan B, Lakshman KM, *et al.* Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. J Clin Endocrinol Metab 2008;93:914-9.
20. Samaras N, Samaras D, Lang PO, *et al.* A view of geriatrics through hormones. What is the relation between andropause and well-known geriatric syndromes? Maturitas 2013;74:213-9.
21. Bachman E, Feng R, Travison T, *et al.* Testosterone suppresses hepcidin in men: A potential mechanism for testosterone-induced erythrocytosis. J Clin Endocrinol Metab 2010;95:4743-7.
22. D'Angelo G. Role of hepcidin in the pathophysiology and diagnosis of anemia. Blood Res 2013;48:10-5.
23. Guo W, Schmidt PJ, Fleming MD, *et al.* Hepcidin is not essential for mediating testosterone's effects on erythropoiesis. Andrology 2020;8:82-90.

24. Artz AS, Stephens-Shields AJ, Bhasin S, *et al.* Markers of Iron Flux duringTestosterone-Mediated Erythropoiesis in Older Men with Unexplained or Iron-Deficiency Anemia. J Clin Endocrinol Metab 2020;105:3396-403.

25. Bachman E, Travison TG, Basaria S, *et al.* Testosterone induces erythrocytosis via increased erythropoietin and suppressed hepcidin: Evidence for a new erythropoietin/hemoglobin set point. J Gerontol A Biol Sci Med Sci 2014;69:725-35.

26. Snyder PJ, Peachey H, Hannoush P, *et al.* Effect of testosterone treatment on bone mineral density in men over 65 years of age. J Clin Endocrinol Metab 1999;84:1966-72.

27. Roy CN, Snyder PJ, Stephens-Shields AJ, *et al.* Association of testosterone levels with anemia in older men a controlled clinical trial. JAMA Intern Med 2017;177:480-90.

28. Eisenga MF, Stam SP, Bakker SJL. Redefining unexplained anemia in elderly. JAMA Intern Med 2017;177:1394-5.

29. Fernández-Balsells MM, Murad MH, Lane M, *et al.* Adverse Effects of Testosterone Therapy in Adult Men: A Systematic Review and Meta-Analysis. J Clin Endocrinol Metab 2010;95:2560-75.

30. Baillargeon J, Urban RJ, Morgentaler A, *et al.* Risk of Venous Thromboembolism in Men Receiving Testosterone Therapy. Mayo Clin Proc 2015;90:1038-45.

31. Martinez C, Suissa S, Rietbrock S, *et al.* Testosterone treatment and risk of venous thromboembolism: Population based case-control study. BMJ 2016;355:i5968.

32. Houghton DE, Alsawas M, Barrioneuvo P, *et al.* Testosterone therapy and venous thromboembolism: A systematic review and meta-analysis. Thromb Res 2018;172:94-103.

33. Ayele HT, Brunetti VC, Renoux C, *et al.* Testosterone replacement therapy and the risk of venous thromboembolism: A systematic review and meta-analysis of randomized controlled trials. Thromb Res 2021;199:123-31.

34. Bhasin S, Brito JP, Cunningham GR, *et al.* Testosterone Therapy in Men with Hypogonadism: An Endocrine Society. J Clin Endocrinol Metab 2018;103:1715-44.

35. Grossmann M, Panagiotopolous S, Sharpe K, *et al.* Low testosterone and anaemia in men with type 2 diabetes. Clin Endocrinol (Oxf) 2009;70:547-53.

8

Association between haematocrit and health outcomes

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Association between haematocrit and health outcomes

Testosterone replacement is indicated in men with low testosterone concentrations such as primary or secondary hypogonadism and adult-onset testosterone deficiency (TD).[1] The United States Food and Drug Administration advocate testosterone therapy (TTh) in classical hypogonadal states only, although other guidelines suggest treatment in TD, a condition increasingly recognised and with a high prevalence (6-12% of men and 40% of men with type 2 diabetes (T2DM)).[2] While some studies have suggested increased cardiovascular disease (CVD) associated with TTh,[3] a recent meta-analysis by Corona *et al.* has not corroborated these controversial findings, especially when TTh is prescribed appropriately.[4] Nonetheless, vigilance must be exercised in clinical care to ensure that variables which might be associated with CVD, such as increased haematocrit (HCT) are monitored. Elevated HCT is the most frequent adverse effect of TTh.[2] As blood oxygen content is linearly and positively associated with HCT, greater tissue oxygenation should result from increased haematocrit. However, a rise in HCT is also related to increased blood viscosity (other contributory factors being plasma viscosity and erythrocyte deformability/aggregation) thereby reducing blood flow.[5] Hence an optimal HCT may exist, in which there is equilibrium between tissue oxygenation and blood flow.

HCT and TTh: what are the guidelines?

Guidelines vary in their suggestions as to the HCT level, above which clinicians should consider ceasing or altering TTh regimens. For example, The British Society for Sexual Medicine,[2] Endocrine Society,[6] European Association of Urology[7] and American Urological Association[8] have all adopted an HCT cut-off of 54%. In contrast, The International Society for the Study of the Aging Male has adopted an HCT cut-off of 52%,[9] whilst the International Consultation for Sexual Medicine has recommended an even more conservative threshold of 50%.[10] We have previously

outlined the pitfalls of using a laboratory reference range to determine the central 95% values of the healthy cohort.[11] Thus, we consider that an action threshold approach based on sensitivity/specificity levels may be better when predicting morbidity and mortality. Because a target HCT value may vary with factors including blood vessel diameter, erythrocyte deformability/aggregation and clinical heterogeneity (*e.g.* diabetes) establishing the ideal HCT for each patient will be problematic.[5]

Increase in HCT associated with TTh: data from studies

We now describe studies that demonstrate increase in HCT associated with TTh. We will not discuss data on HCT changes in placebo groups. A randomised controlled trial (RCT) by Aversa *et al.* showed that following testosterone undecanoate (TU), mean HCT±SD increased (from a baseline of 43.0±3.5%) by 3.5±3.0% and 3.1±3.5%, at 12 and 24 months respectively.[12] Francomano *et al.* studied 20 men with metabolic syndrome (MetS) and TD, treated with TU for 60 months.[13] HCT increased during the follow-up period, from a baseline of 43.8±0.2% to 46.1±0.8% (12 months), 46.1±0.7% (24 months), 46.4±0.6% (36 months), 46.5±0.6% (48 months) and finally 46.6±0.9% (60 months).[13] In another RCT and then open-label phase of 24 months involving 55 obese men (body mass index [BMI] ≥30 kg/m^2) with T2DM and TD, men receiving TU had a mean HCT±SD increase from 42.7±2.0% (baseline) to 43.8±2.6% (3 months), 44.9±2.8% (6 months), 45.3±3.1% (12 months), 45.6±2.7% (15 months), 45.8±3.3% (18 months) and finally 46.0±2.7% (24 months).[14] Importantly a HCT threshold of 52% was not breached in any men given TU. This differed, however, in the T4DM study: a 2-year RCT in which 504 men with waist circumferences >95 cm, impaired glucose tolerance/newly diagnosed T2DM and a serum testosterone ≤14 nmol/L were administered TU.[15] One of the safety triggers was a HCT≥54%. In this study, 106 of 491 (22%) men on TU breached this threshold, compared to 6 of 484 (1%) men on placebo. TU was discontinued in 25 of the 106 men, whilst HCT in the remainder fell below this level on repeat testing, or by the time they had received the final administration of TU.[15]

Increase in HCT associated with TTh: possible mechanisms

The mechanism(s) of HCT increase associated with TTh is unclear. A higher HCT could be due to increased production or decreased breakdown of erythrocytes. Most focus has been on the former process.[5] Coviello *et al.* showed a linear dose-dependent increase in HCT following TTh.[16] The increase was evident in both men aged 19-35 years and 60-75 years, though it was greater in the older cohort.[16] No increase in erythropoietin or soluble transferrin receptor (a marker of bone marrow erythropoiesis) levels was observed. Thus, Coviello *et al.* suggested that testosterone could have a direct bone marrow stimulatory effect by perhaps enhancing

differentiation of erythroid colony forming units into erythropoietin sensitive cells. However Bachman *et al.* demonstrated that the increase in HCT following TTh, was associated with higher erythropoietin concentrations.[17] Following TTh, this increase was noted for just 1-3 months, whilst after 6 months there was a return to pre-TTh levels (no suppression in erythropoietin was found despite the increase in HCT and haemoglobin [Hb]).[17] Interestingly, though TU was significantly associated with lower glucose levels in the T4DM study, HbA1c was not decreased.[15] The T4DM investigators suggested the mechanism for an elevation in HCT was via an increase in erythrocyte lifespan.[15] If confirmed, this suggestion has implications for the use of HbA1c as a marker for glycaemic control in men receiving TTh. This is important given that TD is associated with MetS and T2DM.[2, 18]

HCT and CVD / mortality

Though to date no available RCT describes the relationship between TTh- associated HCT increase and CVD/mortality outcomes, results from longitudinal observational studies hint at a non-linear relationship. Danesh *et al.* carried out a meta-analysis of 16 studies comprising 8020 individuals with a mean HCT of 44%.[19] In the subjects in the upper tertile (HCT>46.3%), there was a significant association (risk ratio: 1.16, 95% CI: 1.05-1.29) with ischaemic heart disease, compared to those in the lower tertile (HCT<41.7%).[19] The significance was strengthened (risk ratio: 1.81, 95% CI: 1.19-2.76, upper HCT tertile *vs.* lower HCT tertile) by the addition of 3 trials that included subjects with established CVD. However, it should be noted that CVD risk factors varied between the different studies.[19]

In contrast, there was no difference between the upper (HCT>47.0%) and lower (HCT<45.0%) tertiles in terms of CVD over a period of 10 years in the European Prospective Investigation into Cancer and Nutrition - Netherlands (EPIC-NL) study (derived from the MORGEN-EPIC and Prospect-EPIC studies) comprising 16,187 patients with no previous CVD.[20]

The Scottish Heart Health Extended Cohort Study studied the associations between plasma viscosity, HCT and whole blood viscosity (the latter being dependent upon both plasma viscosity and haematocrit), and CVD/mortality in 3386 individuals aged 30-74 years over 10-21 years.[21] Increased plasma viscosity was independently associated with CVD and mortality. Despite HCT (mean±SD, 0.4381±0.0394) also being significantly associated with CVD (Hazard ratio (HR): 1.14, 95% CI: 1.04-1.25, P=0.004) and mortality (HR: 1.22, 95% CI: 1.11-1.33, P<0.001), significance was lost (after adjusting for age and gender), when lipids, blood pressure, diabetes, smoking status, family history of CVD and fibrinogen were included as confounders.[21]

Some studies have suggested a J- or U-shaped non-linear relationship between HCT and CVD.[22, 23] The Framingham cohort of 5209 individuals of both genders

followed up over 34 years showed only the highest quintile to be associated (J-shaped association) with both CVD and mortality.[22] Boffetta *et al.* studied 49,983 Iranian adults and showed a U-shaped association between HCT and mortality in both sexes: low and high HCT levels were associated with increased mortality.[23] The risk thresholds were different for men and women. Cox regression analyses showed that mortality in males was higher when HCT was ≤39% or ≥45% (reference: 40-44%) and ≤34% or ≥40% (reference: 35-39%) in females.[23] The non-linear relationship between HCT and mortality is evident when HCT is increased from a low baseline level, as seen in a study of 5302 patients (Lombardy Registry, mean baseline HCT±SD: 0.301±0.045) with end stage renal failure, treated with erythropoietin.[24] Mortality was negatively associated with an increase in HCT (Odds ratio (OR): 0.95, 95% CI: 0.92-0.97) following erythropoietin.[24]

Elevated HCT and T2DM

Insulin resistance and impaired insulin secretion are factors that could influence the association between HCT and mortality.[25] This is important as MetS and T2DM are associated with adult-onset TD[2, 18] and TTh is commonly used in these men with a resultant greater risk of TTh-related HCT increase. Wannamethee *et al.* noted an independent association between HCT and T2DM incidence in a prospective study of 7193 middle-aged men.[26] This was independent of age, BMI, smoking, physical activity, HDL cholesterol and systolic blood pressure. It was observed that T2DM risk was significantly higher in men with HCT ≥ 48.0% compared with HCT <42.0% (risk ratio: 4.5; 95% CI: 2.5-6.3, regression model adjusted for age and BMI). These findings led the authors to recommend that HCT be considered a risk modifier in the association between T2DM and CVD, in view of its effect of increasing blood viscosity.[26] In T2DM, erythrocytes appear to undergo morphological changes as their shape alters from the expected biconcave disc, to a more elongated structure, thereby increasing blood viscosity.[5, 27] Furthermore, reduced erythrocyte deformability in T2DM has been associated with poorer glycaemic control and microvascular complications.[28] It is possible that the increase in erythrocyte aggregation noted in T2DM can also raise blood viscosity, which in turn could be a risk factor in the development of microvascular disease.[29, 30] This risk is possibly exacerbated further by the increase in HCT following TTh. Thus, with this emergent evidence, it may be appropriate to consider T2DM as a subgroup, within the heterogenous adult-onset TD cohort.[11]

TTh and change in HCT following TTh: data from the BLAST RCT and BLAST screened cohort survey

The BLAST (Burntwood, Lichfield, Atherstone, Sutton Coldfield, Tamworth) RCT (European Union Clinical Trials Register: EudraCT 2008-000931-16, West Midlands

Regional Ethics Committee approval: 08/H1208/30) was a 30-week randomised double-blind placebo-controlled trial (September 2008 - June 2012) carried out in the West Midlands, England, to assess the impact of TTh (1000mg of TU) in 199 men with T2DM and adult-onset TD (serum total testosterone ≤12 nmol/L or calculated free testosterone ≤0.25 nmol/L and symptoms of TD).[31] Data were available in 103 and 86 men receiving placebo and TU respectively. In order to recruit the 199 men with T2DM and adult-onset TD, 857 men with T2DM (from the patient registers of 5 primary care practices), were screened for total testosterone (TT) and calculated free testosterone (cFT) between April 2007-April 2009.[32]

In 2014, following the completion of the BLAST RCT, it was proposed that data be obtained on the 857 men (BLAST screened cohort) screened for the RCT. Based upon early morning TT and cFT levels of the 857 patients screened for the BLAST RCT, men were classified as having either: Normal Testosterone (TT>12 nmol/L and cFT>0.25 nmol/L; 320 men) or Low Testosterone (TT≤12 nmol/L or cFT≤0.25 nmol/L; 537 men). The Low Testosterone group were further stratified by whether they were prescribed TTh (175 men) or remained untreated (362 men) during the follow-up (median [IQR]: 3.9 (3.2, 4.6) years). Between April-September 2014, all available data were collected from the most recent attendance to assess changes from baseline in the 857 men. The long-term follow-up of the BLAST screened cohort[32] was approved as an audit by the relevant Primary Care Trust Ethics Committees.

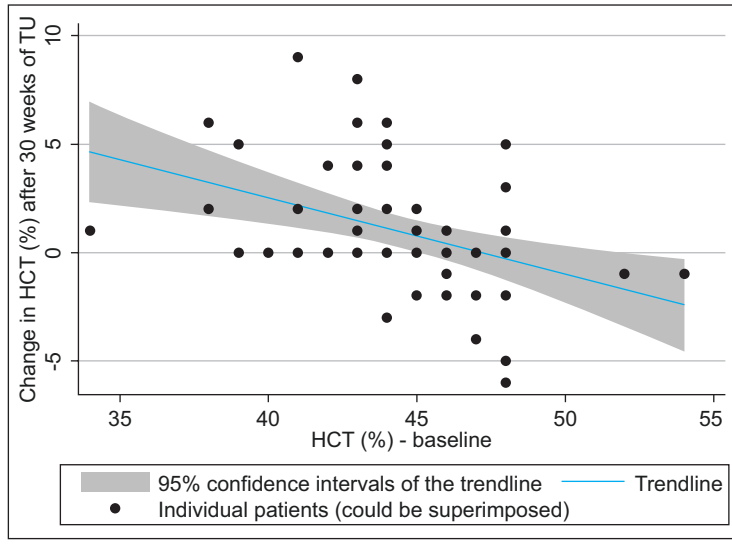
BLAST RCT: change in HCT and factors predicting HCT change

HCT during follow-up was available in 61 of the 86 men on TU at 30 weeks. Whilst HCT did not change significantly in men given placebo (baseline: 43.2%, 30-weeks: 43.5%, P [sign-rank test]=0.15), it increased significantly in men receiving TU (baseline: 44.4%, 30-weeks: 45.4%, P[sign-rank test]=0.015). Furthermore, no patient breached the 54.0% threshold during the follow-up. Figure 8.1 (Model 1 of the attached data) shows that baseline HCT– but not age, total testosterone level, SHBG or HbA1c – was associated with the 1% increase in HCT after 30 weeks of TU. A univariate linear regression analysis showed a similar association (Figure 8.1: model 2 of the attached data) between baseline HCT and change in HCT at the end of the RCT in the 61 men on TU (c:-0.35, 95% CI:-0.56, -0.14, P=0.001). Importantly, the association had a negative coefficient, indicating that the lowest baseline HCT would result in the greatest increase. This may provide reassurance that men receiving TTh with a baseline HCT approaching the upper reference limit are unlikely to undergo a significant rise.

BLAST screened cohort survey

This analysis included 291 men with HCT data at baseline (as 97 of the 175 men started on TU had their treatment discontinued after varying treatment duration, we only included for this analysis, men never prescribed TTh). Firstly, we

Figure 8.1. BLAST RCT: a graphic illustration of the association between baseline HCT and change in HCT after 30 weeks in the 61 men on TU treatment (based on Model 2 of the data presented below the chart). Baseline factors associated with HCT change in men on TU; the above chart is based on model 2.



	c (95% CI)	P	R^2
Change in HCT (%) after 30 weeks - RCT			
Model 1 (N.=55)			
Baseline HCT (%)	-0.35 (-0.60, -0.10)	0.007	0.19
Age (Years)	0.026 (-0.052, 0.10)	0.51	
Total testoseronr (nmol/L)	-0.049 (-0.33, 0.23)	0.73	
SHBG (nmol/L)	-0.046 (-0.11, 0.022)	0.18	
HbA1c (%)	-0.22 (-0.88, 0.43)	0.53	
Model 2 (N.=61)			
Baseline HCT (%)	-0.35 (-0.56, -0.14)	0.001	0.16

confirmed in the BLAST screened cohort, that baseline HCT was associated with change in HCT. This was previously observed in the BLAST RCT in 166 of the 291 men not on TU and with HCT data available at baseline and final assessment. This larger screened cohort with longer follow-up (median [IQR]:4.35 (3.45, 5.16) years) also showed an inverse relationship (linear regression, c:-0.40, 95% CI:-0.58, -0.22, P<0.001, N.=166 men) between baseline HCT (independent variable) and change in HCT (dependent variable). Thus, the association between baseline

HCT and change in HCT is evident in both men on TTh (BLAST RCT) and not on TTh (BLAST screened cohort). We then studied factors related to HCT at baseline, and subsequently studied the association between baseline HCT, change in HCT, final HCT and mortality.

a. Association between baseline factors and HCT

We performed multiple regression analyses on the men with HCT measured at baseline (291 of the 537 men not on TTh). Table 8.I shows that age, serum total testosterone and diastolic blood pressure were independently associated with baseline HCT when entered in a single multiple regression model. It is important to note that since the introduction of the Quality and Outcomes Framework,[33] HbA1c, blood pressure and lipids in individuals with T2DM, would be treated to target, and this could skew the distribution of these parameters.[34]

Table 8.I BLAST screened cohort survey: linear / multiple regression analyses between HCT (dependent variable) and other factors (independent variables) measured at baseline.

	Baseline values		Association with baseline HCT(%)	
	N.	Median (IQR)	c (95% CI)	P
Baseline HCT (%)	291	44.0 (41.0, 46.0)		
Independent variables (HCT: dependent variable)			Separate regression models	
Age (years)	291	66.9 (57.9, 73.7)	-0.14 (-0.17, -0.10)	<0.001
Total testosterone (nmol/L)	280	12.6 (9.4, 16.4)	0.16 (0.076, 0.24)	<0.001
Sex hormone binding globulin (nmol/L)	236	35.8 (27.5, 52.3)	-0.015 (-0.040, 0.0094)	0.23
HbA1c (%)	272	7.0 (6.5 - 7.7)	0.32 (-0.052, 0.68)	0.092
BMI (kg/m^2)	289	30.3 (26.9, 34.0)	0.08 (-0.00098, 0.17)	0.053
Systolic blood pressure (mmHg)	291	138 (128, 148)	-0.16 (-0.043, 0.011)	0.26
Diastolic blood pressure (mmHg)	291	78 (70, 84)	0.074 (0.033, 0.11)	<0.001
			Single regression model	
Age (years)			-0.12 (-0.16, -0.083)	<0.001
Total testosterone (nmol/L)			0.14 (0.065, 0.22)	<0.001
Diastolic blood pressure (mmHg)			0.044 (0.0038, 0.085)	0.032

b. Association between HCT change and mortality

HCT at baseline and final assessment was available in 166 men, with 146 men surviving (12.0% mortality). Baseline HCT was similar to the larger cohort seen in Table 8.I (median HCT (IQR): 44.0 (41.0, 46.0)%) and no significant difference was observed between the men who died and those who survived (Table 8.II). Age, as expected was the only baseline factor to be associated with

Table 8.II. BLAST screened cohort survey: data, stratified by mortality of the men not on TTh followed by a Cox regression analysis examining the association between HCT and survival.

	Median (IQR)		
Baseline values	**Alive (N.=146)**	**Dead (N.=20)**	**P (rank-sum)**
HCT (%)	44.0 (41,46)	43.0 (39.0, 46.0)	0.35
Age (years)	66.5 (58.9, 72.8)	76.7 (72.0, 79.8)	0.0001
Total testosterone (nmol/L)	12.6 (9.1, 15.8), N.=140	10.5 (8.9, 12.5)	0.056
Sex hormone binding globulin (nmol/L)	35.4 (27.1, 49.7), N.=127	41.6 (33.8, 63,4)	0.063
HbA1c (%)	7.1 (6.5, 7.8), N.=134	7.2 (6.4, 7.8)	1.00
BMI (kg/m^2)	30.0 (26.9, 33.7)	27.0 (24.2, 32.0),n=19	0.057
Systolic blood pressure (mmHg)	138 (126, 149)	139 (130, 156)	0.33
Diastolic blood pressure (mmHg)	78 (70, 84)	70 (62, 80)	0.069
Values at final assessment			
HCT at final assessment (%)	43.0 (39.0, 45.0)	36.0 (31.5, 42.5)	0.0009
Change in HCT (%)	-1.0 (-3.0, 1.0)	-3.5 (-10, 0.5)	0.038
Cox regression analyses	**Hazard Ratio (95 % CI)**		**P**
Model 1			
Baseline age (years)	1.10 (1.03, 1.18)		0.005
Baseline HCT (%)	0.85 (0.76, 0.95)		0.006
Change in HCT (%)	0.82 (0.75, 0.90)		<0.001
Model 2			
Baseline age (years)	1.11 (1.05, 1.17)		<0.001
HCT at final assessment (%)	0.85 (0.79, 0.92)		<0.001

There were insufficient data for the regression analyses to include change in testosterone, sex hormone binding globulin, HbA1c, BMI and blood pressure as confounders.

mortality. Interestingly, change in HCT as well as HCT at final assessment were both associated with mortality; the values being higher in the men who survived. Cox regression analyses were performed to confirm the above findings (Table 8.II: models 1 and 2) with age included as a confounder. Age, baseline HCT and change in HCT were seen to be significantly associated with survival (Table 8.II: model 1), and as expected from these results, HCT at final assessment showed a similar result (Table 8.II: model 2). All HCT parameters were inversely related to mortality.

Further research is required to evaluate this association. Unfortunately, data on change in serum TT, SHBG and Hb concentrations were scarce and their inclusion as confounding factors within the regression models would have been valuable. Whilst HCT may therefore not be causative, and perhaps represent a surrogate factor for disease states, such as anaemia, our analyses demonstrate a pattern in which low HCT may provide a useful marker of mortality in men with adult-onset TD and T2DM.

Conclusions

We have reviewed the recommendations offered by various guidelines regarding monitoring HCT levels when using TTh. Furthermore, the increased HCT associated with TTh in numerous studies is summarised, with possible mechanisms described. The T4DM study suggests TTh may be associated with increased erythrocyte longevity,[15] this being of potential clinical importance when monitoring glycaemic control in men with T2DM on TTh and should be further evaluated. The association between HCT and mortality appears complex in view of effects on oxygen delivery and viscosity. T2DM may also have an impact on all the associations described above and these patients should be considered as an important subgroup. Finally, we have presented data from the BLAST RCT of an inverse relationship between baseline HCT and change in HCT, and from the BLAST screened cohort survey of an inverse association between HCT and mortality in men with T2DM and low testosterone levels. This association requires validation in different subgroups (*e.g.*, men receiving TTh). Possible mechanism(s) whereby elevated HCT can lead to increased CVD and mortality should be explored. We recently noted that peak systolic velocity was associated with coronary heart disease, although we suggested that peak systolic velocity may have been a surrogate for a summation of risk factors that were associated with it.[5, 35] The impact of changing HCT on flow characteristics in different patient subgroups and arteries of varying diameter is also an area for further evaluation. This will hopefully lead to greater understanding of the mechanisms involved and perhaps, may provide a more objective measure of risk. Until then we would agree that the currently recommended HCT of 54% be used as a threshold for caution in men on TTh for adult-onset TD.[2]

References

1. Livingstone M, Kalansooriya A, Hartland AJ, *et al.* Serum testosterone levels in male hypogonadism: Why and when to check – A review. Int J Clin Pract 2017;71:e12995.
2. Hackett G, Kirby M, Edwards D, *et al.* British Society for Sexual Medicine guidelines on adult testosterone deficiency, with statements for UK practice. J Sex Med 2017;14:1504-23.
3. Morgentaler A, Miner MM, Caliber M, *et al.* Testosterone therapy and cardiovascular risk: advances and controversies. Mayo Clin Proc 2015; 90:224-51.
4. Corona G, Rastrelli G, Di Pasquale G, *et al.* Testosterone and cardiovascular risk: meta-analysis of interventional studies. J Sex Med 2018;15:820-38.
5. Konig CS, Balabani S, Hackett GI, *et al.* Testosterone therapy: An assessment of the clinical consequences of changes in haematocrit and blood flow characteristics. Sex Med Rev 2019;7:650-60.
6. Bhasin S, Cunningham GR, Hayes FJ, *et al.* Testosterone therapy in men with androgen deficiency syndromes: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2010;95:2536-59.
7. Dohle GR, Arver S, Bettocchi C, *et al.* European Association of Urology Guidelines: Male Hypogonadism [Internet]. 2018 [cited 21 May 2021]. Available from: https://uroweb.org/guideline/male-hypogonadism/
8. Mulhall JP, Trost LW, Brannigan RE, *et al.* Evaluation and management of testosterone deficiency: AUA guideline. J Urol 2018;200:423-32.
9. Lunenfeld B, Mskhalaya G, Zitzmann M, *et al.* Recommendations on the diagnosis, treatment and monitoring of hypogonadism in men. Aging male 2015;18:5-15.
10. Khera M, Adaikan G, Buvat J, *et al.* Diagnosis and treatment of testosterone deficiency: recommendations from the Fourth International Consultation for Sexual Medicine (ICSM 2015). J Sex Med 2016;13:1787-804.
11. Ramachandran S, Konig CS, Hackett G, *et al.* Managing clinical heterogeneity: An argument for benefit based action limits. Journal of Medical Diagnostics and Therapy 2018;1:034701.
12. Aversa A, Bruzziches R, Francomano D, *et al.* Effects of testosterone undecanoate on cardiovascular risk factors and atherosclerosis in middle-aged men with late-onset hypogonadism and metabolic syndrome: results from a 24-month, randomized, double-blind, placebo-controlled study. J Sex Med 2010;7:3495-503.
13. Francomano D, Lenzi A, Aversa A. Effects of five-year treatment with testosterone undecanoate on metabolic and hormonal parameters in ageing men with metabolic syndrome. Int J Endocrinol 2014;2014:527470.
14. Groti Antonič K, Antonič B, Pfeifer M. Effects of Testosterone Therapy on Erythrocytosis and Prostate Adverse Events in Obese Males with Functional Hypogonadism and Type 2 Diabetes in a 2-Year Clinical Trial. Androgens: Clinical Research and Therapeutics 2020;1:85-93.
15. Wittert G, Bracken K, Robledo KP, *et al.* Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021;9:32-45.
16. Coviello AD, Kaplan B, Lakshman KM, *et al.* Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. J Clin Endocrinol Metab 2008;93:914-9.

17. Bachman E, Feng R, Travison T, *et al.* Testosterone suppresses hepcidin in men: a potential mechanism for testosterone-induced erythrocytosis. J Clin Endocrinol Metab 2010;95:4743-7.
18. Wang C, Jackson G, Jones TH, *et al.* Low testosterone associated with obesity and the metabolic syndrome contributes to sexual dysfunction and cardiovascular disease risk in men with type 2 diabetes. Diabetes care 2011;34:1669-75.
19. Danesh J, Collins R, Peto R, *et al.* Haematocrit, viscosity, erythrocyte sedimentation rate: meta-analyses of prospective studies of coronary heart disease. Eur Heart J 2000;21:515-20.
20. Lassale C, Curtis A, Abete I, *et al.* Elements of the complete blood count associated with cardiovascular disease incidence: findings from the EPIC-NL cohort study. Sci Rep 2018;8:1-11.
21. Peters SA, Woodward M, Rumley A, *et al.* Plasma and blood viscosity in the prediction of cardiovascular disease and mortality in the Scottish Heart Health Extended Cohort Study. Eur J Prev Cardiol 2017;24:161-7.
22. Gagnon DR, Zhang TJ, Brand FN, *et al.* Hematocrit and the risk of cardiovascular disease--the Framingham study: a 34-year follow-up. Am Heart J 1994;127: 674-82.
23. Boffetta P, Islami F, Vedanthan R, *et al.* A U-shaped relationship between haematocrit and mortality in a large prospective cohort study. Int J Epidemiol 2013;42:601-15.
24. Locatelli F, Conte F, Marcelli D. The impact of haematocrit levels and erythropoietin treatment on overall and cardiovascular mortality and morbidity--the experience of the Lombardy Dialysis Registry. Nephrol Dial Transplant 1998;13:1642-4.
25. Facchini FS, Carantoni M, Jeppesen J, *et al.* Hematocrit and hemoglobin are independently related to insulin resistance and compensatory hyperinsulinemia in healthy, non-obese men and women. Metabolism 1998;47:831-5.
26. Wannamethee SG, Perry IJ, Shaper AG. Hematocrit and risk of NIDDM. Diabetes 1996;45:576-9.
27. Pasquini G, Albanese B, Manescalchi PG, *et al.* Relation of blood viscosity, plasma viscosity and haematocrit. Ric Clin Lab 1983;13:327-31.
28. Moon JS, Kim JH, Kim JH, *et al.* Impaired RBC deformability is associated with diabetic retinopathy in patients with type 2 diabetes. Diabetes Metab 2016;42:448-52.
29. Cho YI, Mooney MP, Cho DJ. Hemorheological Disorders in Diabetes Mellitus. J Diabetes Sci Technol 2008;2:1130-8.
30. Lin T, Rechenmacher S, Rasool S, *et al.* Reduced Survival in Patients with "Coronary Microvascular Disease". Int J Angiol 2012;21:89-94.
31. Hackett G, Cole N, Bhartia M, *et al.* Testosterone replacement therapy improves metabolic parameters in hypogonadal men with type 2 diabetes but not in men with coexisting depression: the BLAST study. J Sex Med 2014;11:840-56.
32. Hackett G, Heald AH, Sinclair A, *et al.* Serum testosterone, testosterone replacement therapy and all-cause mortality in men with type 2 diabetes: retrospective consideration of the impact of PDE5Inhibitors and statins. Int J Clin Pract 2016;70:244-53.
33. Clinical Knowledge Summaries: Diabetes-Type 2: QOF Indicators [Internet]. National Institute for Health and Care Excellence; c2008-2021 [cited 2021, May 21]. Available from: https://cks.nice.org.uk/topics/diabetes-type-2/goals-outcome-measures/qof-indicators/

34. Clarke EL, Richardson JR, Bhartia M, *et al.* Convergence of HbA1c values towards target in 272 primary care patients following nine years of target-driven care. Qual Prim Care 2013;21:285-90.
35. König CS, Atherton M, Cavazzuti M, *et al.* The association of peak systolic velocity in the carotid artery with coronary heart disease: A study based on portable ultrasound. Proc Inst Mech Eng H 2021;235:663-75.

9

Testosterone therapy in diabetes

Geoffrey I. Hackett

Key messages

Approximately 40% of men with type 2 diabetes (T2DM) have hypogonadatrophic hypogonadism (HH) with associated increased risk of cardiovascular and all-cause mortality.[1] Men with HH are at significantly increased risk of developing incident T2DM. During the development of the British Society for Sexual Medicine (BSSM) guidelines for testosterone therapy, we conducted MEDLINE, EMBASE and COCHRANE reviews on T2DM, HH, testosterone deficiency, cardiovascular and all-cause mortality from May 2005 to December 2020. This yielded 1820 articles, 54 clinical trials and 33 randomised clinical trials involving testosterone therapy in men with T2DM or Metabolic Syndrome (MetS). Studies with testosterone therapy suggest significant benefits in glycaemic control, anaemia, bone density, fat and lean muscle mass, sexual function and quality of life.[2] Meta-analyses of RCTs, rather than providing clarification, have further confused the issue by including under-powered studies of inadequate duration, multiple therapy regimes, some discontinued, and inbuilt bias in terms of studies included or excluded from analysis.

Introduction

Type 2 Diabetes is a major world-wide health and economic concern. Currently 420 million people in the world live with T2DM. In the UK in 2017, 26% of the population over 65 have T2DM, and 56% of these were men. The prevalence is 6 times greater in men of South East Asian origin and 3 times greater in men of Afro-Caribbean background.[3] The mechanisms behind these increased rates are complex, involving increased visceral fat, decreased metabolism of fat during exercise and the identification of several genetic links to this predisposition. In the US, two-thirds of men over 65 have T2DM, predominantly linked to obesity and dietary factors.[4]

Obesity accounts for 80-85 per cent of the overall risk of developing Type 2 diabetes and underlies the current global spread of the condition.[3] Other risk factors are sedentary lifestyle, family history and gestational diabetes. In men, there is now

strong evidence linking low testosterone to obesity, components of the metabolic syndrome and subsequent T2DM.[3] Several studies have shown high levels of hypogonadism (HG) in men with type 2 diabetes with around 20% being overtly hypogonadal with TT below 8 nmol/L and around 50% falling below the 12 nmol/L level for mild HG.[5] The American Association of Clinical Endocrinologists (AACE), American Diabetes Association (ADA) recommend that all men with T2DM should be screened for hypogonadism along with all men with BMI >30 or waist circumference over 104 cm.[6] The 2018 Endocrine Society guidelines, in contrast, advise against any form of testosterone screening.

Recent re-classification of HG by the Endocrine Society refers to T2D related HG as "functional" and some endocrine guidelines[7] suggest that only "classical" HG be treated. Evidence suggests that men classified as "functional HG" form the majority showing benefit from clinical trials.[8]

Evidence that low testosterone levels predispose to development of type 2 diabetes

The link between T2DM and HG is considered bi-directional and conventional management has centred around lifestyle strategies of weight and exercise which would appear to be failing as the worldwide prevalence of T2DM continues to rise.[9] The evidence suggests that low testosterone leads to new onset T2DM and contributes to worsening co-morbidities.[10-12]

In a study of 1,413 men, those in the first (lowest) tertile of low free testosterone (FT) and TT were four times more likely to have diabetes than those in the third tertile of low TT and FT.[10] Furthermore, low FT and sex hormone binding globulin (SHBG) have been shown to predict the onset of diabetes in men for up to 10 years of follow up.[11] A meta-analysis of prospective studies showed men with total testosterone levels above 15.5 nmol/L had a 42% lower risk of incident diabetes compared with men with a total testosterone of 15.5 nmol/L or less.[13] A 2011 meta-analysis by Corona *et al.*,[13] found baseline total testosterone was 2.08 nmol/L lower in men who developed incident T2DM compared with those who did not. They concluded that a major reason for this diminished relationship in some studies was failure to adjust for central adiposity by waist circumference.

Several longitudinal studies have shown that low levels of TT and FT independently predict development of T2DM or metabolic syndrome.[14-20]

Holmboe *et al.*[21] reported on 5250 men from the Danish population followed up for 29 years and found that low TT and low SHBG were strongly associated with incident T2DM. They reported no effect of LH, concluding that primary hypogonadism was not a risk factor for T2DM, but that low TT should be considered a risk marker for T2DM. As there were no data on T therapy, a causal relationship could not be established.

Increased cardiovascular and all-cause mortality and low testosterone

Several long-term studies, reviews and meta-analyses, provide evidence to support the association between TD and increased cardiovascular (CV) and diabetes associated all-cause mortality,[22-27] although evidence for a pathogenic link is lacking.[28, 29]

One systematic review concluded that low levels of endogenous testosterone are associated with an increased risk of all-cause and CV death in community-based studies of men, with a reduction of 2.1 standard deviations in TT being associated with a 25% increase in mortality. However, most of the studies had issues with cohort selection and choice.[22]

Another meta-analysis evaluating the association between endogenous testosterone and all-cause mortality and cardiovascular disease (CVD) mortality[23] reported a protective effect of increased total testosterone. Research examining the data from 1954 subjects, in terms of several statistical models, found that even after strict adjustment for comorbidities, there was a consistent link between testosterone level and mortality risk throughout, without proving causation.[30]

In a prospective study involving 581 men with T2DM, patients were followed up for a mean of 5.81 years. Low testosterone was defined as TT<10.4 nmol/L. Fifty-one men received T Therapy for at least 2 years. The mortality rates were 20% in the low testosterone group *versus* 9.1% in the normal testosterone group, independent of comorbidities and therapies. Mortality was 9.4% in those with TD in the treated group.[25]

In a 10-year Australian study involving 3690 older men, TT and FT levels in the normal range were associated with reduced all-cause and CV mortality. This was the first evidence to suggest that that both low and high levels of testosterone were associated with all-cause mortality.[26]

A longitudinal study from Sweden involving 1109 subjects aged 40 years and over, with a mean follow up of 14.1 years, suggested a strong association between low baseline testosterone and incident myocardial infarction (MI).[27]

Although these studies suggest a consistent association between low testosterone and CVD incidence and mortality, this did not prove a pathogenic link. Some authors suggest that low testosterone could be a "marker" of illness.[29, 30]

CV risk reduction and glycaemic control in T2DM

The mainstay of modern therapy for T2DM is cardiovascular risk reduction. Smoking cessation, weight reduction and increased exercise have been shown to improve insulin sensitivity and should always be offered in conjunction with other therapies.[31] Low testosterone has been demonstrated to be closely linked with components of the metabolic syndrome in T2DM, but testosterone therapy is not routinely considered as integral to diabetes control.[13]

The main target to improve outcome in T2DM has traditionally been improved glycaemic control as assessed by HbA1c or more recently by IFCC.[31] Insulin resistance, as measured by the Homeostatic Measurement of Insulin Resistance (HOMA-IR) is known to be an independent risk factor for cardiovascular events[32] and the main therapeutic target and is regarded as the gold standard for assessment of change in HOMA-IR. Bonara *et al.*[32] reported that each unit change in HOMA-IR was associated with a 50% increase in CV events. In the UKPDS trial, each 1% increase in HbA1c was associated with a 21% increase in death, 14% increase in myocardial infarction and 43% increase in PVD.[33] Intensive management with metformin *versus* conventional management was shown to reduce HbA1c from a mean of 7% at baseline to 6.2% at 12 months, but long-term follow-up over 10 years plus have shown that HbA1c levels rise with time and that weight loss is not maintained.[34] Aggressive targets for hypertension and dyslipidaemia have led to NICE guidance that all men with T2DM should be offered Metformin, Statin and ACE inhibitor at initial diagnosis unless contraindicated.[31] We therefore need alternative strategies as eventually many patients fail with a regime dedicated to lifestyle change and hypoglycaemic drugs.

The role of testosterone therapy in glycaemic and metabolic control

Prospective, observational studies show that visceral adiposity, smoking and the MetS predict the development of low testosterone levels.[35, 36] Large prospective cohort studies in the U.S.[37] and in Germany[38] men showed that weight gain and development of diabetes or the MetS accelerated the age-related decline in testosterone. In men with T2DM followed longitudinally, changes in testosterone levels over time correlated inversely with changes in insulin resistance, suggesting that improved lifestyle factors or altered pharmacological management that improved insulin sensitivity may also contribute to increased testosterone levels.[39] Observational studies found that both weight loss and exercise increase testosterone levels.[40] Bariatric surgery improves glycaemic control and testosterone levels but there are clearly implications of cost and the selection of appropriate patients.[40]

Early trials in obese men without T2DM yielded variable results. However, a 4.3 year follow up from EMAS[41] found that biochemical reversal of secondary hypogonadism was not associated with improvement in symptoms. A placebo controlled RCT in obese hypogonadal men treated with severe weight reduction and either long-acting testosterone undecanoate (TU) injections or placebo for 12 months, found that although weight and BMI loss was achieved in both groups, only the TU group preserved lean muscle and achieved symptomatic benefit.[42, 43] This study provides strong evidence for the benefits of T therapy for longer than 6 months combined with lifestyle for men with HG and MetS.

A large- scale placebo-controlled study on the possible reduction of incident diabetes by treating younger obese men with MetS with Testosterone Undecanoate v

placebo, without both groups receiving intensive lifestyle and exercise intervention reported in 2021.[44] TU produced a 40% reduction in incident diabetes over 2 years with associated greater reductions in visceral adiposity, and improvement in ED.

Kapoor *et al.*[45] found that Testosterone Enanthate every 2 weeks for 12 weeks lowered HOMA-IR by 1.7 units and HbA1c by 0.37%, with slight reduction in total cholesterol.

The Moscow study involved 184 obese men randomised to long-acting TU for 26 weeks found marked reductions in weight, BMI, waist circumference and inflammatory markers but no reduction in fasting glucose.[46]

The TIMES2 study involved 220 men with either T2DM or MetS treated for 26 weeks with 2% testosterone gel followed by an open label phase. There was a significant reduction in HOMA-IR at 12 months (P=0.006) but although the HbA1c fell by 0.4% at 12 months, in men with T2DM, this just failed to reach significance. As the randomisation phase lasted only 6 months, there was possible selection bias for those men continuing to the open label phase and the drop-out rate was rather high at 29%.[47]

The largest RCT conducted to date exclusively in men with T2DM is the BLAST study (an acronym of the towns and cities involved – Birmingham, Lichfield, Tamworth, Atherstone and Sutton Coldfield). This involved 857 men screened from 7 UK general practice diabetes registers to provide 200 men detected with TT<12 nmol/L or FT<0.25 nmol/L (MILD HG group) or TT<8 nmol/L or FT<0.18 nmol/L (SEVERE HG) group. Men were randomised to TU 1000 mg or placebo injections, initially after 6 weeks and then 12 weeks for a 30- week intervention period.[48] There was a significant treatment effect in HbA1c in the poorly controlled men (HbA1c≥7.5%) of 0.4% at 30 weeks and a significant effect in the well- controlled (<7.5%) at 18 weeks (P=0.002) but not at 30 weeks. A likely explanation for this was the impact of diet and lifestyle advice given at baseline for all patients and the likelihood that the initial impact was diminished at 30 weeks. Effect on HbA1c was most marked in men achieving sustained levels of testosterone in the mid to upper range. There were significant reductions in weight, BMI and waist circumference, plus marked improvement in sexual function and quality of life scores. There was little effect seen in men diagnosed or treated for depression (23%). These parameters continued to improve in the 12-month open label phase. HOMA-IR was unchanged during the 30- week RCT but fell by 1.5 units in the 12 months open label phase, along with marked falls in insulin levels in the open label phase. Selection bias was likely in those proceeding to the open label phase, as analysis showed that the decisions to proceed to the open label phase was driven by improvements in sexual function.[48]

Dhindsa *et al.*[49] studied 94 men with T2DM, 50 eugonadal and 44 with HH, randomised to either testosterone cypionate 250mg every 2 weeks or placebo injection for 24 weeks. Men with HH had higher subcutaneous and visceral fat mass than eugonadal men. Insulin Sensitivity measured by Glucose infusion rate (GIR) was 36% lower in men with HH. GIR increased by 32% after 24 weeks of testosterone

therapy but did not change after placebo (P=0.03 for comparison). HOMA-IR improved by 1.4% with treatment (P=0.03) and was unchanged with placebo. There was a decrease in subcutaneous fat mass (3.3 kg) and increase in lean mass (3.4 kg) after testosterone treatment (P<0.01) compared with placebo. Visceral and hepatic fat did not change.

Ghanim *et al.* investigated 32 men with well-controlled T2DM and HH and 32 matched men with T2DM and normal T levels. They were randomised to either TU or placebo every 2 weeks for 23 weeks with repeat visceral fat and vastus medialis muscle biopsy. Men with HH were found to have 35% additional insulin resistance. Total visceral subcutaneous fat was deceased by 3.3 kg with TU and lean muscle mass increased by 2.9 kg. Adenosine 5′-monophosphate-activated protein kinase-α (AMPK α) expression was 33% and 29% lower in subcutaneous fat and skeletal muscle at baseline and increased by 41% and 46% with TU therapy. Testosterone modulated the expression of AMPKα and phosphorylated AMPKα. The authors suggest that this is the likely mechanism by which T therapy improves insulin resistance.[50]

A 5-year study of 40 obese hypogonadal men, 20 treated with TU and 20 randomised to control, showed reductions in weight (15%), waist circumference (10 cm) and HbA1c (1.5%) and 2.15-point reduction in HOMA-IR.[51]

Long term registry studies have shown reductions in weight, BMI, waist circumference and HbA1c maintained for up to eight years. In 316 men with pre-diabetes 90% reverted to normoglycemia with testosterone therapy whereas 40% in the control group progressed to frank diabetes.[52]

Gianetti *et al.* conducted a 40-week study of TU *vs.* Placebo in 88 obese men with T2DM (mean age 62) and concluded that there was no significant impact on HOMA IR or HbA1c but there was a reduction in fat mass and increase in lean muscle mass. However, the treatment group were notably less obese (93 kg *vs.* 101.5 kg) with baseline HOMA-IR of 2.1 falling to 1.75 (P=0.08). Baseline HbA1c was 6.8%. This suggests that patients were well controlled with minimal insulin resistance suggesting that the study might have been under-powered for this cohort.[53]

Table 9.I. Treatment effect of Testosterone Undecanoate *vs.* Placebo in 199 men with type 2 diabetes – the BLAST study.

	HbA_{1c} >75 (%)	Weight (kg)	BMI (kg/m^2)	WC (cm)	TC (mmol/L)	EF (IIEF)	AMS (points)	HADS - D (points)	GEQ (% improved)
30 weeks	-0.41	-0.7	-0.3	-2.5	-0.25	+3.0	-5.3	-1.01	46
P value	0.007	0.13	0.01	0.012	0.025	0.006	0.095	0.64	<0.001
82 weeks	-0.87	-2.7	-1.00	-4.2	-.0.19	+4.31 +9.57 PDE5I	-8.1	-2.18	67-70
P value	0.009	0.016	0.019	<0.001	0.035	0.003	0.001	0.001	0.0001

AMS: aging males' symptoms scale; BMI: body mass index; EF: ejection fraction; GEQ: glopal efficacy question; HAD-S: hospital anxiety and depression, scale-depression; HbA1c: gycated haemoglobin; IIEF: intemational index of erectile function; PDE5I: phosphodiesterase type 5 inhibitor; TC: total cholesterol; WC: waist circumference.

In the T trial,[54] involving 788 men randomised to either testosterone gel 1% or placebo for 1 year, there was a significant reduction in fasting insulin, -1.6 *vs.* + 1.8 µU/mL (P=0.02) and HOMA-IR, adjusted difference -0.3 *vs.* -0.2 (P=0.03). There were no significant changes in fasting glucose or HbA1c. It is interesting that 37% in each cohort had type 2 diabetes and no treatment effect was seen in those not on anti-diabetes medication, which suggests that the predominant effects were seen in those men with type 2 diabetes. As baseline HbA1c was 6.3% and HOMA-IR 5.8, this suggests that these improvements in Insulin and HOMA-IR were seen in patients who were not particularly Insulin resistant.

Recently, the T4DM from Australia[44] was published in January 2021. The 2-year study of 1007 obese men with pre-diabetes involved randomisation to either TU or placebo injections, plus intensive weight, and exercise interventions for both groups. The cut off total testosterone level was 14 nmol/L which would be regarded as "normal" according to most guidelines.

This study established conclusively that TU was associated with a 40% reduction in progression to T2DM compared with intensive lifestyle intervention. The reduction in post prandial blood glucose was 1.7 *versus* 0.95 nmol/L. There were also significant reductions in total and abdominal fat and waist circumference with TU in addition to increases in lean muscle mass, arm muscle mass, grip strength and modest improvement in sexual function. Interestingly despite men on placebo plus intensive lifestyle losing 3.5 kg and 4.85 cm in waist circumference, there was no improvement in clinical symptoms, ED or free testosterone. The TU group lost 3 times as much visceral fat and increased muscle mass, whereas the lifestyle plus placebo group lost 1.75 of muscle mass. Importantly, the clinical improvements with TRT were unrelated to baseline testosterone levels.

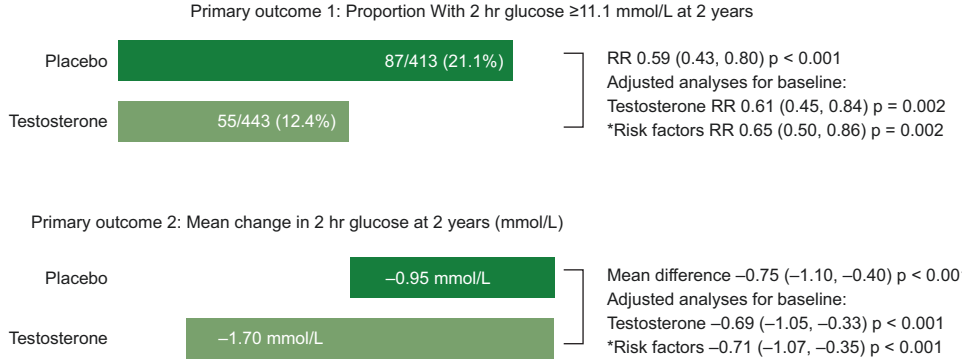
Despite 25% of patients showing a raised haematocrit (>54%) at some stage in the 2 years, only 5% were withdrawn due to 2 elevated readings (Figure 9.1, Figure 9.2).

The conclusions from all these studies are that properly conducted RCTs, involving men with T2DM and clear evidence of IR, treated for sufficient duration (preferably over 12 months) with TRT to achieve consistent levels in the upper normal range, improve glycaemic control. There would appear little justification to recommend lifestyle intervention as sole therapy in symptomatic men with HG and T2DM. Poorly designed studies including men without T2DM or IR, treated with insufficient dose or short duration, have merely caused confusion. The conclusions of these studies are summarised in Table 9.II.

Lipid metabolism and biomarkers

Trials of testosterone injection therapy on lipids have repeatedly demonstrated small reductions in total, HDL and LDL levels. Meta-analyses involving variable

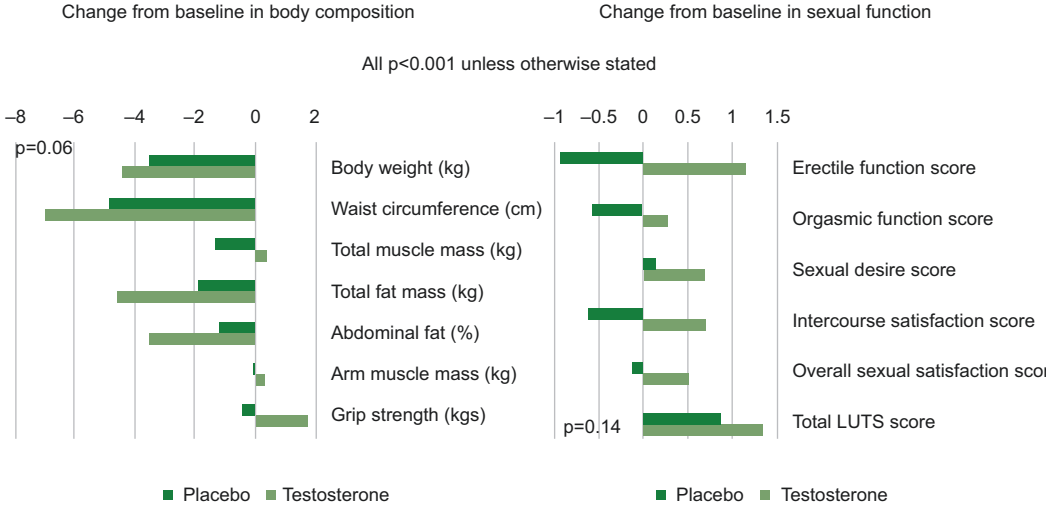
Figure 9.1. Primary results from the T4DM study (Wittert *et al.*).



*Center, age group (50-59, 60–74y), WC (95–100,101–115, >115cm), 2-h glucose on OGTT (7.8–9.5, 9.6–11.0, 11.1–15.0mmol/L), smoking (yes, no), and first-degree family history of T2D (yes, no), baseline serum testostrone (≤8 (230ng/dL), 8–11, ≥11mmol/L (317 ng/dL))

There was no relationship between baseline testosterone and the treatment effect (p=0.26)

Figure 9.2. Secondary results from the T4DM study (Wittert *et al.*).



entry criteria, routes of administration, doses and duration have generally shown inconsistent effects.

In the T trial,[54] with 69% of men over 65 on statin therapy, the effects of testosterone gel v placebo over 12 months was, for total cholesterol, 4.18-4.00 and 4.33-4.24

Table 9.II. Published RCT and observational studies demonstrating the effect of TRT on glycaemic control.

Reference	Glycaemic parameters treatment effect	Conclusions
Kapoor *et al.* 2007 N.=27 RCT Men with T2DM.[45]	HOMA-IR 4.2-3.5 (P=0.02) INSULIN 13.68-11.76 HbA1c 7.28-6.91 (P=0.03)	TTh significantly improved insulin sensitivity, HbA1c and insulin levels.
Kalinchenko *et al.* 2010. N.=184 RCT TU v placebo. Obese men with and without T2DM. 30 weeks.[46]	HOMA-IR 5.4-4.3 (P=0.04) INSULIN 18.9-15.6 (P=0.07)	TTh significantly improves insulin sensitivity, lowers insulin levels, CRP, and IL-6. reduces BMI and waist circumference.
Jones *et al.* 2011. N.=220. RCT. Men with T2DM and MetS. 2% gel.[47]	HOMA-IR 5.9-4.93. Not powered for HbA1c as well controlled T2DM	TTh improves insulin sensitivity, reduces Lipoprotein-A. Improved sexual function.
Hackett *et al.* 2013 N.=200. RCT. TU *vs.* placebo Men with T2DM. 30 weeks plus 52 weeks open label.[59]	HOMA-IR 3.52-2.48 (after 82 weeks) HbA1c 8.85-8.42 in poorly controlled T2DM	TTh significantly reduced HbA1c, HOMA-IR, BMI, waist circumference and sexual function. All responses reduced in men with baseline depression.
Francomano *et al.* 2014 N.=20. TU *vs.* control. 5- year follow-up, Men with T2DM.[51]	HOMA-IR 4.25-1.4 (P=0.0001) HbA1c 7.7-6.1 (P=0.0001)	TTh significantly improved insulin sensitivity, HbA1c, BMA, waist circumference, metabolic and endothelial markers.
Gianetti *et al.* 2014 N.=88. 40 weeks RCT. TU *vs.* placebo. Men with T2DM.[53]	HOMA-IR 2.11-1.75 (P=0.08) HbA1c 6.8-7.1 (P=0.05)	No improvement in insulin sensitivity or HbA1c in men with essentially well controlled T2DM.
Dhindsa *et al.* 2016 N.=44 (20 active *vs.* 14 placebo). 24 weeks. Testosterone injection 250 mg per week *vs.* placebo. Men with T2DM.[49]	INSULIN 13.6- 9.9 (P=0.04) HbA1c 6.8-7.2 (P=0.27) (short duration and well controlled T2DM)	TTh in men with T2DM and HH increases insulin. Sensitivity, lean muscle mass, and decreases subcutaneous fat.
Grotti *et al.* 2018 N.=55. RCT TU *vs.* placebo. 12-months follow-up. Obese men with T2DM.[78]	INSULIN 26.03-17.51 (P<0.001) HOMA-IR 11.45-6.81 (P<0.001) HbA1c 8.12-7.18 (P<0.001)	Reduced insulin levels, improved insulin sensitivity Improved HbA1c. Improved flow mediated dilatation (P=0.005).
Mohler *et al.* 2018. N.=788. Placebo controlled RCT. 1% gel.[60] 12-month follow-up. Men over 55 with HG. 37.6% with T2DM.[38]	INSULIN 19.6-17.9 (P=0.02) HOMA-IR 5.8-5.5 (P=0.03 HbA1c 6.3-6.3. (NS)	Improved insulin sensitivity but not HbA1c (well controlled – only 30% with T2DM). No effect on men without T2DM medication suggesting greater effect in men with T2DM.

(*to be continued*)

Table 9.II. Published RCT and observational studies demonstrating the effect of TRT on glycaemic control *(continues)*.

Reference	Glycaemic parameters treatment effect	Conclusions
Yassin *et al.* and Haider *et al.* N.=316, control=87. 9-11-year follow-up. Registry study. Single centre.[52]	HOMA-IR 8.7-1.8 HbA1c 7.8-5.4	Improvement in HOMA-IR and HbA1c. Remission in T2DM in reduced by 34.3% with 40% reduction in progression from pre-diabetes to frank diabetes.
Wittert et al (T4DM) 2020 Double blind RCT 2 years. TU *vs.* placebo in obese men with pre-diabetes (N.=1007, combined with aggressive diet and exercise intervention).[44]	2 hr post prandial glucose reduced by 1.70 *vs.* 0.95 nmol/L (P<0.001).	40% reduction in progression to type 2 diabetes, reduced waist circumference, reduced total and abdominal fat, increased lean muscle mass, grip strength and improved sexual symptoms (all P<0.001). No improvement in symptoms with lifestyle change alone, despite significant weight loss.

(P<0.001), for HDL-C 1.15-1.11 and 1.17-1.17 (P<0.001), for LDL-C 2.27-2.19 and 2.37-2.31 (P<0.05). Eleven men in the testosterone arm and 4 in the placebo, initiated lipid-lowering medication after baseline, which appears contradictory to a finding of higher baseline plaque burden in the placebo group.

Reduction in TC and LDL-C will result in more patients reaching conventional targets for CHD prevention,[31] but the impact of TRT on HDL-C has usually been considered to neutralise the positive effects on TC and LDL-C. The role of HDL-C as a predictor of CVD is not well established. The Framingham Heart Study[55] showed an inverse relationship between HDL-C and CVD, but, in contrast, the Dallas Heart Study[56] demonstrated that cholesterol efflux, not HDL-C was associated with CVD. Studies with Niacin and the cholesteryl ester transfer protein (CETP) inhibitors both raise HDL-C but did not show CVD benefits.[57]

In the T trial,[44] there were no significant effects on d-dimer, CRP, IL-6 and Troponin in line with other studies showing minor and inconsistent changes in biomarkers. The Moscow trial[46] demonstrated significant reduction on CRP and IL-6 in a cohort of obese men randomised to either TU or placebo for 6 months.

The TIMES2 trial[47] showed similar reductions in TC, HDL-C and LDL-C cholesterols but also a significant reduction in lipoprotein A (P<0.008), an independent risk marker for cardiovascular disease.

Testosterone and sexual function in type 2 diabetes

Men with T2DM have ED due to multiple co-morbidities, most notably macro and microvascular disease, autonomic and peripheral neuropathy, depression, and

multiple medications in addition to HG[31] Zitzmann *et al.*[58] found that falling T levels significantly impact on ED around 8 nmol/L and Buvat *et al.*[59] described a threshold of 10.4 nmol/L where T therapy might salvage men who fail with PDE5 inhibitors. Men with T2DM also have significantly reduced responses to conventional ED therapy, due to multiple co-morbidities.[2] Hackett *et al.*[28] screened a UK population of men with T2DM and found that the impact of HG on ED was greatest in men with TT below 8 nmol/L, in agreement with the findings of Zitzmann *et al.* These findings suggest that there are pitfalls in drawing conclusions from studies of T therapy where men with T2DM form only a minor subgroup. Correcting low T levels will usually only address the impact of HG on ED, but not the burden due to other co-morbidities,[28] which will need to be addressed by additional specific therapies.[28] Some trials only assessed ED using IIEF-5, which only evaluates erections, whereas T is involved in desire, sexual frequency, orgasm, ejaculation, sensation and sexual satisfaction.

The BLAST study[28, 48] found that the improvement from baseline in the SEVERE group (TT 8 nmol/L or less) at 30 weeks was 3.9 points (5.8 points *vs*. placebo), equated to the difference between the groups at baseline screening, suggesting that this was correcting the disease burden due to HG. Improvements reached highest significance at 6 months, suggesting that studies of less than 6 months were of insufficient duration. In the sub-group of men taking a PDE5 inhibitor, the improvement at the end of the open label phase was 9.5 points, stressing the importance of treating the multiple co-morbidities. In the MILD HG group, there was no improvement in EF score, *versus* placebo. There were marked improvements in sexual desire in both groups, most marked in the SEVERE group and prompt improvement in desire predicted later improvement in EF. Men with depression, 23% of the group, showed little response by 30 weeks,[28, 48] which is in line with other studies looking at multiple parameters in T2DM where therapeutic responses were strong influenced by depression. Meta-analyses have shown depression to be strongly linked with sexual dysfunction in T2DM.[30, 31]

The TIMES-2 study[47] involved 12 months RCT of 2% testosterone gel in 220 men (mean age 59) with either T2DM (62%) or MetS with a 4.87-point improvement in EF score from a baseline of 12 points. Unfortunately, the drop-out rate was 29% which might reflect an issue related to gel, whereas BLAST involved injections administered by the nurse involved in the patient's normal diabetes care.

In the T trials,[54] involving older mean (mean age 72) 37% of 780 recruited patients had T2DM. The IIEF score increased by 2.64 points *vs.* placebo, but sexual activity increased to 4 times per week, along with significant increase in sexual desire. The authors described this improvement as "modest". The disproportionate increase in desire and sexual activity in proportion to EF scores is probably related to age associated co-morbidities as shown by the high coronary atheroma burden in the cardiovascular arm of the study. The authors concluded that PDE5 inhibitors might have produced a greater effect than T therapy, quoting Spitzer *at al.*[60] The authors, might, however, have underestimated the benefits of increased sexual activity and desire

in the context of the relationship of the couple. It is unlikely that PDE5 inhibitors alone would have been effective in this group, without the benefits in sexual desire associated with T therapy in men with levels below 8 nmol/L.[28] PDE5 inhibitor studies were conducted in highly motivated patients who were required to be in stable heterosexual relationships and make multiple sexual attempts prior to inclusion in clinical trials, leading to placebo responses that were not seen in studies involving TRT. Patients with HG, and poorly controlled diabetes were excluded from these regulatory studies such that it is inappropriate to extrapolate response rates from studies in the eugonadal population.[57]

The evidence suggests that older men with T2DM need a PDE5 inhibitor plus normalisation of testosterone levels.[61, 48] The study by Spitzer *et al.*[55] involved a population who had an excellent response to pre-treatment with Sildenafil with an increase of over 3 nmol/L from baseline, such that the mean TT at randomisation to T therapy was 12.63 nmol/L (up from 8.61 at baseline) meaning that 50% did not fit their criteria for HG and therefore would not expect to benefit from T therapy. Despite these weaknesses, the effect of TRT was close to significance, even though the exposure to T was only 3 months. Paradoxically, despite recognising the effect of PDE5 inhibitors in the Spitzer study, only 1 of the long- term longitudinal studies of testosterone included data on PDE5 inhibitor use, reporting a 12% use at baseline, increasing to 50% in 4 years of follow-up in the men taking testosterone.[63, 64] Some recent reviews have suggested that, in view of the high rates of ED and BPH in men with T2DM, plus other multiple benefits, daily PDE5i therapy with tadalafil is indicated.[62, 63]

Meta-analyses of testosterone and metabolism

RCTs assessing the impact of testosterone therapy on metabolism[64] are of insufficient duration, often less than 26 weeks (mean 33.5 weeks).[65] Many involve mixed populations with minimal IR such that it is doubtful that changes in insulin resistance or HbA1c can be achieved.[65, 66] Such studies are unlikely to be powered to assess cardiovascular safety of MACE,[67] especially where multiple delivery systems are involved.[67] Longitudinal studies often have inbuilt bias related to inaccurate initial diagnosis and lack of evidence of compliance with therapy.[70] PDE5 inhibitors are often co-prescribed with TRT and these have multiple potential benefits in men with T2DM, such as improvements in ED and LUTS.[68] Longitudinal studies have largely ignored the possible metabolic impact of PDE5 inhibitors.[69, 70] The inclusion or exclusion of a handful of controversial studies can profoundly alter the conclusions.[71]

A meta-analysis of 156 RCTS by Huo *et al.*[69] was at STARK variance, concluding that there was no evidence from clinical trials to support the use of TRT for cardiovascular health, sexual function, physical function, mood, or cognitive function.

Ponce *et al* concluded that testosterone therapy improved sexual desire, erectile dysfunction, sexual satisfaction but increased erythrocytosis.[70] Corona *et al.*

conducted a meta-analysis of 59 RCTs involving 3029 treated and 2049 controls concluded that there was clear evidence that TRT reduced fat mass and increased lean muscle mass.[65, 66] They concluded that TRT improved, sexual function, insulin sensitivity and glycaemic control in men with metabolic disease,[65, 66] with no suggestion of increased cardiovascular risk. They suggested that there was limited evidence of a reduction in all-cause mortality in men with cardiometabolic disease.[66] Benefits in men with T2DM and MetS might be underestimated when meta-analyses combine these men with lower-risk cohorts.

Testosterone therapy, cardiovascular and all-cause mortality in type 2 diabetes

Numerous long-term studies, and various reviews and meta-analyses, have provided evidence to support the association between HG and increased cardiovascular (CV) and all-cause mortality. This topic in considered at length in another chapter. A systematic review and meta-analysis evaluating the association between endogenous testosterone and mortality concluded that low levels of endogenous testosterone are associated with an increased risk of all-cause and CV death in community-based studies of men, with a reduction of 2.1 standard deviations in TT being associated with a 25% increase in mortality.

Most of the studies involving testosterone therapy had issues with cohort selection and choice.[69, 70] Quang *et al.* evaluated 12 RCTs (only 4 exclusively in T2DM) and 13 Non-RCTs (N.=729,927), concluding protective effects of TRT against all-cause mortality and major adverse cardiac events in hypogonadal men with T2DM and/or MetS. The authors suggest that taking no action, is likely to have severe implications for men with T2DM and low testosterone.[71]

Conclusions

The low levels of testosterone frequently seen in men with type 2 diabetes are associated with increased co-morbidity and mortality. Studies with testosterone therapy suggest significant benefits in sexual function, quality of life, glycaemic control, anaemia, bone density, fat and lean muscle mass. Longitudinal studies on cardiovascular and all-cause mortality have several logistic problems, related to potential increased mortality associated with inadequately treated patients and possible selection bias, but those restricted to men with T2DM shown clear evidence of reduced cardiovascular and all-cause mortality. Future studies need to address the impact of PDE5 inhibitor use, due to the growing evidence that these drugs impact cardiovascular and all-cause morbidity and mortality. Men with T2DM usually require PDE5 inhibitors as well as testosterone therapy to improve sexual function by addressing the multiple co-morbidities. The T4DM study also showed a high prevalence of

voiding and storage LUTS symptoms that increased in severity over the course of the 2-year study. This reinforces the strong case for including daily PDE5 inhibitors as a routine therapy in men with T2DM.

Restricting testosterone therapy to men with classical HG is not supported by recent evidence. The results of lifestyle intervention as sole therapy for HG in T2DM are disappointing. The balance of evidence suggests that men with T2DM and MetS associated with HG are likely to benefit from testosterone therapy combined with lifestyle intervention. Recent findings from the T4DM study conclusively demonstrate the benefits of treating even moderately low testosterone levels to prevent progression to frank T2DM. Meta-analyses of RCTs, rather than providing clarification, have further confused the issue by including under-powered studies of inadequate duration, non-homogenous cohorts, multiple regimes, discontinued medication, and inbuilt bias in terms of studies included or excluded from analysis.

References

1. Dhindsa S, Ghanim H, Batra M, *et al.* Insulin Resistance and Inflammation in Hypogonadotropic Hypogonadism and Their Reduction After Testosterone Replacement in Men With Type 2 Diabetes. Diabetes Care 2016;39:82-91.
2. Kloner RA, Carson C 3rd, Dobs A, *et al*. Testosterone and Cardiovascular Disease. J Am Coll Cardiol 2016;67:545-57.
3. American Diabetes Association. Available from: https://www.diabetes.org.uk/Professionals/Position-statements-reports/Statistics [cited 2018, April 1].
4. Available from: http://www.diabetes.org/diabetes-basics/statistics/ (cited 2018, April 1).
5. Hackett G, Cole N, Deshpande A. Biochemical hypogonadism in men with type 2 diabetes in primary care practice. Br J Diabetes Vasc 2009;9:226-31.
6. Goodman N, Guay A, Dandona P, *et al*. American Association of Clinical Endocrinologists and American College of Endocrinology position statement on the association of testosterone and cardiovascular risk. Endocr Pract 2015;21:1066-73.
7. Bhasin S, Brito JP, Cunningham GR, *et al.* Testosterone therapy in men with Hypogonadism, An Endocrine Society Guideline. J Endocrinol Metabolism 2018:103:1-30.
8. Corona G, Rastrelli G, Maggi M. Diagnosis, and treatment of late onset hypogonadism: systematic review and meta-analysis of TRT outcomes. Best Pract Res Clin Endocrinol Metab 2013;27:557-79.
9. Kelly DM, Jones TH. Testosterone: a vascular hormone in health and disease. J Endocrinol 2013;217930:R47-71.
10. Selvin E, Feinleib M, Zhang L, *et al.* Androgens and diabetes in men: results from the Third National Health and Nutrition Examination Survey (NHANES III). Diabetes Care 2007;30:234-8.
11. Haffner SM, Shaten J, Stern MP, *et al.* Low levels of sex hormone-binding globulin and testosterone predict the development of non-insulin-dependent diabetes

mellitus in men. MRFIT Research Group. Multiple Risk Factor Intervention Trial. Am J Epidemiology 1996;143:889-97.
12. Ding EL, Song Y, Manson JE, *et al.* Sex Hormone–Binding Globulin and Risk of Type 2 Diabetes in Women and Men. N Engl J Med 2009;361:1152-63.
13. Corona G, Monami M, Rastrelli G, *et al.* Testosterone and metabolic syndrome: a meta-analysis study. J Sex Med 2011;8:272-83.
14. Stellato RK, Feldman HA, Hamdy O, *et al.* Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. Diabetes Care 2000;23:490-4.
15. Oh JY, Barrett-Connor E, Wedick NM, *et al.* Endogenous sex hormones and the development of type 2 diabetes in older men and women: The Rancho Bernardo Study. Diabetes Care 2002;25:55-60.
16. Laaksonen DE, Niskanen L, Punnonen K, *et al.* Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. Diabetes Care 2004;27:1036-41.
17. Vikan T, Schirmer H, Njølstad I, *et al.* Low testosterone and sex hormone-binding globulin levels and high estradiol levels are independent predictors of type 2 diabetes in men. Eur J Endocrinol 2010;162:747-54.
18. Lakshman KM, Bhasin S, Araujo AB. Sex hormone-binding globulin as an independent predictor of incident type 2 diabetes mellitus in men. J Gerontol a Biol Sci Med Sci 2010;65:503-9.
19. Li C, Ford ES, Li B, *et al.* Association of testosterone and sex hormone-binding globulin with metabolic syndrome and insulin resistance in men. Diabetes Care 2010;33:1618-24.
20. Antonio L, Wu FCW, O'Neill TW, *et al.* Associations Between Sex Steroids and the Development of Metabolic Syndrome: A Longitudinal Study in European Men. J Clin Endocrinol Metab 2015;100:1396-404.
21. Holmboe S, Jensen T, Linneberg A, *et al.* Low Testosterone: A Risk Marker Rather Than a Risk Factor for Type 2 Diabetes. JCEM 2016:101:3180-90.
22. Araujo AB, Dixon JM, Suarez EA, *et al.* Endogenous testosterone and mortality in men: a systematic review and meta-analysis. J Clin Endocrinol Metab 2011;96:3007-19.
23. Ruige JB, Mahmoud AM, De Bacquer D, *et al.* Endogenous testosterone and cardiovascular disease in healthy men: a meta-analysis. Heart 2011;97:870-5.
24. Haring R, Volzke HV, Steveling A, *et al.* Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20-79. Eur Heart J 2010;31:1494-501.
25. Muraleedharan V, Marsh H, Kapoor D, *et al.* Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. Eur J Endocrinol 2013;169:725-33.
26. Yeap B, Alfonso H, Chubb S, *et al.* In older men an optimal plasma testosterone is associated with reduced all-cause mortality and higher dihydrotestosterone with reduced ischemic heart disease mortality, while estradiol levels do not predict mortality. J Clin Endocrinol Metab 2014;99:E9-18.
27. Daka P, Langer RD, Larsson CA. Low concentrations of serum testosterone predicts acute myocardial infarction in men with type 2 diabetes mellitus. BMC Endocr Disorder 2015;15:1-12.

28. Hackett GI. Testosterone replacement therapy and mortality in older men. Drug Saf 2016;39:117-30.
29. Muraleedharan V, Jones TH. Testosterone and mortality. Clin Endocrinol 2014;81:477-87.
30. Oskui PM, French WJ, Herring MJ, *et al*. Testosterone and the cardiovascular system. A comprehensive review of the clinical literature. J Am Heart Assoc 2013;2:e000272.
31. Home PD, Mant J, Diaz J, *et al*. Management of type 2 diabetes: Updated NICE guidance BMJ 2008;336:1306-8.
32. Bonora E, Formentini G, Calcaterra F, *et al.* HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects: prospective data from the Verona Diabetes Complications Study. Diabetes Care 2002;25:1135-41.
33. Stratton IM, Cull CA, Adler AI, *et al*. Additive effects of glycaemia and blood pressure exposure on risk of complications in type 2 diabetes: a prospective observational study (UKPDS 75). Diabetologia 2006;49:1761-9.
34. Holman RR, Sanjoy P, Bethel A, *et al.* 10-Year Follow-up of Intensive Glucose Control in Type 2 Diabetes. N Engl J Med 2008;359:1577-89.
35. Laarksonen D, Niskonen L, Punnonen K, *et al.* The Metabolic Syndrome and Smoking in Relation to Hypogonadism in Middle-Aged Men: A Prospective Cohort Study. JCEM 2005;90:712-9.
36. Tsai EC, Boyko EJ, Leonetti DL, *et al.* Low serum testosterone level as a predictor of increased visceral fat in Japanese American men. Int J Obes Relat Metab Disord 24:485-91.
37. Travison TG, Araujo AB, O'Donnell AB, *et al.* A population-level decline in serum testosterone levels in American men. J Clin Endocrinol Metab 2007;92:196-202.
38. Haring R, Ittermann T, Völzke H, *et al*. Prevalence, incidence and risk factors of testosterone deficiency in a population-based cohort of men: results from the study of health in Pomerania. Aging Male 2010;13:247-57.
39. Kumagai H, Zenipo-Miyaki A, Yoshikawa T, *et al.* Lifestyle modification increases serum testosterone level and decreases central blood pressure in overweight and obese men. Endocr J 2015;62:423-30.
40. Gloy VL, Briel M, Bhatt DL, *et al.* Bariatric surgery *versus* non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. BMJ 2013;347:f5934.
41. Rastrelli G, Carter EL, Ahern T, *et al.* Development of and Recovery from Secondary Hypogonadism in Aging Men: Prospective Results from the EMAS. J Clin Endocrinol Metab 2015;100:3172-82.
42. Ng Tang Fui M, Hoermann R, Prendergast LA, *et al.* Symptomatic response to testosterone treatment in dieting obese men with low testosterone levels in a randomized, placebo-controlled clinical trial. Int J Obes 2017;41:420-6.
43. Ng Tang Fui M, Prendergast LA, Dupuis P, *et al.* Effects of testosterone treatment on body fat and lean mass in obese men on a hypocaloric diet: a randomised controlled trial. BMC Medicine 2016;14:153.
44. Wittert G, Bracken K, Robledo KP, *et al.* Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021;9:32-45.

45. Kapoor D, Clarke S, Stanworth R, *et al.* The effect of testosterone replacement therapy on adipocytokines and C-reactive protein in hypogonadal men with type 2 diabetes. Eur J Endocrinol 2007;156:595-602.

46. Kalinchenko SY, Tishova YA, Mskhalaya GJ, *et al.* Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. Clin. Endocrinol (Oxf) 2010;73:602-12.

47. Jones T, Arver S, Behre H, *et al.* TIMES2 Investigators. Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). Diabetes Care 2011;34:828-37.

48. Hackett G, Cole N, Bhartia M, *et al.* Testosterone Replacement Therapy Improves Metabolic Parameters in Hypogonadal Men with Type 2 Diabetes but not in Men with Coexisting Depression: The BLAST Study. J Sex Med 2014; 11:840-56.

49. Dhindsa S, Ghanim H, Batra M, *et al.* Insulin Resistance and Inflammation in Hypogonadotropic Hypogonadism and Their Reduction After Testosterone Replacement in Men with Type 2 Diabetes. Diabetes Care 2016;39:82-91.

50. Ghanim H, Dhindsa S, Batra M, *et al.* Testosterone Increases the Expression and Phosphorylation of AMP kinase α in Men with Hypogonadism and Type 2 Diabetes. J Clin Endocrinol Metab 2020;105:1169-75.

51. Francomano D, Lenzi A, Aversa A. Effects of five-year treatment with testosterone undecanoate on metabolic and hormonal parameters in ageing men with metabolic syndrome. Int J Endocrinol 2014;2014:527470.

52. Yassin A, Haider A, Haider KS, *et al.* Testosterone Therapy in Men with Hypogonadism Prevents Progression from Prediabetes to Type 2 Diabetes: Eight-Year Data from a Registry Study. Diabetes Care 2019;42:1104-11.

53. Gianatti EJ, Dupuis P, Hoermann R, *et al.* Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: a randomized controlled trial. Diabetes Care 2014;37:2098-107.

54. Snyder PJ, Bhasin S, Cunningham GR, *et al.* Effects of Testosterone Treatment in Older Men. N Engl J Med 2016;374:611-24.

55. Mahmood SS, Levy D, Vasan RS, *et al.* The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. Lancet 2014;383:999-1008.

56. Powell-Wiley TM, Cooper-McCann R, Ayers C, *et al.* Change in Neighbourhood Socioeconomic Status and Weight Gain: Dallas Heart Study. Am J Prev Med 2015;49:72-9.

57. Keene D, Price C, Shun-Shin MJ, *et al.* Effect on cardiovascular risk of high density lipoprotein targeted drug treatments niacin, fibrates, and CETP inhibitors: meta-analysis of randomised controlled trials including 117 411 patients. BMJ 2014;349:g4379.

58. Zitzmann M, S Faber, E Nieschlag. Association of specific symptoms and metabolic risks with serum testosterone in older men. J Clin Endocrinol Metab 2006;91:4335-43.

59. Buvat J, Montorsi F, Maggi M, *et al.* Hypogonadal men nonresponses to the PDE5 inhibitor tadalafil benefit from normalization of testosterone levels with a 1% hydroalcoholic testosterone gel in the treatment of erectile dysfunction (TADTEST study). J Sex Med 2011;8:284-93.

60. Spitzer M, Bhasin S, Travison TG, *et al.* Sildenafil increases serum testosterone levels by a direct action on the testes. Andrology 2013;1:913-8.

61. Lowe G, Bahnson R. Non-invasive management of primary phosphodiesterase type 5 inhibitor failure in patients with erectile dysfunction Ther Adv Urol 2010;1:235-42.

62. Hackett G, Heald AH, Sinclair A, *et al.* Serum Testosterone, Testosterone Replacement Therapy and All- cause Mortality in men with Type 2 Diabetes: Retrospective Consideration of the impact of PDE5 Inhibitors and Statins. Int J Clin Pract 2016;70:244-53.

63. Hackett G. Should PDE5 inhibitors be prescribed to all men with newly diagnosed type-2 diabetes? Br J Diabetes and Vasc Dis 2015;15:184-90.

64. Grossmann M, Hoermann R, Wittert, *et al.* Effects of testosterone treatment on glucose metabolism and symptoms in men with type 2 diabetes and the metabolic syndrome: a systematic review and meta-analysis of randomized controlled clinical trials. Clin Endocrinol (Oxf) 2015;83:344-51.

65. Corona G, Maseroli E, Rastrelli G, *et al.* Cardiovascular risk associated with testosterone-boosting medications: a systematic review and meta-analysis. Expert Opin Drug Saf 2014;13:1327-51.

66. Corona G, Rastrelli G, Morgentaler A, *et al.* Meta-analysis of Results of Testosterone Therapy on Sexual Function Based on International Index of Erectile Function Scores. Eur Urol 2017; 72:1000-11.

67. Borst S, Yarrow J. Injection of testosterone may be safer and more effective than transdermal administration for combating loss of muscle and bone in older men. Am J Physiol Endocrinol Metab 2015;308:E1035-E1042.

68. Hackett G, Jones PW, Strange RC, *et al.* Statin, testosterone and phosphodiesterase 5-inhibitor treatments and age-related mortality in diabetes. World J Diabetes 2017;8:104-11.

69. Huo S, Scialli AR, Mc Garvey S, *et al.* Treatment of Men for "Low Testosterone": A Systematic Review. PLoS One 2016;11:e0162480.

70. Ponce O, Spencer-Bonilla G, Alvarez-Villalabos N, *et al.* The efficacy and adverse events of testosterone replacement therapy in hypogonadal men: A systematic review and meta-analysis of randomized, placebo-controlled trials. J Clin Endocrinol Metab 2018.

71. Quang LM, Kalhan A. Cardiovascular benefits and risks of testosterone replacement therapy in hypogonadal men with type 2 diabetes mellitus and/or the metabolic syndrome: a systematic review. British J Diabetes 2018;18.

10

Management of hypogonadism in diabetes

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Introduction

This chapter reviews the practical aspects of management of hypogonadism in men with diabetes. The majority of older men with low testosterone have secondary hypogonadism with low or low-normal LH. This secondary hypogonadism is associated with obesity, type 2 diabetes and cardiovascular risk factors. For decades, the approach to these men has been lifestyle advice, irrespective of how many times such advice has failed and despite meta-analyses confirming this lack of effect. Since 1996, in the UK, the number of people diagnosed with diabetes has increased from 1.4 million to 2.6 million. By 2025 it is estimated that over four million people will have diabetes.[1] Several studies have shown benefits from T therapy in type 2 diabetes above and beyond the effects of diet and lifestyle change,[1-6] although many await the perfect study to definitively answer all these questions. There is now conclusive evidence for TRT in terms of sexual function, improvement and prevention of type 2 diabetes, weight loss and prevention of osteoporosis.[2] Conclusive long term safety studies are still awaited, but the results of uncontrolled longitudinal studies over many years, suggest an overall trend in favour of TRT.

Here we review the practical aspects in the management of such patients.

Recommendations for the clinic

Current guidelines recommend that all men with type 2 diabetes and pre-diabetes should have their testosterone and SHBG measured as part of routine care.[1] Blood tests should be taken, ideally fasted and between 8 and 11am (Table 10.I). To further complicate the process, there are also seasonal variations, and considerable debate about the ideal method of measurement, but in reality the family physician will be restricted to the equipment used by his local laboratory.

All men with sexual dysfunction, not just ED, should also have their testosterone assessed prior to treatment. Low testosterone is a common cause of failure of ED therapies,[1] and much time and resources can be wasted if low testosterone is

not excluded. Several studies have shown that TRT alone produces an improvement in EF score of 2-3 points at 3 months. The BLAST study showed a 9-point improvement with a PDE5 inhibitor was given, especially tadalafil 5mg daily, which may be effective in 50% of men who fail with on-demand therapy.[1] A daily PDE5i is more likely to be effective in hypogonadism because of diminished libido decreasing motivation to take on-demand medication.[1]

Table 10.I. Screening for hypogonadism.

Recommendations–screening	LoE	Grade
screen for TD in adult men with consistent and multiple signs of TD	3	C
screen all men presenting with ED, loss of spontaneous eractions, or low sexual desire	1	A
screen for TD in all men with T2DM BMI >30 kg/m^2 or waist circumference >102 cm	2	A
screen for TD in all men on long-term opiate, antipsychotic, or anriconvulsant medication	2	B

The BSSM guidelines[1] recommend that all men with TT<8 nmol/L (FT <180 pmol/L) should be treated and that men with TT 8-12 nmol/L (FT 180-225 pmol/L) should be considered for a minimum 6- month trial of testosterone, based on symptom severity. Recently the T4DM study[2] found that treating TT of up to 14 nmol/L potentially reduces progression from pre-diabetes to frank diabetes. This is an important finding as a recent meta-analysis of dietary interventions over many years suggest poor outcomes in terms of diabetes prevention.[3] These findings suggest that lifestyle advice alone is insufficient as sole therapy, especially when previous interventions have been tried and clearly failed. A low T may contribute to fatigue and low motivation leading to treatment failure. T therapy should always be given in conjunction to lifestyle advice.[1]

Making the diagnosis of hypogonadism

On physical examination, features suggestive of testosterone deficiency include deceased body hair, decreased testicular size and gynaecomastia, but these are often absent. Fine wrinkling of the skin around the mouth may also be apparent. Measuring testicular volume can be very helpful in case the patient mentions this as a possible treatment side effect to another doctor.[1]

Questionnaires such as AMSS (Ageing Male Symptom Score) and IIEF-5 (International index of erectile function) can be useful for assessing change in symptoms over time.[1]

Before initiating TRT, measure Hb, haematocrit, TT, SHBG, FT, PSA. Repeat if TT 12 nmol/L or less (AM fasting).

Isotope dilution mass spectrometry (MS) is the reference method for total testosterone measurement, but most labs rely on direct chemiluminescent immunoassays

that displace bound forms of testosterone from SHBG and albumin. Results of these immunoassays depend on the effectiveness of displacement. In practice, the physician will need to accept the method available from the local laboratory.[1]

Measurements should be repeated at 3, 6, 12 months aiming at a target level of 15-30 nmol/L.[1] These can usually be performed at the time of routine diabetes and cardiovascular checks.

Current guidelines suggest that hypogonadism should only be treated after 2 low levels of either total (less than 12 nmol/L) or free testosterone (less than 220 pmol/L) on samples taken at least a month apart, with the patient being free of acute illness likely to acutely lower levels (Table 10.II). The COVID-19 pandemic has made the accurate diagnosis of hypogonadism challenging.

Testosterone therapy: contraindications

Traditionally guidelines have recommended caution in elderly patients but recent studies[4] suggest that outcomes might be better in older patients, especially in terms of mortality. We need to be aware of making our own subjective decisions as to what is important to elderly patients, especially in relation to sexual function. The purpose of treatment is to maintain a level of testosterone in the normal (preferably upper normal) range.[1]

Prostate cancer

TRT is contraindicated in locally advanced or metastatic prostate or breast cancer.[1] Some experts, however, have suggested that maximal prostate cancer growth occurs at low testosterone concentrations.[1] It is also strongly recommended that a PSA

Table 10.II. Making the diagnosis of hypogonadism.

Recommendation–diagnosis	LoE	Grade
Restrict diagnosis of TD to men with persistent symptoms suggestion TD and confirmed low T	3	C
Measure fasting T levels in the morning before 11 A.M. acknowledging that, in normal life, non–fasting levels could be up to 30% lower	2	A
Repeat TT assessment on ≥2 occasions by a reliable method; in addition, measure FT in men with levels close to the lower normel range (8-12 nmol/L) or those with suspected or known abnormal SHBG levels	1	A
Measure LH serum levels to differentiate primary from secondary TD	2	A
Base decisions on therapy on published action levels rather than laboratory reference ranges	4	B

and/or DRE be undertaken prior to TRT in men over 55 (less with a family history, and at 3 months and 6 months after initiation, thereafter annually.[1] A PSA of over 4 ng/mL (Europe) of 3 ng/mL (UK) especially with a strong family history. A finding of a nodule on DRE would demand urgent referral. In terms of long-term follow-up, current guidelines do not require regular DRE, unless clinically indicated by standard practice.[1] In clinical trials PSA may increase by up to 30%, after initiating TRT such that the level at 6 months should be considered as baseline with subsequent follow-up according to standard guidelines.[1] See Guidelines chapter for discussion on international variations.

Heart failure

Despite evidence from clinical trials that TRT may improve heart failure by 1 NYHA classification point in the longer term,[5] it is contra-indicated in NYHA 4 (very severe) heart failure due to possible short term fluid retention.[1] TRT is also listed as contra-indicated within 6 months of an acute MI or stroke.[1]

Haematocrit

Guidelines recommend caution if the baseline Hct is above 50% and discontinuation above 54%.[1] The T trial demonstrated a high incidence of anaemia in elderly hypogonadal men at baseline, that was reversed with TRT, even if a prior diagnosis had been made and the patient had received treatment.[6] In T4DM,[2] although 106/504 (22%) had 1 elevated haematocrit at some stage over 2 years, only 25/504 (5%) had 2 elevated readings, necessitating withdrawal according to protocol. There were no significant adverse events in the testosterone treated group.

Obstructive sleep apnoea

Despite evidence that TRT can improve obesity leading potentially to long term improvement, TRT is contraindicated in severe OSA even though any adverse effect is usually transitory.[1]

Fertility

All guidelines suggest that TRT should be avoided in those men wishing to father children in the next 12 months. Delay in fertility is likely to be greater with long acting- injections.[1] Some clinics advocate daily "micro-dosing" with testosterone enanthate (off label), but there is little published evidence to support this. The effects of TRT are usually reversible with time after stopping, but sexual symptoms such as ED and low libido are likely to return when therapy is stopped.[1]

Choice of testosterone therapy

Effectively the choice of therapy in the majority of patients is either a 1% or 1.62% gel (Testogel, also known as Androgel), a 2% gel (Tostran or Testavan) administered daily to either the upper arms and shoulders or the groin, or alternatively as an intramuscular injection given into the buttock. Gels are formulated in either sachets or pump dispenser.[1] The Testavan applicator eliminates the need to apply gel to the hands.[1] Patients usually make their own decision after a brief discussion as to the pros and cons of each treatment. Compliance is likely to be greater when patients have been involved in the selection process.

Gels

The 1.62% gel (Testogel/Androgel) is applied as 2 pump actuations (40.5 mg of testosterone) to the shoulders and upper arms in the morning after washing. The dose range is 20.25 mg/day (minimum) to 81 mg/day (maximum). After allowing 3-5 minutes to dry, clothing may be applied. In some countries, including the UK, the 1.62% gel is also available in sachet.

The 1% gel (Testogel/Androgel) is supplied in sachets in the majority of countries but also available in a pump dispenser in the U.S. and Australia. The starting dose is 50 mg/day and a maximum of 100 mg/day applied to the upper arms, shoulders or abdomen.

A licensed 2% gel (Testavan/Testarzon) comes with an applicator. The starting dose is 1 pump actuation which delivers 23 mg testosterone. If a therapeutic level is not achieved the dose can be titrated to a maximum of 3 pump actuations (69 mg testosterone). The gel is applied via the applicator to the upper arms and shoulders.

Another 2% gel (Tostran/Fortesta/Tostrex) is applied to the abdomen and inner thighs in the morning. In the UK, the starting dose is 6 x 10 mg pumps and the maximum dose is 80 mg. In the U.S. the starting dose is 40mg with a maximum dose of 70 mg/day.

A 5% testosterone cream, Androforte, is available in Australia and may shortly be available in Europe.

Injections

Short acting injections of T propionate and Cypionate have been used since the 1950s. They can be associated with wide fluctuations in T levels with mood swings and abrupt changes in libido, along with local swelling and pain at injection sites.[1] A combination of 4 esters (Sustanon) has done little to solve these problems even with the addition of the longer acting enanthate.[1]

The most widely used injection is Testosterone Undecanoate, which is licensed as a 4 mL injection 250 mg/mL. After an initial dose, a second is given at 6 weeks

and a 3rd 12 weeks after the second. The larger volume of an oil- based injection can be more painful, but this is more tolerable with a dose interval of 12 weeks.[1] There is a rare side effect of micro-emboli and anaphylaxis (POME) that can cause breathlessness and an oily taste in the mouth.[1] Risk can be significantly reduced by withdrawing the syringe plunger to ensure the injection is not made into a vein and by giving the injection slowly over several minutes. Patients are advised to wait in the clinic for 15 minutes.[1] In the BLAST study, not a single case of POME was seen during over 2000 injections.[4]

What does this mean for the clinic?

In reality, the day to do day management of hypogonadism is between gel and long-acting testosterone undecanoate and physicians would be advised to be adept at using familiar medications rather than trying them all. By watching the patient as you explain the options, it becomes very clear, as soon as you state the pros and cons, such that the physician can see which the patient favours. Most guidelines recommend commencing with a topical gel on the basis that, in the event of any adverse events, these will resolve quickly. As a health care professional is usually required to administer the TU injections, this might have cost or resource implications, especially during the COVID pandemic. Short acting injections, usually enanthate and cypionate, often sourced through private clinics, have few published trials and unacceptably high rates of polycythaemia. We would recommend that patients receive licensed formulations through a regulated medical source. Lifestyle advice and management of associated co-morbidities are equally important.

Morales *et al.*[7] reported the time to improvement of various symptoms are shown in Table 10.III. It may be that the patient is more interested in prompt symptom benefit where the physician is more interested in cardio-metabolic outcomes.

Table 10.III. Time to improvement with TRT.[7]

Time to improvement		
Symptom	At 3 months	• Increase energy • Improved emotional well-being • Enhanced libido • Reduced erectile dysfunction
	At 6 months	• Increased strength • Metabolic improvement • Improved cognition • Enhanced CV health • Decreased body fat
	At 12 months	• Enhanced bone mineral density

It is probably better to be conservative in telling patients about these timelines as most will only get one trial of TRT. Most manufacturers recommend conservative starting doses[1] such that Mulligan *et al.*[8] *et al* found that 39% of men still had levels below 10.4 nmol/L at 3 months. For these reasons the BSSM guidelines recommend that trials of TRT should be for a minimum of 6 months. In view of modest early response in erectile function in the first 3 months of TRT, the co-prescription of a PDE5 inhibitor in conjunction with TRT is strongly recommended. Ideally this should be daily tadalafil 5 mg, as hypogonadal men with low desire have less motivation to take on-demand medication.

Oral testosterone formulations

Older oral preparations of natural testosterone are rapidly metabolised by first pass lipid-metabolism, making oral T less effective.[1] The efficacy is limited because of bioavailability, fluctuating serum levels and short half-life. For multiple reasons, older oral T formulations have virtually disappeared from European markets. In recent years, a new generation of oral testosterone preparations have been launched in the U.S. These newer agents avoid first pass liver metabolism due to intestinal lymphatic absorption. Finally, a buccal formulation (Striant) twice daily was developed but has between withdrawn because of oral side-effects such as irritation, gingivitis and dysgeusia.[1]

Nasal testosterone

A nasal pump gel formulation (Natesto) delivering 5.5 g of T delivered as 2 pumps 3 times daily is licensed in the United States, Canada and some Asia Pacific countries. Absorption is through the nasal mucosa and first pass metabolism is avoided. Trials suggest that this led to therapeutic levels in over 90% of cases without suppressing the HPG axis, preserving fertility. However, longer term studies are required to confirm this.[9]

Subdermal testosterone pellets

Subdermal pellets have been available since the 1940s, involving a small incision over the hip area every 6 months. Side effects of fibrosis, skin infections and extrusion have limited their use. Polycythaemia rates were reported at around 50%, necessitating surgical removal in many cases. A licensed formulation is only available in the USA and Australia.[1]

Aromatase inhibitors

Anastrazole and letrozole (AIs) have been used off label to treat functional hypogonadism along with selective estrogen receptor modulators such as clomiphene and enclomiphene citate.[10] Both classes increase testosterone by negative hypothalamic-pituitary feedback on T. Oral AIs necessitate long term follow-up as they significantly raise testosterone and also lower oestradiol. Low oestrogen levels may have

potentially detrimental effects on bone health. In addition, clinical improvement in symptoms do not match the results seen with testosterone preparations. Outcomes of AIs appear better in younger men where fertility is likely to be preserved. AIs have been shown to slightly improve body composition but showed no significant improvements in lean muscle mass or glucose homeostasis.

Enclomiphene was rejected for licence for hypogonadism in 2015. Although T levels improved and E2 levels improved, authorities felt that there was insufficient information on clinical improvement. Further phase 3 safety studies were requested. There has been considerable off-label use of both classes of drugs, both alone or in conjunction with testosterone to mitigate adverse effects and preserve fertility in younger men.[10]

Patient monitoring

Blood should also be taken for TT, SHBG, Free T and PSA. It is suggested that blood be repeated at 3 months, 6 months and 12 months with the aim to maintain levels between 15 nmol/L and the upper level of normal for the laboratory. For monitoring purposes with gels, blood testing should be performed 2-4 hours after application (Table 10.IV). Patients need to thoroughly wash their hands to avoid contamination of the venepuncture sites, as this is a common cause of erroneously high levels.[1] These high levels can cause panic and confusion, with therapy being stopped, causing patients to lose confidence. Low levels, especially with poor clinical response may indicate poor absorption requiring switch to injections.

With long-acting injections, a blood test should be taken just before the 4th injection is due and the interval (rather than the dose) between injections can be adjusted up or down by 2 weeks according to the trough level. As the blood can be taken at the same time as an injection visit, there are fewer "false interpretations" of low or high results due to timing of samples in relation to application. Several studies have recently shown that COVID-19 severely lowers T levels and that low T levels are associated with increased mortality.[11]

Consider measuring oestradiol levels in men who develop gynaecomastia, or in those with a poor response in sexual function or depression. Raised oestradiol is usually due to excessive aromatisation in obese men. Conversion to oestrogen is necessary for bone health but unwanted symptoms may merit a change in testosterone formulation or addition of an aromatase inhibitor (off label use).[1]

Conclusions

TRT prescribing has increased significantly over the last 2 years as an inevitable result of routine screening in men with ED and T2DM, rather than the influence of advertising. As most studies show that response to ED therapies is greatly reduced in hypogonadal men, especially those with T2DM, these patients usually require TRT in addition to appropriate ED therapy according to published guidelines. In patients

Table 10.IV. British society for sexual medicine recommendations for UK practice with levels of evidence and grades of recommendation.

	LoE	Grade
Recomendations–screening		
Screen for TD in adult men with consistent and multiple signs of TD	3	C
Screen all men presenting with ED, loss of spontaneous erections,or low sexual desire	1	A
Screen for TD in all men with type 2 diabetes, BMI >30 kg/m^2, or waist circumference >102 cm	2	A
Screen for TD in all men on long-term opiate, antipsychotic, or anticonvulsant medication	2	B
Recommendations–diagnosis		
Restrict diagnosis of TD to men with persistent symptoms suggesting TD and confirmed low T level	3	C
Measure fasting T levels in the morning before 11 A.M. acknowledging that, in normal life, non-fasting levels could be up to 30% lower	2	A
Repeat TT assessment on ≥2 occasions by a reliable method; in addition,measure free T in men with levels close to lower normal range (8-12 nmol/L) or those with suspected or known abnormal SHBG level	1	A
Measure LH serum levels to differentiate primary from secondary TD	2	A
Base decisions on therapy on published action levels rather than laboratory reference ranges	4	B
Recommendations–initiating T therapy		
Perform cardiovascular, prostate, breast,and hematologic assessments before start of treatment	1a	A
Offer T therapy to symptomatic men with TD syndrome for treated localized low-risk prostate cancer (Gleson score <8, stages 1-2, preoperative PSA level <10 ng/mL, and not starting before 1 year of follow-up) and without evidence of active disease (based on measurable PSA level, DRE result, and evidence of metastatic disease)	3	B
Assess cardiovascular risk factors before commencing T therapy and optimize secondary prevention in men with established disease	1a	A
Recommendations–benefits and risks and T therapy		
Beyond 6 months there is evidence of benefit for T therapy in body composition, bone mineralization, and features of metabolic syndrome	3	A
T therapy improves sexual desire, erectile function, and sexual satisfaction	1	A

(*to be continued*)

Table 10.IV. British society for sexual medicine recommendations for UK practice with levels of evidence and grades of recommendation *(continues)*.

	LoE	Grade
Decreases in BMI and waist size and improved glycemic control and lipid profile are observed in hypogonadal men receiving T therapy	2	A
Trials of T therapy should be ≥6 months and maximal benefit is often seen beyond 12 months	2	A
Fully inform the patient about expected benefits and side effects of therapy and facilitate a joint decision by an informed patient and physician	3	A
Fully discuss the adverse effects of T therapy and its future reversiblity on future fertility for each patient and his partner and offer alternative treatment as necessary	1b	B
In patients with adult-onset TD, when TRT is prescribed, offer weight-loss and lifestyle adivce as standard management	2	A
In severely symptomatic patients with TT levels <8 nmol/L, lifestyle and dietary advice alone is unlikely to produce meaningful clinical improvement within a relevant clinical period	2	B
Recommendations–follow-up		
Assess response to therapy at 3, 6, and 12 months and every 12, months thereafter	4	C
Aim for a target TT level of 15-30 nmol/L to achieve optimal response	4	C
Monitor hematocrit before treatment, at 3-6 months, and every 12 months thereafter; decrease dosage, or switch preparation, if hematocrit is >0.54; if hematocrit remains high, consider stopping and reintroduce at a lower dose	4	C
Assess prostate health by PSA and DRE before commencing TRT followed by PSA at 3-6 months, and every 12 months, and every 12 months thereafter	4	C
Assess cardiovascular risk before TRT is initiated and monitor cardiovascular risk factors throughout	1b	A

BMI: body mass index; DRE: digital rectal examination; ED: erectile dysfunction; LH: luteinizing hormone; LoE: level of evidence; PSA: prostate specific antigen; T: testosterone; TD: testosterone deficiency; TRT: testosterone replacement therapy; TT: total testosterone.

who have presented with sexual dysfunction, prompt improvement of these symptoms are likely to be a top priority. On demand ED therapies do not improve libido, whereas TRT has been repeatedly shown to be effective.

Recent studies also suggest that the prevalence of hypogonadism is high in men (40-50%) with T2DM[2] and that cardiovascular outcomes in men with T2DM are worse. The T4DM study strongly suggests that TRT reduces diabetes progression in

patients with TT<14 nmol/L and these findings are likely to further increase TRT prescribing as general practitioners will have to manage TRT as part of routine diabetes care.

References

1. Diabetes UK Association. Available from: https://www.diabetes.org.uk/professionals/position (accessed 1/1/2022).
2. Hackett G, Kirby M, Jones TH, *et al.* British Society for Sexual Medicine Guidelines on Adult Testosterone Deficiency. J Sex Med 2017;14:1504-23.
3. Wittert G, Bracken K, Robledo KP, *et al.* Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021;9:32-45.
4. Hackett G, Jones PW, Strange RC, *et al.* Statin, testosterone and phosphodiesterase 5-inhibitor treatments and age-related mortality in diabetes. World J Diabetes 2017;8:104-11.
5. Pugh PJ, Jones RD, West JN, *et al.* Testosterone treatment for men with chronic heart failure. Heart 2004;90:446-7.
6. Snyder PJ, Bhasin S, Cunningham GR, *et al.* Effects of Testosterone Treatment in Older Men. N Engl J Med 2016;374:611-24.
7. Morales A, Bella AJ, Chun S, *et al.* A practical guide to diagnosis, management, and treatment of testosterone deficiency for Canadian physicians. Can Urol Assoc J 2010;4:269-75.
8. Mulligan T, Frick MF, Zuraw QC, *et al.* Prevalence of hypogonadism in males aged at least 45 years: the HIM study. Int J Clin Pract 2006;60:762-9.
9. Gronski MA, Grober ED, Gottesman IS, *et al.* Efficacy of Nasal Testosterone Gel (Natesto®) Stratified by Baseline Endogenous Testosterone Levels. J Endocr Soc 2019;3:1652-62.
10. Awouters M, Vanderschueren D, Antonio L. Aromatase inhibitors and selective estrogen receptor modulators: Unconventional therapies for functional hypogonadism? Andrology 2020;8:1590-7.
11. Dhindsa S, Zhang N, McPhaul MJ, *et al.* Association of Circulating Sex Hormones with Inflammation and Disease Severity in Patients With COVID-19. JAMA Netw Open 2021;4:e2111398.

11

Guidelines of testosterone in type 2 diabetes

Geoffrey I. Hackett

Introduction

Evidence based guidelines form the basis of modern clinical practice. The field of sexual medicine, however, presents unique problems, in comparison with guidelines on, say, hypertension and dyslipidaemia, where outcomes are clearly defined. The most common symptoms associated with hypogonadism are:

1. loss of sexual desire;
2. reduced erection with sexual activity;
3. loss of morning erection.

For this reason, it is essential that testosterone guidelines include patient related outcomes (PROs) for sexual dysfunction in general and erectile dysfunction in particular. Likewise, ED guidelines need to consider the role of testosterone therapy in terms of desire, ejaculation and sexuality, taking into consideration the impact on the man and his partner. Committees often comprised exclusively of urologists fail to consider the impact of therapy on important subjects such as cardiovascular risk, insulin sensitivity and cognitive impairment. By focusing on "hard end points" such as IIEF scores, they often overlook the fact that adult couples usually have sex for pleasure for themselves and their partner, such that "softer end points" such as "satisfaction and preference" might actually be more valid outcome measures.

What do the current guidelines say?

The 2018 Endocrine Society (ES) Guidelines reported that there are no adequately powered Randomised Controlled Clinical Trials (RCTs) on the effects of T replacement on Major Adverse Coronary Events (MACE).[1] The few RCTs that have reported were limited by their small size, short intervention durations, variable quality of adverse event reporting, and failure to prespecify and adjudicate cardiovascular events. The trials included in multiple meta-analyses suffered from multiple limitations, including heterogeneity of eligibility criteria, dosing, formulations, and

therapy durations; variability in the quality of adverse event recording; lack of large trial cohorts; failure to adjudicate cardiovascular outcomes; and inadequate MACE events. The ES recommend T therapy for men with symptomatic T deficiency to induce and maintain secondary sex characteristics and correct bothersome symptoms of hypogonadism after discussing the potential benefits and risks of therapy and of implications for follow-up. The authors recommended against screening for low testosterone, even in potential high-risk groups.

In contrast with AACE (American Association of Clinical Endocrinologists) guidelines on obesity,[2] recommending screening for all men with T2DM plus those with BMI >30 or waist circumference >102 cm. In a separate guideline AACE concluded the evidence does not indicate increased CV risk with TTh. The BSSM guidelines advise testosterone measurement in all men with Erectile Dysfunction or symptoms suggestive of hypogonadism (Figure 11.1).[3]

The 2018 American Urology Association (AUA) guideline recommends that prior to initiating treatment, clinicians should inform testosterone deficient patients that low testosterone is a risk factor for cardiovascular disease and that, at this time, it cannot be stated definitively whether testosterone therapy increases or decreases the risk of cardiovascular events (*e.g.*, myocardial infarction, stroke, cardiovascular related death, all-cause mortality]. They also advised that clinicians should inform patients of the absence of evidence linking testosterone therapy to the development of prostate cancer.[4] Urological expert guidelines recommend regular Digital Rectal Examination (DRE) but endocrine panels list DRE "according to clinical indication", relying on PSA to exclude baseline prostate cancer. The BSSM guidelines involved multidisciplinary authors, recommending baseline DRE but relying on PSA for follow-up.

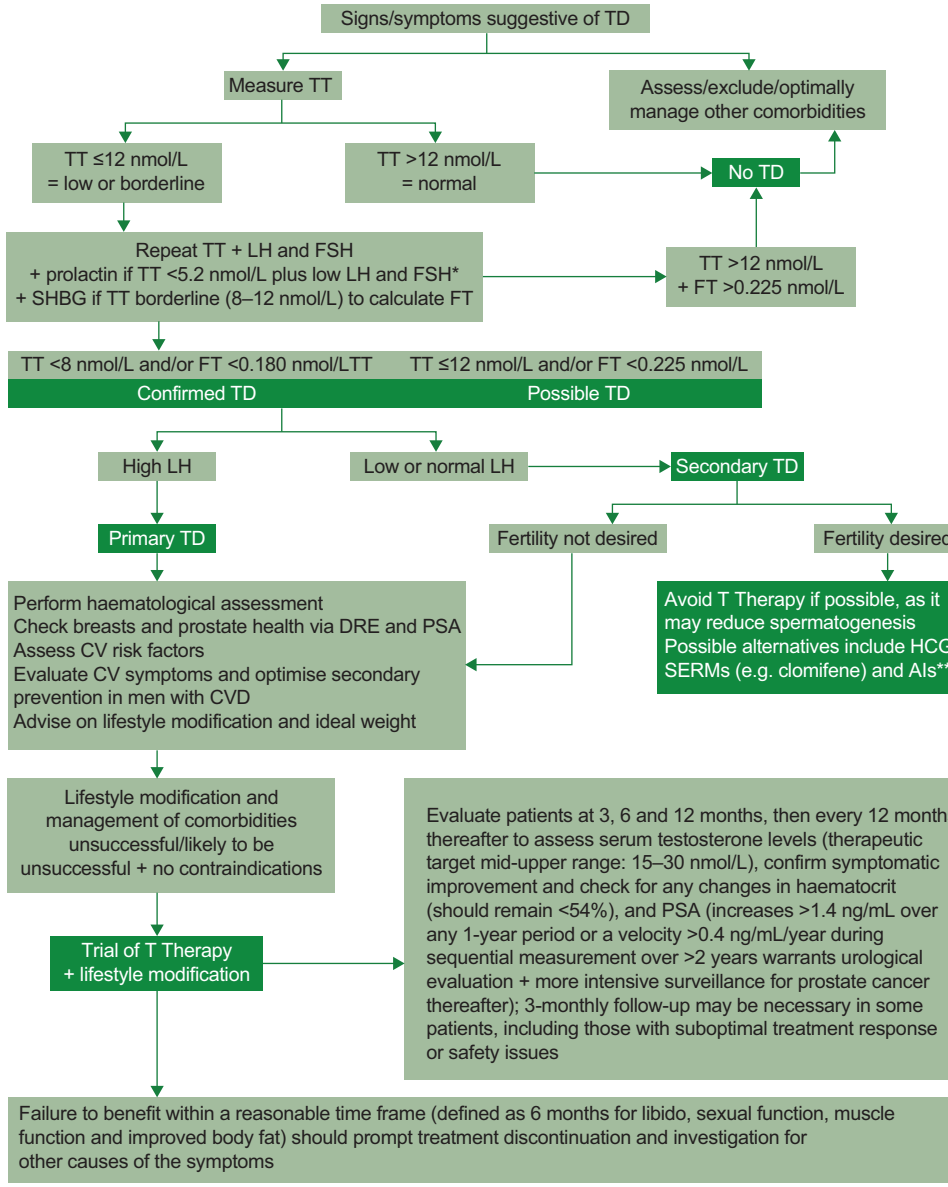
The International Expert Consensus Conference on Testosterone Deficiency concluded that the weight of evidence does not support the contention that TRT increases CV risk.[5] They comment that, for approximately two decades there was suggestive evidence that a normal endogenous serum T, or TTh itself, provided protective benefits against adverse CV outcomes. Observational studies showing reduction in cardiovascular events with TTh over 8 years were not considered.[6]

What does this mean for clinical practice?

A recent review by Grossmann[7] concluded that randomized controlled clinical trials (RCTs), showed that testosterone treatment does not reduce body weight, but modestly reduces fat mass and increases muscle mass. He also concluded that short-term studies have shown that testosterone treatment in carefully selected obese men may have modest benefits on symptoms of androgen deficiency and body composition, additive to diet alone.

The T trial also confirmed positive outcomes in sexual function, walking distance, quality of life, mood, depression, anaemia, and bone density.[8] These accumulated multiple benefits, may equate to significant quality of life benefits for patients. The

Figure 11.1. The BSSM Guidelines of Testosterone Deficiency in Adult Men.



conclusion of Grossmann was that longer term, larger RCTs designed for patient-important outcomes and potential risks are required. Until such trials are available, he suggested that testosterone treatment cannot be routinely recommended for men with obesity-associated nonclassical hypogonadism and that therapy should be restricted to men with "classical hypogonadism". Lifestyle measures or, where

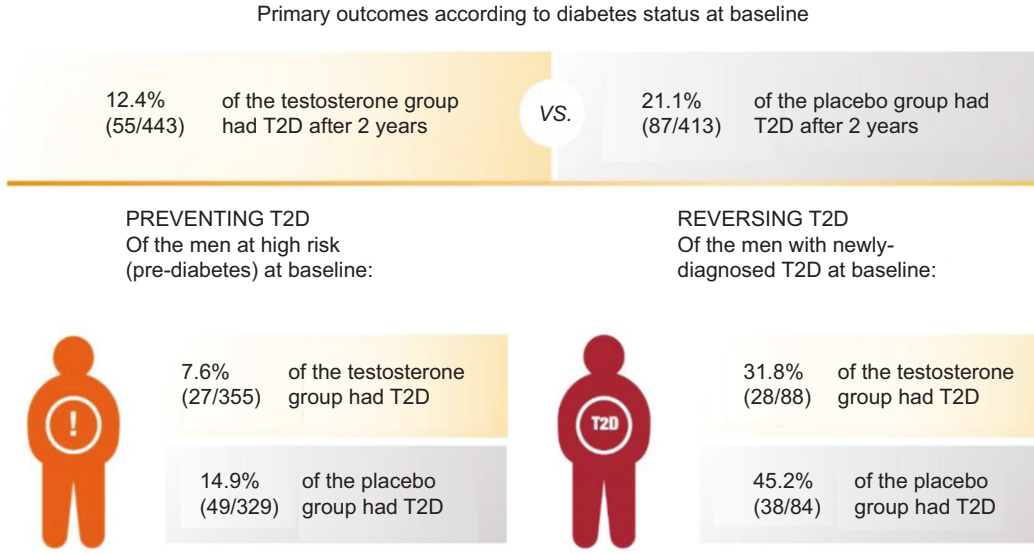
indicated, bariatric surgery to achieve weight loss, and optimization of comorbidities should remain first line treatment, despite limited evidence of symptomatic improvement for these measurements alone. These conclusions are questioned by Corona *et al.* who pointed out that data from RCTs show that clinical benefits from TTh are almost exclusively conducted in men with "non-classical hypogonadism" and not a single RCT has ever been conducted on long term safety in men exclusively with "classical" hypogonadism, nor is there a logical reason why TTh should be safer in men with classical aetiology.[8]

Men with HG present with distressing symptoms associated with poor quality of life. They rightly expect their physician to be motivated to treat their symptoms rather than wait for decades until the perfect RCT addresses statistical risk. TRT is associated with modest improvements in a range of symptoms that might add up to huge benefit to the patient.[9] This might be underestimated by specialists with a restricted area of interest. The symptoms of HG cross many specialties and the clinical decision maker needs to be aware of the impact of HG and the benefits of TRT outside the comfort area of their own specialty. It is worthy to mention that there is a current Phase 4 clinical trial (Registration number NCT03518034),[10] referred to as the Testosterone Replacement Therapy for Assessment of Long-term Vascular Events and Efficacy ResponSE in Hypogonadal Men (TRAVERSE) Study, across 389 centres in the United States and Puerto Rico. This study attempts to more definitively determine whether and to what degree TTh impacts risk of incurring a major CV event, defined as nonfatal stroke or MI. The study randomizes 6,000 middle-aged/elderly male hypogonadal subjects (serum T <300 ng/dL) to either topical TTh or placebo and assesses the mean time between T administration and initial major CV event over 60 months.

The T4DM study, published in Jan 2021,[11] involved over 1000 men obese men with pre-diabetes, randomised to either long-acting TU injection or placebo and followed up for 2 years. Both groups were subject to intensive diet and lifestyle intervention. The investigators found a 40% reduction in progression to type 2 diabetes over 2 years with TU (Figure 11.2).

TU combined with intensive diet and lifestyle advice produced significant reduction in visceral fat and improvement in lean muscle and sexual function, above lifestyle alone. The study was not powered to definitively address the issue of cardiovascular safety. In common with other studies, physical symptoms and sexual function actually deteriorated in the placebo arm despite weekly involvement in a weight watch programme, regular follow-up and effective weight loss. Likewise, in the BLAST RCT and T4DM, despite active lifestyle intervention over 2 years, sexual function and clinical symptoms deteriorated in the placebo group. These findings do not support the reliance of lifestyle advice alone as an effective therapeutic approach for men with hypogonadism.[12] A recent review of current guidelines by Bhasin *et al.*[12] demonstrated the current state of confusion around the subject, as they concluded: Testosterone treatment of older men with low

Figure 11.2. Reduction in onset diabetes with TRT (T Undecanoate 1000 mg) over 2 years (from Wittert G, *et al.* Lancet Diabetes Endocrinol 2021;9:32-45).[11]



3.5 million UK men have pre-diabetes with 40% (1.4m) with low testosterone. T4DM suggests that 23k cases of male T2DM/2 years could be prevented.

testosterone levels improves overall sexual activity, sexual desire and erectile function, as well as improving real and volumetric bone density, as well as estimated bone strength in the spine and hip: corrects unexplained anaemia of aging: increases skeletal muscle mass, strength and power, self-reported mobility, and some measures of physical function: and modestly improves depression. [Recent evidence also shows prevention of progression to, and reversal of diabetes].

Imagine a highly symptomatic patient, who has eventually achieved a specialist consultation and presented with this huge list of life changing evidence-based benefits, only the hear that: the endocrine society recommends against testosterone therapy of all older men with low testosterone levels on major cardiovascular events and prostate cancer risk remain unclear (although the balance of available evidence for both *suggests* positive benefit) but suggests *consideration* of treatment on an individualized basis in men who have consistently low testosterone levels (now suggested to be 14 nmol/L to prevent diabetes progression) and symptoms or conditions suggestive of testosterone deficiency.

It is worthy of note that the need to be diagnosed with "classical hypogonadism" has disappeared." How many patients presented with this huge list of potential benefits are likely to reply, "thank you doctor, I will tolerate all these symptoms until we are completely clear as to all the long-term safety issues?".

A recent review[13] highlighted several differences between the AUA and ES guidelines, showing the different priorities of panels comprising entirely of either urologists or endocrinologists (Table 11.I). It is also of interest that the ES also recommend against screening for hypogonadism in high- risk groups such as T2DM where the prevalence is 40-50% (with a cut-off of 12 nmol/L, and even higher if a cut-off of 14 nmol/L is taken. Presumably, the thinking behind this is that these symptoms will not be an issue if we ignore them (Table 11.II).

Table 11.I Overview of practice guidelines from the 2018 AUA and ES for diagnosis and treatment of low testosterone.

	American Urological Association	Endocrine Society
Diagnonsis definition	Patient having signs/symptoms of low testosterone plus < 300 ng/dl on two morning samples	Patients having signs/symptoms of low testosterone using two separate morning readings. Recommend using CDC cutoff as <264 ng/dl
Distinguishing primary *vs.* secondary hypogonadism	After determining low testosterone physicians should measure LH	Recommend to measure LH and FSH after low testosterone measurements to distinguish primary vs secondary
When to test for prolactin levels	After determining low testosterone on two separate measurements and low LH, a prolactin level should be measured	Recommends further workup to determine cause of low testosterone including prolactin, iron overload syndromes, head trauma, etc.
HCT measurment	Before starting testosterone, if patient has elevated HCT above 50%, testosterone should be withheld, During treatment if HCT rises above 54%, should decrease or discontinue testosterone	Should not give testosterone to patients with elevated HCT. During therapy if patients develop erythrocytosis, they should stop testosterone therapy and resume at a lower dose when levels return to normal
Reproductive evaluation	Before starting therapy, patients should have reproductive evalution before therapy and should not be prescribed if trying to conceive	Men who wish to conceive in 6-12 months should not take testosterone and patient may wish to bank sperm
Age cutoff	No mention	Recommend against replacement in men over 65 except on an individualized basis
Monitoring testosterone levels	Goal to achive minimal dose of testosterone to 450-600 ng/dl	Monitor levels at 3-12 months and should be at mid- normal

CDC: Centres for Disease Control and Prevention; LH: luteinizing hormome; FSH: follicle-stimulating hormone; HCT: hematocrit.

Table 11.II. Cut-off values of testosterone for laboratory diagnosis.

	Cut-off values	Year of release and update
Expert opinion	Total T (TT) or free T below the lower limits of normal	Before Official Guideline
ISSAM	TT<231 ng/dL (8 nmol/L) TT:231-346 ng/dL (8-12 nmol/L) or free T<52 pg/mL	2005
	TT<230 ng/dL (8 nmol/L) TT:230-350 ng/dL (8-12 nmol/L) or free T<52 pg/mL, SHBG	2008
	TT<350 ng/dL (12 nmol/L) or free T<65 pg/mL	2015
Endocrine Society	TT<300 ng/dL or free T<5ng/dL	2006
	TT<280-300 ng/dL or free T<5-9 ng/dL	2010
	TT<300 ng/dL or free T<5 ng/dL	2018
ISSM	TT<350 ng/dL (12 nmol/L)	ICSM 2015
AUA	TT<300 ng/dL	2021
BSSM	TT<12 nmol/L FT<180 pmol/L	2017
EAU	12 nmol/L	2021
Society for Endocrinology	<8 nmol/L high risk >12nmol/L low risk <11 nmol/L cut-off For treatment	2021

The recent Canadian Urological Association guidelines on ED,[14] recommend against the routine use of daily tadalafil, despite pointing out that, in trials the IIEF score was slightly better for daily medication, when 35 mg total doses (7 x 5 mg) *versus* 50-60 mg in on-demand trials. The authors stressed the importance of "patient centred" decision making but decided that 80% of men and 79% of their partners preferring daily therapy, as being of low significance. They also concede that up to 50% of men with ED will have bothersome LUTS where daily tadalafil would be appropriate to treat both conditions. They also felt that the combination of PDE5is plus testosterone was of little additional benefit, quoting improvement of less than 2 IIEF points, whilst totally ignoring the global improvement in desire, ejaculation and orgasm with the addition of testosterone therapy.

Digital rectal examination

The 2021 EAU guidelines recommend PSA plus DRE before TRT, after 3 and then 12 months, *i.e.* 3 DREs in 12 months, in contrast the 2021 Society for Endocrinology Guidelines 2021 state:

"Historically, prostate cancer screening has been conducted during testosterone treatment, in the form of serum PSA measurement and digital rectal examination (DRE) (since 1% of prostate cancers are non- PSA-secreting). However, endocrinologists generally have no experience recognising the features of prostate cancer during DRE, which makes this practice likely to be ineffective and ***potentially harmful****. Major risk factors for prostate cancer are increased age, black ethnicity and family history, and all men (regardless of testosterone treatment) should undergo screening according to local practice. Theoretically, prostate screening might exclude a pre-existing tumour during testosterone treatment, but there is insufficient evidence to support the efficacy or safety of such an approach. In the absence of robust evidence, we do not recommend that mandatory screening for prostate cancer be performed during testosterone treatment".*

We do not believe that EAU guidance is practical. As there is no evidence that TRT is associated with increased prostate cancer risk, then 3 DREs in 12 months is unnecessary, inconvenient and not cost effective, especially in the post Covid era. It is also likely to lead to patients discontinuing important medical treatment because of inconvenience and embarrassment. Patients on TRT are likely to benefit by early detection of prostate cancer unrelated to their T levels by routine PSA on TRT follow-up.

Conclusions

In summation, while there are some variations and certain topics not discussed within each individual set of guidelines, all organisations are in general agreement over most key areas. All agree that Testosterone Deficiency is a clinical syndrome, closely related to type 2 diabetes and pre-diabetes, that requires a low testosterone level as well as signs and/or symptoms for a diagnosis to be made. Unfortunately, there is no formal NICE guidance, just a short document on their website, based largely on BSSM guidance. The exact threshold varies or is not provided, but the organisations suggest a threshold level between 300 and 350 ng/dL. All societies recommend routine laboratory monitoring within the first year and annually thereafter. The guideline committees acknowledge limited data on cardiovascular disease and TTh. The consensus is to withhold TTh within 3-6 months of an MI or stroke or in patients with severe heart failure. The findings of the T4DM study in 2021, with a cut-off of 14 nmol/l, are likely to influence future guidelines significantly.

Prostate cancer is another grey area. Although the consensus is that there are no data to suggest TTh causes prostate cancer, no studies to data have been sufficiently powered to provide evidence of long-term safety.

The major problem is that the guidelines in this paper have been developed by specialist "expert" groups and are unlikely to be accessed by family doctors. Until these guidelines are endorsed by the authorities that regulate primary care, or, in the UK, formal NICE guidance is published, then family doctors are unlikely to take on the burden of screening for or managing hypogonadism in the way that they manage other chronic conditions.

References

1. Bhasin S, Brito JP, Cunningham GR, *et al.* Testosterone therapy in men with hypogonadism: An endocrine society clinical practice guideline. J Clin Endocrinol Metab 2018;103:1715-44.
2. Garvey WT, Mechanick JI, Brett EM, *et al.* American Association of Clinical Endocrinologists and American College of Endocrinology Comprehensive Clinical Practice Guidelines for Medical Care of Patients with Obesity. Endocr Pract 2016;22 Suppl 3:1-203.
3. Hackett G, Kirby M, Edwards D, *et al.* British Society for Sexual Medicine Guidelines on adult testosterone deficiency, with statements for UK practice. J Sex Med 2017;14:1504-23.
4. Mulhall JP, Trost LW, Brannigan RE, *et al.* Evaluation and management of testosterone deficiency: AUA guideline. J Urol 2018;200:423-32.
5. Khera M, Adaikan G, Buvat J, *et al.* Diagnosis and treatment of testosterone deficiency: Recommendations from the fourth international consultation for sexual medicine (ICSM 2015). J Sex Med 2016;13:1787-804.
6. Haider RS, Haider A, Saad F. Remission of type 2 diabetes following long-term treatment with injectable testosterone undecanoate in patients with hypogonadism and type 2 diabetes: 11-year data from a real-world registry study Diabetes Obes Metab 2020;22:2055-68.
7. Grossmann M. Hypogonadism and male obesity: Focus on unresolved question. Clin Endocrinol (Oxf) 2018;89:11-21.
8. Snyder PJ, Bhasin S. Cunningham GR. Lessons from the Testosterone Trials 2018;39:369-86.
9. Corona G, Goulis DG, Huhtaniemi I, *et al.* European Academy of Andrology (EAA) guidelines on investigation, treatment and monitoring of functional hypogonadism in males: Endorsing organization: European Society of Endocrinology. Andrology 2020;8:970-87.
10. Good Clinical Practice Network. Testosterone Replacement Therapy for Assessment of Long-term Vascular Events and Efficacy Response in Hypogonadal Men (TRAVERSE) Study. Available from: https://ichgcp.net/clinical-trials-registry/NCT03518034
11. Wittert K, Bracker K, Robledo KP, *et al.* Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021;9:32-4.
12. Rodrigo dos Santos M, Bhasin S. Benefits and Risks of Testosterone Treatment in Men with Age-related decline in Testosterone. Annu Rev Med 2021;72:75-91.
13. Zucker JI, Masterson TA. Comparison of the American Urology Association and Endocrine Society Guidelines on Testosterone Deficiency. Int J Impot Res 2021.
14. Domes T, Najafabadi BT, Roberts M, *et al.* Canadian Urological Association guideline: Erectile dysfunction. Can Urol Assoc J 2021;15:310-22.

12

Cardiovascular benefits and risks of testosterone therapy

Geoffrey I. Hackett

Introduction

In January 2014, the US Food and Drug Administration (FDA) convened an advisory committee meeting to review cardiovascular (CV) risks of testosterone therapy (TTh), after publication of two observational studies that reported increased CV risks with TTh.[1, 2] Several months later, the FDA expanded the stated purpose of this advisory committee to include a review of the suitable populations for TTh.[3] On 17 September 2014, the advisory committee voted to restrict therapeutic indications for TTh and requested that the pharmaceutical industry perform a cardiovascular safety study. In March 2015, all US commercial T products underwent a mandatory label change that: 1) restricted the indicated population and 2) warned against the possible risk of myocardial infarction (MI) and stroke.[4] The European Union and Health Canada, also issued warnings regarding TTh and potential cardiovascular risk. A review by the European Medicines Agency's (EMA) Pharmacovigilance Risk Assessment Committee (PRAC) found no evidence to support increased risk with TRT but recommended updating the product information warning about the potential increased CV risk in hypogonadal men using TTh.[5] These publications have received extensive media coverage and led to many, often flawed, observational studies, that have produced variable results.

Low testosterone and increased cardiovascular and all-cause mortality

Several long-term observational studies and, reviews have provided evidence to support an association between hypogonadism [HG] and increased cardiovascular (CV) and all-cause mortality[6-10] in the general population but especially in men with type 2 diabetes[11, 12] and proven cardiovascular disease.[13] Several meta-analyses confirmed these findings[14-17] although evidence for a pathogenic link is lacking. Recent guidelines from the American Urology Association,[18] recommended that physicians inform patients that low testosterone is an independent risk factor for cardiovascular

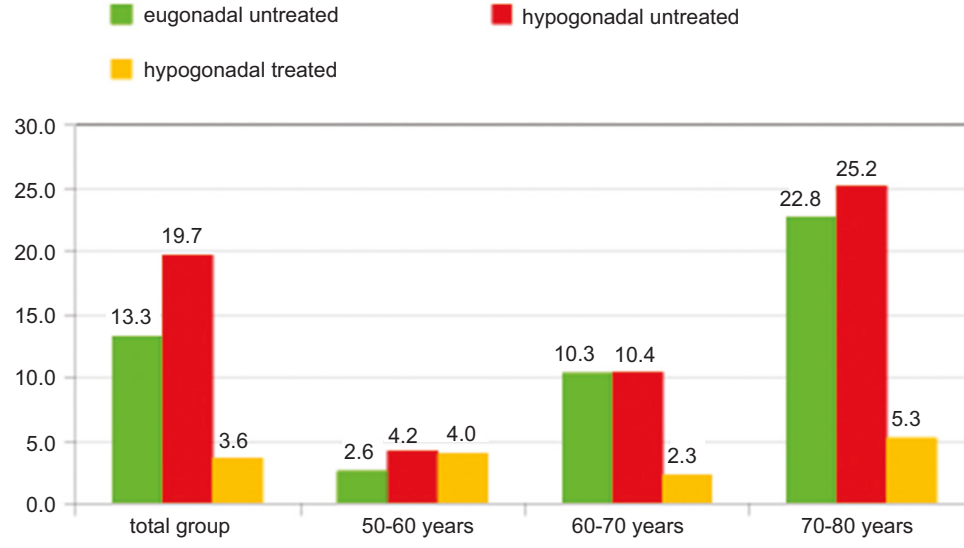
disease as well as the possible risks of therapy. In contrast the Endocrine Society recommend against screening, even for men with T2DM.[19]

A systematic review and meta-analysis evaluating the association between endogenous testosterone and mortality concluded that low levels of endogenous testosterone are associated with an increased risk of all-cause and CV death in community-based studies of men, with a reduction of 2.1 standard deviations in TT being associated with a 25% increase in mortality.[15] However, most of the studies had issues with cohort selection bias.[14, 16]

Three systematic reviews and meta-analyses evaluating the association between endogenous testosterone and all-cause mortality and cardiovascular disease (CVD) mortality[20-22] reported a protective effect of increased total testosterone (Figure 12.1). Research examining the data from 1954 subjects, in terms of several statistical models, found that even after strict adjustment for comorbidities, there was a consistent link between testosterone level and mortality risk throughout, without proving causation.[6]

In a prospective study involving 581 men with T2DM, patients were followed up for a mean of over 5 years. Low testosterone was defined as TT<10.4 nmol/L. The mortality rates were 20% in the low testosterone group *versus* 9.1% in the normal testosterone group, independent of comorbidities and therapies, and 9.4% in those with TD in the treated group.[11]

Figure 12.1. Mortality data of men with Type 2 diabetes mellitus not receiving PDE5 Inhibitors followed for approximately 4 years (from: Hackett G, *et al.* Int J Clin Pract 2016;70:244-53).



In a 10-year Australian study involving 3690 older men, TT and FT levels in the normal range were associated with reduced all-cause and CV mortality. This was the first evidence to suggest that both low and high levels are associated with all-cause mortality, and higher levels of dihydrotestosterone (DHT) reduce CV risk.[7]

A study from Sweden involving 1109 subjects aged 40 years and over, with a mean follow-up of 14.1 years, suggested a strong association between low baseline testosterone and incident myocardial infarction (MI).[8]

Although these studies demonstrated a consistent association between low testosterone and CVD incidence and mortality, this did not prove a pathogenic link. The conclusions from some reviews were that low testosterone could be a "marker" of illness.[16, 17]

Mechanisms for possible decreased cardiovascular risk with testosterone therapy

Insulin resistance (IR) is an independent predictor of cardiovascular risk[23, 24] and a major target for risk reduction.[24, 25] Testosterone therapy in men with T2DM reduced IR and markers of inflammation.[26, 27] Low testosterone is associated with increased visceral adiposity.[28] Testosterone therapy has been shown to reduce visceral fat and increase lean muscle mass, improve exercise capacity, and reduce IR in contrast to diet alone.[29] The T4DM study recently reported a 40% reduction in progression to diabetes in 1007 obese men with pre-diabetes treated with TU *vs.* placebo.[30]

Hypogonadism has been demonstrated in 26% to 37% of male patients with chronic heart failure (HF) with reduced ejection fraction.[31, 32] Chronic HF patients with low testosterone have been shown to have higher rates of hospital admissions and increased morbidity and mortality.[33] Reduced T levels are associated with increased systemic vascular resistance, lower heart rate variability, and depleted baroreflex sensitivity.[34, 35] In chronic HF, hepatic congestion leads to an increase in SHBG levels, with subsequent decrease in free T levels.[36] Low T is frequently associated with impaired exercise tolerance and can exacerbate symptoms in patients with HF with reduced ejection fraction. Testosterone therapy has been shown to improve functional exercise capacity, skeletal muscle performance, insulin resistance and baroreceptor sensitivity in elderly men with chronic HF.[37] Testosterone administered intravenously, acutely increases cardiac output and reduces peripheral vascular resistance through calcium channel blockade.[38] Long-term treatment with TTh prolonged time to develop ischaemia on a treadmill.[38] Toma *et al*.[39] published a meta-analysis of randomized controlled trials evaluating the effect of TRT on exercise capacity in men and women with heart failure. New York Heart Association functional class improved by 1 grade in 9.8% of patients in the placebo groups *versus* 35% of patients in the TRT group. There were no significant differences in major adverse cardiac events between the TRT and placebo.

During chronic replacement, testosterone may reduce circulating levels of inflammatory mediators, including tumour necrosis factor alpha (TNF-alpha) and interleukin-1beta, thus potentially leading to a reduction of left ventricular muscle fibrosis.[40]

Mechanisms for possible increased cardiovascular risk with testosterone therapy

Adverse CVD events associated with TTh may stem from a 6% increased rate of polycythaemia related to multiple mechanisms. The conversion of testosterone to oestradiol stimulates erythropoiesis in the bone marrow. Supraphysiologic levels of testosterone observed with short-acting injections may amplify this effect as may the conversion of testosterone gels to dihydrotestosterone (DHT), via the action of 5-alpha reductase in skin. Increased DHT levels may be associated with an increased risk of fluid retention and thrombosis, resulting in hypertension and heart failure.[41-43] No studies, however, have convincingly demonstrated increase risk of deep vein thrombosis.[45-46] Studies with high doses of testosterone showed reduced levels of HDL cholesterol and adiponectin.[47] Studies suggest that effects on erythrocytosis are more marked with pellets and injections (especially short-acting) compared with gels. Unfortunately, these reports were based on retrospective prescribing data rather than RCTs.[47] Luo *et al.* used data from UK Biobank participants to demonstrate that endogenous testosterone genetically predicted by the JMJD1C gene region was positively associated with thromboembolism, postulating this as a mechanism to explain possible gender differences in cardiovascular events.[48]

Meta-analyses of RCTs

Several meta-analyses have considered the impact of testosterone therapy on metabolism but, far from clarifying the issue, in some cases they create confusion.[49-52] The highest level of evidence comes from RCTs, but many are of insufficient duration, often less than 26 weeks and sometimes only 12 weeks (mean 33.5 weeks).[51] Many involve mixed populations such that it is doubtful that changes in insulin resistance or HbA1c can be achieved in men without significant IR.[51] These analyses are likely to be of insufficient duration to answer questions as to cardiovascular safety of MACE.[50] Many studies on this subject exclude the first 6 months on the basis that events in this period are likely to be due to the untreated condition rather than the impact of therapy.[9-11, 13] Different therapies are analysed together, often patches, oral medication or implants that are largely obsolete or withdrawn. Injections of different esters are grouped together, and U.S. studies do not include long-acting Testosterone Undecanoate, a major therapy in Europe for over a decade.[53, 54] The inclusion of longitudinal studies creates bias related to inaccurate initial diagnosis, lack of follow up diagnosis and little evidence of therapy compliance.[55] Recent

evidence suggests a potential cardiovascular benefit from taking PDE5 inhibitors, often co-prescribed with testosterone therapy although this is also likely to be associated with men in stable long- term relationships associated with health and socio-economic benefits.[56-59]

A comprehensive meta-analysis of 156 RCTS by Huo *et al.*[60] concluded that the prescription of testosterone supplementation for low-T for cardiovascular health, sexual function, physical function, mood, or cognitive function is without support from randomized clinical trials. There was no analysis of data related to insulin resistance and no attempt to conduct a sub-analysis of men with cardiometabolic disease. PDE5 inhibitor use was not recorded.

Two recent meta-analyses concluded that testosterone therapy improved sexual desire, erectile dysfunction, sexual satisfaction but increased erythrocytosis.[61, 62] A meta-analysis by Corona *et al.* involving 59 RCTs involving 3029 treated and 2049 controls concluded that there was clear evidence that TRT reduced fat mass and increased lean muscle mass.[63] They also concluded that TRT improved insulin resistance and glycaemic control with results being more marked in men with metabolic disease. There were non-significant effects on lipids or blood pressure.[63] Further meta-analyses from Corona *et al.* concluded that treatment with T is not effective in reducing CV risk, however, when TTh is correctly applied, it is not associated with an increase in CV risk and it may have a beneficial effect in some sub-populations, especially men with T2DM and MetS. The authors pointed out that the mean duration of follow-up in RCTs was less than 6 months.[64]

These findings suggest that men with T2DM, MetS, and IR are likely to see the greatest benefits from TRT and that this effect might be underestimated when meta-analyses combine these men with younger cohorts without significant co-morbidities. It is likely that the multiple benefits in these high-risk patients outweigh a small potential risk of short-term fluid retention or erythrocytosis, which should be readily detected with appropriate monitoring according to published guidelines. Elliott *et al.*[65] carried out a meta-analysis of 18 RCTs and concluded that TRT significantly improved quality of life, depression, erectile function, and libido. They found no differences between the method of delivery and increased risk of major harm (Table 12.I).

Unfortunately, no comprehensive meta-analysis of RCTs will overcome the issue of short duration studies involving small numbers of patients of mixed patients treated with different formulations. In Table 12.I, having reviewed 35 RCTs we consider only those recruiting >100 subjects and of 26 weeks duration or longer. Within these limitations, the conclusion would be that there is no evidence of increased MACE or all-cause mortality. Although usually contributing a higher level of evidence, sometimes RCTs involve screening large populations to achieve eligible cohorts, such these the subjects might not be truly representative of the general population. For example, the T trials screened 51,085 subjects to recruit 780 for randomization (Table 12.I).

Table 12.I. Results from RCTs of >100 patients with >26 weeks follow-up.

Study	Patients/ medication	Duration (weeks)	Comorbidities	T levels	Findings
Emmelot-Vonk 2008[93]	120/117 TU 160 mg/d	26	67.3 Mixed	10.7	No difference on MACE
Legros 2009[94]	237/79 TU 80-240 mg	52	58.7 Mixed	12.8	No increased MACE
Basaria 2010[95]	106/103	26	74 Frail	<12	23 possible cardiac related events on T *vs.* 5 on placebo
Kalinchenko 2010[85]	113/71 TU 1000 mg/12 w	30	52.1 MetS	<12	No difference in MACE
Srnivas-Shankar 2010[96]	136/138 TG 50 mg/d	26	73.8 Frail	11	
Jones 2011[97]	108/112 TG 60 mg/d	52	59.9 MetS/ T2DM	9.5	No difference in MACE
Behre 2012[98]	183/179 TG50-75 mg	48	62 Frail	10.5	No difference in MACE
Hackett 2013[99]	96/103 TU 1000 mg/12 w	30	61.5 T2DM	<12	No difference in MACE
Hildreth 2013[100]	96/47 TG 100 mg/d	52	66.5 Frail	<12 nmol/L	10 MACE on placebo *vs.* 2 on TTh
Basaria 2015[101]	155/151 TG 75 mg/d	156	67.6 Elderly	10.5	Non-significant increase in Coronary artery Calcium in placebo
Snyder 2015[70]	395/395 TG 50-100 mg	52	72.2 Elderly	<9.5	No difference in MACE. & deaths
Wittert 2020[31]	503/504 TU 1000 mg/12 w	104	Pre-diabetes	<14 nmol/L	No difference in MACE

Longitudinal studies suggesting increased cardiovascular risk

Longitudinal studies are usually of longer duration with larger numbers of patients. Potential selection bias relate to recruitment of patients based on severity of symptoms who influence physicians to treat plus the requirement in many countries to be able to afford long term treatment. Conversely more symptomatic patients often

have lower testosterone levels with greater baseline risk. Men with severe ED are more likely to be treated and the presence of ED is known to confer an additional 50% CV risk. Evidence of compliance is often lacking, as is evidence of patients being treated to therapeutic levels (Table 12.II).

A retrospective US study[1] involved 8709 men with a baseline TT of ≤10.4 nmol/L who were undergoing angiography. During a mean follow-up of 840 days, raw data results showed 681 of the 7486 patients not receiving T therapy died, 420 suffered MIs and 486 experienced strokes. Of the 1223 patients receiving T therapy, 67 died, 23 suffered MIs and 33 experienced strokes. These findings suggested a benefit from T therapy. Complex statistical analysis, using more than 50 covariates, concluded that there was a greater risk in the TTh group. However, there were concerns regarding the exclusion of 1132 patients who experienced events because they were prescribed TTh after the cardiac event, when they should have been included in the untreated group, increasing the events by 70%. There were also no data confirming a correct diagnosis of TDS before TTh and treated men had a mean a mean baseline TT 1.1 nmol/L lower than the untreated group, which would have compared greater risk, but this vaiable was not considered. Starting on T did not ensure that it would be continued: 17.6% of patients in the T group had only 1 prescription filled, and only 60% of patients had follow-up T levels determined after therapy had started. In those patients that did have post-treatment levels determined, the average treatment level was only 332 ng/dL, which is lower than the usual therapeutic target level. Using patches in a large number of patients (63% of those on T) is problematic, as many patients suffer local reactions to the patches and higher discontinuation rates. When challenged, the authors revised the number to 132, but admitted that 104 women had been mistakenly included in the analysis.

A study analysing prescribing data in men treated with TTh,[2] without records of blood results or symptoms, defined non-fatal coronary events as the major endpoint, assessed in the 12 months before and 3 months after therapy. However, the benefits of T Therapy take longer than this to appear and other studies have excluded the first 3 months treatment from analysis due to the likelihood of events relating to the pre-existing condition. Crucially, data on fatal CV events and all-cause mortality data was not collected, despite TTh in other studies having a major impact on mortality rather than event numbers. Twelve-month post-treatment data were collected but not presented. Prior to treatment, the event rates within the groups were strangely identical. There was a small increase in non-fatal cardiac events in men taking TTh, which was more marked in those with increased risk. Overall, events were lower than predicted from comparable research. The lack of mortality data demonstrates a failing to realise that a treatment that reduced mortality was likely to increase non-fatal events. In addition, the study design was not prospective, which casts doubts on the validity of retrospective assessment for the 12-month pre-treatment period. The selection of a comparison cohort of men initiated on

PDE5 inhibitors may have been unwise in the light of studies showing potential cardiovascular benefits with these drugs.[56-59]

Budoff *et al.*[66] published results of CT angiography in a subset of 138 men from the T Trials. Compared with placebo, TTh was associated with a significantly greater increase in noncalcified and total plaque volume, but not in calcified plaque. No major cardiovascular events occurred in either treatment group. No medication changes or interventions reported as a result of these findings of high baseline plaque levels, predominantly in the placebo arm. This appears surprising as standard practice would have been to increase statin doses and refer for possible intervention in such cases. Although these results and the title of the paper would appear concerning, it is difficult to conclude that this study demonstrated increased CV risk.

Table 12.II. Results from observational studies.

Reference	Population	Sample	Findings	Benefit *vs.* risk
Shores 2012[72]	Elderly men	398/633 T *vs.* No Rx Testosterone cypionate/ enanthate	Reduced all-cause mortality with TTh, especially T2DM	+
Muraleedharan 2013[11]	Men with T2DM	64/174 T *vs.* No Rx Mixed	Reduced mortality with TTh	+
Vigen *et al.* 2014[1]	Men with CVD	1223/7486 TTh *vs.* No Rx Mixed		-
Finkle *et al.* 2013.[2]	General population. Patients prescribed PDE5Is used as comparative group.	55,593/141031	Increased non- fatal MACE in first 3 months (fatalities not recorded, no long-term follow-up)	-
Wallis *et al.* 2016[76]	38,340	Elderly men receiving testosterone therapy	Patients treated with testosterone had lower mortality rates and fewer CV events than controls	+ but possible early increase in events. Reduction in prostate cancer diagnosis
Cheetham *et al.* 2017[74]	44,335	Men aged 40 y with low serum testosterone levels	Fewer CV events in the testosterone treatment group; HR, 0.67 (95% CI, 0.62-0.73) Higher rate of CV events in patients with higher baseline testosterone levels (>400 ng/ dL); HR, 1.64 (95% CI, 1.06-2.54)	+ in patients with low serum testosterone levels, caution in patients with normal serum testosterone levels

(*to be continued*)

Table 12.II. Results from observational studies *(continues)*.

Reference	Population	Sample	Findings	Benefit *vs.* risk
Alexander *et al.* 2017[22]	Men aged 18 years receiving testosterone therapy	Meta-analysis reviewing 39 RCTs and 10 observational studies	Compared with placebo use, exogenous testosterone treatment did not significantly increase MI (OR, 0.87; 95% CI, 0.39-1.93; 16 RCTs), stroke (OR, 2.17; 95% CI, 0.63-7.54; 9 RCTs), or mortality (OR, 0.55-1.41; 20 RCTs). Evidence was rated low quality due to high risk of bias, imprecision, and inconsistency. No definitive conclusion on CV effects of testosterone therapy	+
Hackett *et al.* 2016[71]		Men with T2DM treat *vs.* TU 1000 mg/12 weeks *vs.* no Rx		No risk
Sharma *et al.* 2016[75]	71,407	Patients with low serum testosterone levels	No significant difference in rates of DVT/PE in patients receiving testosterone	No risk
Anderson *et al.* 2016[73]	5695	Men with low testosterone -electronic medical records search	Testosterone supplementation in men with low testosterone levels was associated with a reduced incidence of MACE and death over 3 years compared with no or ineffective supplementation	+
Elliott *et al.* 2017[79]	18 RCTs of less than 2 years	Men with low testosterone levels	No increase in MACE or all-cause mortality	No risk
Loo *et al.* 2019[70]	15401	Men over 45. UK database study	All-cause mortality per hundred patient years was 1.84(1.74-1.95) for non-use v v 1.00 (0.82-1.22) for current use equating to an adjusted HR for death of 0.64	+ but with possible increased risk in first 6 months to 2 years
Etminam *et al.* 2015[78]	934 283	Case control study of men aged 45 to 80	Current use of TRT was not associated with an increased risk of MI (RR 1.01, 95% confidence interval [CI] 0.89-1.16)	No risk

As the volume of noncalcified plaque has not been associated with CV outcomes, so the significance of this finding is unclear.[67] In addition, coronary calcium scores do have a well-established association with CV outcomes, and this measure actually showed non-significant improvement with T administration.[68]

There were no adverse CV events in this subgroup over 12 months, and for the entire study population of 790 men in the T trials there were a greater number of MACE in the placebo arm than the T arm (16 *vs.* 9, respectively).[69] Noncalcified plaque volumes increased in the T arm from 204 to 232 mm^3 compared with 317 to 325 mm^3 in the placebo arm (estimated difference 41 mm^3; 95% CI, 14 to 67 mm^3; P=.003). Although the increase was greater in the T arm, the placebo group had a greater burden of noncalcified plaque by 55% at baseline and 40% at the end of 12 months. This difference between groups was larger than the observed changes over time for either group, indicating that the two treatment populations were substantially different at baseline. It is therefore impossible to know whether the observed results were due to baseline population differences or the drug intervention.[69]

Loo *et al* used the UK Clinical Research Datalink to study 15,401 men and concluded that the HR for composite CV events for men over 45 prescribed TTh *vs.* non-use was 1.21 (C.I 1.00 to 1.46), especially in the first 2 years. No pre-or post-treatment levels were described. Paradoxically the incident for all-cause mortality per hundred patient years was 1.84 (1.74-1.95) for non-use *vs.* 1.00 (0.82-1.22) for current use equating to an adjusted HR for death of 0.64. Despite this clear finding of a 36% reduction in all-cause mortality, the conclusion of the authors was that TRT may increase the risk of CV events in the first 2 years. TRT should be used with caution in ageing men with low testosterone levels.[70]

Longitudinal studies suggesting no-increase or reduction in cardiovascular events

The various metabolic benefits in terms of improved insulin resistance, reduced fat mass, increased lean muscle mass and reduction in inflammatory markers might be expected to translate into a reduction in cardiovascular risk. Inadequate therapy might leave the patient at increased risk of undertreated HG, such that trials need to be assessed in terms of adequate compliance and evidence of sustained treatment to therapeutic levels.[52, 71]

In a retrospective study involving 1031 hypogonadal men, 372 on TTh, the cumulative mortality was 21% in the untreated group *versus* 10 % in the treated group. The greatest effect was observed in younger men and those with T2DM.[72]

In a virtual controlled study, researchers examined electronic medical records between 1996 and 2011 to identify 5695 men with a low initial TT level, a subsequent testosterone level, and up to 3 years of follow-up.[73] Testosterone levels were correlated with the use of testosterone supplementation. The primary outcomes were a

composite of death, nonfatal MI, and stroke (MACE) and death alone. Testosterone supplementation in men with low testosterone levels was associated with a reduced incidence of MACE and death over 3 years compared with no or ineffective supplementation. The results suggest that the positive impact of T Therapy was mainly on mortality as opposed to the number of events, and the benefits were associated with the achievement of therapeutic levels of testosterone. There was no suggestion of increased risk with sustained higher serum levels.

Cheetham *et al.*[74] compared CV outcomes in men aged 40 and older (mean age 59) with documented low testosterone values in a healthcare database. There were 8808 men (19.8%) who received T therapy and 35 527 men (80.2%) did not. The median follow-up was 3.4 years. The hazard ratio for adverse CV events (MI, coronary revascularization, unstable angina, stroke, transient ischaemic attack, or sudden cardiac death) was lower by one- third in the T-treated group (HR 0.67, 95% CI 0.62- 0.73).

Sharma *et al.*[75] retrospectively evaluated 83,010 male veterans with documented low TT levels. The subjects were categorised into three groups: T Therapy with resulting normalisation of TT levels (group 1); TTh without normalisation of TT levels (group 2); and did not receive T Therapy (group 3). The all-cause mortality (HR 0.53, 95 % CI 0.50-0.55), risk of MI (HR 0.82, 95 % CI0.71-0.95) and stroke (HR 0.70, 95 % CI 0.51-0.96) were significantly lower in group 1 *versus* group 2 (N.=25,701, median age 66 years, mean follow-up 4.6 years).

Wallis *et al.*[76] reported a 5 year follow up of 10,311 men on long term T replacement compared with a 28,029-control group and found a decreased risk in all-cause mortality (HR 0.67. 95% CI 0.62-0,73), cardiovascular events (HR 0.84 95% CI 0.72-0.98) and new cases of prostate cancer (HR 0.60 95% CI 0.45-0.80). There appeared to be slight increased risk of CV events in the first 6 months that could have been related to increased risk caused by low T before patients reached therapeutic targets.

Testosterone's Effects on Atherosclerosis Progression in Aging Men (TEAAM) was a 3-year placebo-controlled, double- blind, parallel-group randomized trial involving 308 men aged 60 years or older with low or low- normal T levels (100-400 ng/dL; free T<50 pg/mL).[77] One hundred and fifty- six participants were randomized to receive 75 mg of T, and 152 were randomized to receive placebo gel daily for 3 years, with dose adjustment targeted to achieve T levels between 500 and 900 ng/dL. Co-primary outcomes included common carotid artery intima-media thickness and coronary artery calcium; secondary outcomes included sexual function and health-related quality of life. Results were that T administration for 3 years *vs.* placebo did not result in significant difference in the rates of change in either common carotid artery intima-media thickness or coronary artery calcium. No MACE was reported because this trial was only powered to evaluate atherosclerosis progression, and therefore, although reassuring, these findings should not be interpreted as establishing cardiovascular safety of testosterone use in older men.

Etminan *et al.*[78] performed a case-control study in a cohort of 934 283 men aged 45-80 from a Health Claims Database. For each case with MI, four controls

were identified using density-based sampling. They identified 30 066 MI cases and 120 264 corresponding controls. Current use of TRT was not associated with an increased risk of MI (RR 1.01, 95% confidence interval [CI] 0.89-1.16); first-time users did show an increased risk (RR 1.41, 95% CI 1.06-1.87 with a number needed to harm of 305; this was deemed a relatively small absolute risk). These results should be viewed in the light of evidence suggesting the condition of T deficiency may itself confer increased risk of CV events. Baseline levels were not available for controls, nor was there evidence of effective therapeutic levels during treatment. There was no association between MI and past TRT use and no differences among the different formulations. The Relative Risks for current use and first- time use of TRT in men with a previous history of coronary artery disease were 1.05 (95% CI 0.79-1.41) and 1.78 (95% CI 0.93-3.40), respectively, and not clinically significant. The study has several strengths: 1) due to the large sample size, it includes the largest number of cases of MI with TRT exposure (N.=515); 2) analyses controlled for the most common confounders and time dependent risk patterns; 3) sensitivity analyses effectively removed between- subject variability; 4) the study had adequate statistical power to potentially show an increase in the risk of MI. A large US study of database claims also found no increase in CV events with TRT.[79]

The confounding effect of PDE5 inhibitors

ED, loss of morning erections and low sexual desire are the most common and most specific symptoms that define a diagnosis of hypogonadism.[80] Patients reasonably expect these symptoms to be treated and PDE5Is are the most common first line treatment for ED and are commonly co-prescribed with TRT.[71] Response rates to PDE5Is are lower in men with untreated hypogonadism and therefore discontinuation is more likely.[81] The above longitudinal studies have failed to consider that PDE5Is were developed as cardiovascular drugs and therefore likely to impact cardiovascular outcomes (see chapter 13).[82]

Importance of the method of testosterone delivery

Many early studies reported results from oral TRT, patches, short-acting injections and implants. The current market is largely long-acting injections and topical gel or solution. Layton and colleagues retrospectively analysed 544,115 TRT initiations based on computerized data from the US and UK healthcare systems and concluded that short-acting injections had higher event rates, followed by patches, and then gels.[83] Once again there were no baseline levels to confirm diagnosis and no evidence of treatment compliance, only initiation. It is therefore possible that treatment choice may have reflected many clinical issues, including the severity of deficiency. Therapy discontinuation is highest with short-acting injections followed by patches and then gels,[55] suggesting that the increased mortality might

be related to the severity and undertreatment of testosterone treatment than the therapy itself.

A meta-analysis by Borst and Yarrow came to the opposite conclusion.[53] Placebo-controlled studies involving injection therapy were associated with a significant reduction in mortality compared with patch and gel studies. They postulated that this was associated with more sustained serum levels and reduced conversion to dihydrotestosterone (DHT) by 5α reductase in skin. They reported that transdermal testosterone (patch and gel) elevates serum DHT 5.46-fold, while intramuscular testosterone injection elevates serum DHT only 2.2-fold.[53] Shores *et al.* had earlier described potential adverse cardiovascular risk associated with DHT.[84] This surprising phenomenon occurs despite the fact that transdermal and intramuscular TRT elevated serum testosterone to a roughly similar degree and may be explained by relatively high expression of 5α reductase in skin *versus* lower expression in skeletal muscle.[84]

Many of the these more positive recent publications in Europe are related to the use of long-acting TU, which produces constant sustained levels for up to 12 weeks with improvement in metabolic and sexual parameters and markedly lower withdrawal rates in clinical trials.[29, 30, 71, 85] A major advantage is the avoidance of early compliance issues as a single dose ensures adequate dosing to assess response. Follow-up blood tests can be performed at any time and are easier to assess in relationship to dosing time, ensuring simplified dosing adjustment.[86, 87] There is no risk of partner transfer and less rise in haematocrit due to avoidance of peaks and troughs. Studies suggest a greater improvement in sexual and general wellbeing, related to the achievement of sustained therapeutic levels.[75] Some earlier guidelines suggest 3-6 months as an adequate trial of therapy[86] but recent evidence suggests 6- and probably 12-month trials are required.[87, 88] Improvement in sexual symptoms are seen early[52,69] and these benefits might be underestimated in importance by physicians without an interest in sexual function.

What do we learn from guidelines?

The 2018 Endocrine Society (ES) Guidelines reported that there are no adequately powered RCTs on the effects of T replacement on MACE.[19] The few RCTs that have reported cardiovascular events were limited by their small size, short intervention durations, variable quality of adverse event reporting, and failure to prespecify and adjudicate cardiovascular events. The trials included in multiple meta-analyses suffered from multiple limitations, including heterogeneity of eligibility criteria, dosing, formulations, and therapy durations; variability in the quality of adverse event recording; lack of large trial cohorts; failure to adjudicate cardiovascular outcomes; and inadequate MACE events. Thus, there are insufficient data to establish a causal link between T therapy and cardiovascular events.

Published observational studies often reproduce fatal flaws, not considered in statistic adjustments, namely that treated patients often have lower baseline

testosterone levels. Araujo *et al* showed that a 2.5 SD drop in total testosterone was associated with a 25-35% increase in mortality.[15] More severe symptoms, especially ED, likely to be associated with a decision to treat is known to be an independent risk factor for CVD, associated with a 40-50% additional CV risk.[90] Some authors exclude the first 6 months of therapy from the assessment, as events are more likely to be related to the pre-existing severe hypogonadism. In contrast other authors chose to only report an increase in events in the first 6 months, whilst ignoring clear benefits at 2 years and beyond.[70] Compliance studies suggest that, at best, 34% of patients remain on treatment at 6 months, suggesting that early events are more likely to reflect the underlying condition rather than therapy.[55] Papers involving follow-up testosterone levels to confirm response to therapy, usually show positive benefit from TRT.[71, 73-76] Unfortunately reviews that fail to assess these issues invariable conclude that "results show variable outcomes and caution is advised".

Conclusions

Studies with testosterone therapy suggest significant benefits in sexual function, quality of life, glycaemic control, anaemia, bone density, fat, and lean muscle mass, which should result in reduced cardiovascular risk reduction. Meta-analyses of RCTs, rather than providing clarification, have further confused the issue by including under-powered studies of inadequate duration, heterogeneous medication regimes, and inbuilt bias in terms of studies included or excluded from analysis. Well-conducted longitudinal and registry studies suggest reduction in all-cause and cardiovascular mortality with testosterone therapy, especially in those at high risk through T2DM and metabolic syndrome, when treatment is to target levels and for sufficient duration. There is no justification to support the view that only younger men or those with "classical" hypogonadism should be treated. In the meantime, we need to offer our patients tailored advice that is relevant for their symptoms and circumstances, based on current best evidence. With the global escalation of type 2 diabetes, the positive findings from the T4DM study suggest an important role for testosterone in the prevention of T2DM.

References

1. Vigen R, O'Donnell CI, Barón AE, *et al.* Association of testosterone therapy with mortality, myocardial infarction, and stroke in men with low testosterone levels. JAMA 2013;310:1829-36.
2. Finkle WD, Greenland S, Ridgeway GK, *et al.* Increased risk of non-fatal myocardial infarction following testosterone therapy prescription in men. PLoS One 2014;9:e85805.
3. FDA evaluating risk of stroke, heart attack and death with FDA approved testosterone products. [Internet] 2014. Available from: www.fda.gov/drugs/drugsafety/ucm383904.htm 4

4. Drug Safety Communication: FDA cautions about using testosterone products for low testosterone due to aging; requires labeling change to inform of possible increased risk of heart attack and stroke with use. [Internet] 2015. Available from: http://www.fda.gov/ Drugs/DrugSafety/ucm436259.htm
5. PRAC review does not confirm increase in heart problems with testosterone medicines. [Internet] 2014. Available from: 2014.http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Testosterone_31/Recommendation_provided_by_Pharmacovigilance_Risk_Assessment_Committee/WC500175213.pdf
6. Haring R, Volzke HV, Steveling A *et al.* Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20-79. Eur Heart J 2010;31:1494-501.
7. Yeap B, Alfonso H, Chubb S *et al.* In older men an optimal plasma testosterone is associated with reduced all-cause mortality and higher dihydrotestosterone with reduced ischemic heart disease mortality, while estradiol levels do not predict mortality. J Clin Endocrinol Metab 2014;99:E9-18.
8. Tivesten A, Vandenput L, Labrie F, *et al.* Low serum testosterone and estradiol predict mortality in elderly men. J Endocrinol and Metab 2009;94:2482-8.
9. Shores MM, Matsumoto AM, Sloan KL *et al.* Low serum testosterone and mortality in male veterans. Arch Intern Med 2006;166:1660-5.
10. Khaw KT, Dowsett M, Folkerd E, *et al.* Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men: European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) prospective population study. Circulation 2007;116:2694-701.
11. Muraleedharan V, Marsh H, Kapoor D, *et al.* Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. Eur J Endocrinol 2013;169:725-33.
12. Daka P, Langer RD, Larsson CA. Low concentrations of serum testosterone predicts acute myocardial infarction in men with type 2 diabetes mellitus. BMC Endocr Disorder 2015;15:1-12.
13. Malkin CJ, Pugh PJ, Morris PD, *et al.* Serum testosterone and increased mortality in men with coronary heart disease. Heart 2010;96:1821-5.
14. Ruige JB, Mahmoud AM, De Bacquer D, *et al.* Endogenous testosterone and cardiovascular disease in healthy men: a meta-analysis. Heart 2011;97:870-5.
15. Araujo AB, Dixon JM, Suarez EA, *et al.* Endogenous testosterone and mortality in men: a systematic review and meta-analysis. J Clin Endocrinol Metab 2011;96:3007-19.
16. Oskui PM, French WJ, Herring MJ, *et al.* Testosterone and the cardiovascular system. A comprehensive review of the clinical literature. J Am Heart Assoc 2013;2:e000272.
17. Muraleedharan V, Jones TH. Testosterone and mortality. Clin Endocrinol 2014;81:477-87.
18. Mulhall JP, Trost LW, Brannigan RE, *et al.* Evaluation and Management of Testosterone Deficiency: AUA Guideline. J Urol 2018;200:423-32.
19. Bhasin S, Brito JP, Cunningham GR, *et al.* Testosterone therapy in men with Hypogonadism, An Endocrine Society Guideline. J Endocrinol Metabolism 2018:103:1-30ES
20. Hackett G, Kirby M, Ramachandran S. Testosterone, and the Heart. Eur Cardiol 2019;14:103-10.
21. Corona G, Sforza A, Maggi M. Testosterone Replacement Therapy: Long-Term Safety and Efficacy. World J Mens Health 2017;35:65-76.

22. Alexander GC, Iyer G, Lucas E, *et al.* Cardiovascular risks of exogenous testosterone use among men: a systematic review and meta-analysis. Am J Med 2017;130:293-305.

23. Bonora E, Formentini G, Calcaterra F, *et al.* HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects: prospective data from the Verona Diabetes Complications Study. Diabetes Care 2002;25:1135-41.

24. Stratton IM, Cull CA, Adler AI, *et al.* Additive effects of glycaemia and blood pressure exposure on risk of complications in type 2 diabetes: a prospective observational study (UKPDS 75). Diabetologia 2006;49:1761-9.

25. Home PD, Mant J, Diaz J, *et al.* Management of type 2 diabetes: Updated NICE guidance. BMJ 2008;336:1306.

26. Dhindsa S, Ghanim H, Batra M, *et al.* Insulin Resistance and Inflammation in Hypogonadotropic Hypogonadism and Their Reduction After Testosterone Replacement in Men with Type 2 Diabetes. Diabetes Care 2016;39:82-91.

27. Gianatti EJ, Dupuis P, Hoermann R, *et al.* Effect of testosterone on glucose metabolism in men with type 2 diabetes: a randomized controlled trial. Diabetes Care 2014;37:2098-107.

28. Fernandez CJ, Chacko EC, Pappachan JM. Male Obesity-related Secondary Hypogonadism – Pathophysiology, Clinical Implications and Management. Eur Endocrinol 2019;15:83-90.

29. Ng Tang Fui M, Prendergast LA, Dupuis P, *et al*. Effects of testosterone treatment on body fat and lean mass in obese men on a hypocaloric diet: a randomised controlled trial. BMC Med 2016;14:153.

30. Wittert G, Bracken K, Robledo KP, *et al.* Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021;9:32-45.

31. Naghi JJ, Philip KJ, DiLibero D, *et al.* Testosterone therapy: treatment of metabolic disturbances in heart failure. J Cardiovasc Pharmacol Ther 2011;16:14-23.

32. Aukrust P, Ueland T, Gullestad L, *et al.* Testosterone: a novel therapeutic approach in chronic heart failure? J Am Coll Cardiol 2009;54:928-9.

33. Santos MR, Sayegh AL, Groehs RV, *et al.* Testosterone Deficiency Increases Hospital Readmission and MortalityRates in Male Patients with Heart Failure. Arq Bras Cardiol 2015;105:256-64.

34. Chen Q, Fu Z, Wu X, *et al.* Association of serum androgen concentrations with cardiovascular risk factors in elderly male patients with chronic systolic heart failure in China. Aging Male 2014;17:155-60.

35. Rydlewska A, Maj J, Katkowski B, *et al.* Circulating testosterone and estradiol, autonomic balance and baroreflex sensitivity in middle-aged and elderly men with heart failure. Aging Male 2013;16:58-66.

36. Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 2005;26:833-76.

37. Caminiti G, Volterrani M, Iellamo F, *et al.* Effect of long-acting testosterone treatment on functional exercise capacity, skeletal muscle performance, insulin resistance, and baroreflex sensitivity in elderly patients with chronic heart failure a double-blind, placebo-controlled, randomized study. J Am Coll Cardiol 2009;54:919-27.

38. Pugh PJ, Jones TH, Channer KS. Acute haemodynamic effects of testosterone in men with chronic heart failure. Eur Heart J 2003;24:909-15.

39. Toma M, McAlister FA, Coglianese EE, *et al.* Testosterone supplementation in heart failure: a meta-analysis. Circ Heart Fail 2012;5:315-21.

40. Malkin CJ, Pugh PJ, Jones RD, *et al*. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. J Clin Endocrinol Metab 2004;89:3313-8.

41. Bachman E, Travison TG, Basaria S, *et al.* Testosterone induces erythrocytosis via 267 increased erythropoietin and suppressed hepcidin: evidence for a new 268 erythropoietin/hemoglobin set point. J Gerontol A Biol Sci Med Sci 2013;69:725-35.

42. Coviello AD, Kaplan B, Lakshman KM, *et al.* Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. J Clin Endocrinol Metab 2008;93:914-19.

43. Basaria S, Coviello AD, Travison TG, *et al.* Adverse events associated with testosterone 218 administration. N Engl J Med 2010;363:109-22.

44. Baillargeon J, Urban RJ, Morgentaler A, *et al.* Risk of venous thromboembolism in men receiving testosterone therapy. Mayo Clin Proc 2015;90:1038-4.

45. Martinez C, Suissa S, Rietbrock S, *et al.* Testosterone treatment and risk of venous thromboembolism: population-based case-control study. BMJ 2016;355:i5968.

46. Walker RF, Zakai NA, MacLehose RF, *et al.* Association of Testosterone Therapy with Risk of Venous Thromboembolism Among Men with and Without Hypogonadism. JAMA Intern Med 2019;180:190-7.

47. Pastuszak AW, Gomez LP, Scovell JM, *et al.* Comparison of the Effects of Testosterone Gels, Injections, and Pellets on Serum Hormones, Erythrocytosis, Lipids, and Prostate-Specific Antigen. Sex Med 2015;3:165-73.

48. Luo S, Yeung SLA, J Zhao JV, *et al.* Association of genetically predicted testosterone with thromboembolism, heart failure, and myocardial infarction: mendelian randomisation study in UK Biobank. BMJ 2019;364:l476.

49. Grossmann M, Hoermann R, Wittert G, *et al.* Effects of testosterone treatment on glucose metabolism and symptoms in men with type 2 diabetes and the metabolic syndrome: a systematic review and meta-analysis of randomized controlled clinical trials. Clin Endocrinol (Oxf) 2015; 83:344-51.

50. Corona G, Maseroli E, Rastrelli G, *et al.* Cardiovascular risk associated with testosterone-boosting medications: a systematic review and meta-analysis. Expert Opin Drug Saf 2014;13:1327-51.

51. Corona G, Rastrelli G, Maggi M. Diagnosis and treatment of late onset hypogonadism: systematic review and meta-analysis of TRT outcomes. Best Pract Res Clin Endocrinol Metab 2013 ;27:557-79.

52. Hackett G. Metabolic Effects of Testosterone Therapy in Men with Type 2 Diabetes and Metabolic Syndrome. Sex Med Rev 2019;7:476-90.

53. Borst S, Yarrow J. Injection of testosterone may be safer and more effective than transdermal administration for combating loss of muscle and bone in older men. Am J Physiol Endocrinol Metab 2015;308:E1035-E1042.

54. Middleton T, Turner L, Fennell C, *et al.* Complications of injectable testosterone 258 undecanoate in routine clinical practice. Eur J Endocrinol 2015;172:511-17.

55. Schoenfeld MJ, Shortridge E, Cu Zi, *et al.* Medication adherence and treatment patterns for hypogonadal patients treated with topical testosterone therapy: a retrospective medical claims analysis. J Sex Med 2013;10:1401-9.

56. Anderson S, Hutchings DC, Woodward M, *et al.* Phosphodiesterase type-5 inhibitor use in type 2 diabetes is associated with a reduction in all-cause mortality. Heart 2016;102:1750-6.

57. Andersson DP, Trolle Lagerros Y, Grotta A, *et al.* Association between treatment for erectile dysfunction and death or cardiovascular outcomes after myocardial infarction. Heart 2017;103:1264-70.

58. Pofi R, Gianfrilli D, Badagliacca R, *et al.* Everything you ever wanted to know about phosphodiesterase 5 inhibitors and the heart (but never dared ask): How do they work? J Endocrinol Invest 2016;39:131-42.

59. Hackett G, Jones PW, Strange RC, *et al.* Statin, testosterone and phosphodiesterase 5-inhibitor treatments and age-related mortality in diabetes. World J Diabetes 2017;8:104-11.

60. Huo S, Scialli AR, McGarvey S, *et al.* Treatment of Men for "Low Testosterone": A Systematic Review. PLoS One 2016;11:e0162480.

61. Xu L, Freeman G, Cowling BJ, *et al.* Testosterone therapy and cardiovascular events among men: a systematic review and meta-analysis of placebo-controlled randomized trials. BMC Med 2013;11:108.

62. Ponce OJ, Spencer-Bonilla G, Alvarez-Villalobos N, *et al.* The efficacy and adverse events of testosterone replacement therapy in hypogonadal men: A systematic review and meta-analysis of randomized, placebo-controlled trials. J Clin Endocrinol Metab. 2018.

63. Corona G, Ganguli VA, Maserati E, *et al.* Therapy of endocrine disease: Testosterone supplementation and body composition: results from a meta-analysis study. Eur J Endocrinol 2016;174:R99-116.

64. Corona G, Rastrelli G, Di Pasquale G, *et al.* Testosterone and Cardiovascular Risk: Meta-Analysis of Interventional Studies. J Sex Med 2018;15:820-38.

65. Elliott J, Kelley SE, Millar AC, *et al.* Testosterone therapy in hypogonadal men: a systematic review and network meta-analysis. BMJ Open 2017;7:e015284.

66. Budoff MJ, Eilenberg SS, Lewis CE, *et al.* Testosterone treatment and coronary artery plaque volume in older men with low testosterone. JAMA 2017;317:708-16.

67. Park HB, Heo R, Ó Hartaigh B, *et al.* Atherosclerotic plaque characteristics by CT angiography identify coronary lesions that cause ischemia. JACC Cardiovasc Imaging 2015;8:1-10.

68. Criqui MH, Denenberg JO, Ix JH, *et al.* Calcium density of coronary plaque and risk of incident cardiovascular events. JAMA. 2014; 311:271-278.

69. Snyder PJ, Bhasin S, Cunningham GR, *et al.* Testosterone Trials Investigators. Effects of Testosterone Treatment in Older Men. N Engl J Med 2016;374:611-24.

70. Loo SY, Azoulay L, Nie R, *et al.* Cardiovascular and Cerebrovascular Safety of Testosterone Replacement Therapy Among Aging Men with Low Testosterone Levels: A Cohort Study. Am J Med 2019;132:1069-77.

71. Hackett G, Heald AH, Sinclair A, *et al.* Serum Testosterone, Testosterone Replacement Therapy and All-cause Mortality in men with Type 2 Diabetes: Retrospective Consideration of the impact of PDE5 Inhibitors and Statins. Int J Clin Pract 2016;70:244-53.

72. Shores MM, Smith NL, Forsberg CW, *et al.* Testosterone treatment and mortality in men with low testosterone levels. J Clin Endocrinol Metab 2012;97:2050-8.

73. Anderson JL, May HT, Lappé DL, *et al.* Impact of Testosterone Replacement Therapy on Myocardial Infarction, Stroke, and Death in Men with Low Testosterone Concentrations in an Integrated Healthcare System. Am J Cardiol 2016;117:794-9.

74. Cheetham TC, An J, Jacobsen SJ, *et al.* Association of testosterone replacement with cardiovascular outcomes among men with androgen deficiency. JAMA Intern Med 2017;177:491-9.

75. Sharma R, Oni OA, Gupta K, *et al.* Normalization of testosterone level is associated with reduced incidence of myocardial infarction and mortality in men. Eur Heart J 2015;36:2706-15.

76. Wallis CJ, Lo K, Lee Y, *et al.* Survival and cardiovascular events in men treated with testosterone replacement therapy: an intention-to-treat observational cohort study. Lancet Diabetes Endocrinol 2016;4:498-506.

77. Basaria S, Harman SM, Travison TG, *et al.* Effects of testosterone administration for 3 years on subclinical atherosclerosis progression in older men with low or low- normal testosterone levels: a randomized clinical trial. JAMA 2015;314:570-81.

78. Etminan M, Skeldon SC, Goldenberg SL, *et al.* Testosterone therapy and risk of myocardial infarction: a pharmacoepidemiologic study. Pharmacotherapy 2015;35:72-8.

79. Li H, Mitchell L, Zhang X, *et al.* Testosterone Therapy and Risk of Acute Myocardial Infarction in Hypogonadal Men: An Administrative Health Care Claims Study. J Sex Med 2017;14:1307-17.

80. Antonio L, Wu FCW, O'Neill TW, *et al.* Low Free Testosterone is Associated with Hypogonadal Signs and Symptoms in Men with Normal Total Testosterone. J Clin Endocrinol Metab 2016;101:2647-57.

81. Buvat J, Montorsi F, Maggi M, *et al.* Hypogonadal men nonresponses to the PDE5 inhibitor tadalafil benefit from normalization of testosterone levels with a 1% hydroalcoholic testosterone gel in the treatment of erectile dysfunction (TADTEST study). J Sex Med 2011;8:284-93.

82. Kloner RA, Goldstein I, Kirby MG, *et al.* Cardiovascular Safety of Phosphodiesterase Type 5 Inhibitors After Nearly 2 Decades on the Market. Sex Med Rev 2018;6:583-94.

83. Layton BL, Meier CL, Sharpless JL, *et al.* Comparative safety of testosterone dosage forms. JAMA Intern Med 2015;175:1187-96.

84. Shores MM, Biggs ML, Arnold AM, *et al.* Testosterone, dihydrotestosterone, and incident cardiovascular disease and mortality in the cardiovascular health study. J Clin Endocrinol Metab 2014;99:2061-8.

85. Kalinchenko SY, Tishova YA, Mskhalaya GJ, *et al.* Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. Clin. Endocrinol (Oxf) 2010;73:602-12.

86. Dohle GR, Arver S, Bettocchi C, *et al.* EAU Guidelines on Male Hypogonadism. [Internet] 2015. Available from: http://uroweb.org/wp-content/uploads/18-Male-Hypogonadism_LR1.pdf. [Accessed 2018, May 22].

87. Hackett G, Kirby M, Jones TH, *et al.* British Society for Sexual Medicine Guidelines on Adult Testosterone Deficiency. J Sex Med 2017;14:1504e1523.

88. Morgentaler A, Zitzmann M, Traish AM, *et al.* Fundamental concepts regarding testosterone deficiency and treatment: international expert consensus resolutions. Mayo Clin Proc 2016;91:881-96.

89. Emmelot-Vonk MH, Verhaar HJ, Nakhai Pour HR, *et al.* Effect of testosterone supplementation on functional mobility, cognition, and other parameters in older men: a randomized controlled trial. JAMA 2008;299:39-52.
90. Legros JJ, Meuleman EJ, Elbers JM, *et al.* Oral testosterone replacement in symptomatic late-onset hypogonadism effects on rating scales and general safety in a randomized, placebo-controlled study. Eur J Endocrinol 2009;160:821-31.
91. Basaria S, Coviello AD, Travison TG, *et al.* Adverse events associated with testosterone administration. N Engl J Med 2010;363:109-22.
92. Srinivas-Shankar U, Roberts SA, Connolly MJ, *et al.* Effects of testosterone on muscle strength, physical function, body composition, and quality of life in intermediate-frail and frail elderly men: a randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab 2010;95:639-50.
93. Jones TH, Arver S, Behre HM, *et al.* Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). Diabetes Care 2011;34:828-37.
94. Behre HM, Tammela TL, Arver S, *et al.* A randomized, double-blind, placebo-controlled trial of testosterone gel on body composition and health-related quality-of-life in men with hypogonadal to low-normal levels of serum testosterone and symptoms of androgen deficiency over 6 months with 12 months open-label follow-up. Aging Male 2012;15:198-207.
95. Hackett G, Cole N, Bhartia M, *et al.* Testosterone replacement therapy with long-acting testosterone undecanoate improves sexual function and quality-of-life parameters vs placebo in a population of men with type 2 diabetes. J Sex Med 2013;10:1612-27.
96. Hildreth KL, Barry DW, Moreau KL, *et al.* Effects of testosterone and progressive resistance exercise in healthy, highly functioning older men with low-normal testosterone levels. J Clin Endocrinol Metab 2013;98:1891-900.
97. Basaria S, Harman SM, Travison TG, *et al.* Effects of testosterone administration for 3 years on subclinical atherosclerosis progression in older men with low or low-normal testosterone levels: a randomized clinical trial. JAMA 2015;314:570-81.

13

The role of PDE5 inhibitors in hypogonadism and diabetes

Geoffrey I. Hackett

Introduction

Type 2 diabetes is a major health and economic concern for the Western World. In the UK in 2018, 26% of the population over 65 are diagnosed, and 56% of these are men. The prevalence is 6 times greater in men of South East Asian origin and 3 times greater in men of Afro-Caribbean background.[1] In the US, two-thirds of men over 65 have T2DM and 1 in 3 are living with pre-diabetes and 84% are unaware. UKPDS confirmed that each increase of 1% in HbA1c increases risk of diabetes related death by 21%, myocardial infarction by 14%, and peripheral vascular disease by 43%.[2] Currently, all men diagnosed with type 2 diabetes over age 40 are routinely commenced on Metformin, Statin and ACE inhibitor or Angiotensin Receptor Blocker (ARB), as preventive strategies according to NICE guidance.[3] In an age of "personalised" care in diabetes, this chapter presents the current evidence supporting the routine use of PDE5 inhibitors, especially in the presence of hypogonadism.

Current NICE, ADA and EAU guidance

Men with T2DM have a 32% higher prevalence of LUTS/BPH due to shared mechanisms of chronic inflammation, insulin resistance, endothelial dysfunction, pelvic atherosclerosis and sympathetic overactivity (Table 13.I)[4] but first line NICE advice for men with minimal prostate enlargement is an alpha-blocker,[5] known to adversely affect ejaculation, which may already be affected by autonomic

Table 13.I. Erectile dysfunction in type 2 diabetes mellitus related to duration, control and number of complications of diabetes.

- Autonomic neuropathy
- Peripheral neuropathy
- Hypertension
- Peripheral vascular disease
- Dyslipidaemia
- Drug side effects
- Benign Prostatic Hyperplasia (LUTS)
- Depression
- Hypogonadism (double risk)
- Psychological factors
- Plus ejaculatory disorders. Retrograde/anejaculation
- Reduced sensation

neuropathy in men with T2DM.[4] In addition, the co-prescribing with an on-demand PDE5 inhibitor with an alpha-blocker is more likely to produce symptomatic hypotensive episodes in men with T2DM,[6] due to co-morbid autonomic neuropathy, co-prescribed anti-hypertensives and hypoglycaemia. Recent evidence suggests that alpha-blockers precipitate heart failure.[7]

EAU and BSSM guidelines[8, 9] do recommend daily tadalafil as first line for men with ED and LUTS but unfortunately this strategy is not even considered by NICE, nor are diabetes specialists likely to motivated to follow urology or sexual medicine guidelines.

Likewise, AUA,[10] ADA,[11] AACE[12] and BSSM guidelines[13] do recommend Testosterone measurement in men with T2DM, with or without ED, but testosterone is not considered in current NICE guidance on T2DM and there is no NICE guidance on ED, even after 22 years of PDE5 inhibitors.

The case for daily tadalafil 5 mg for endothelial dysfunction in men with T2DM

Erectile dysfunction is now acknowledged as an independent risk factor for cardiovascular events and mortality,[14] increasing the risk by 50% in men with type 2 diabetes. ED has now been introduced into cardiovascular risk calculators.[15] Modern cardiovascular risk strategies revolve around reducing the impact of the modifiable risk factors as demonstrated in the INTERHEART study.[16] It is therefore logical that ED should be approached in the same fashion as other independent risk factors. The chronic pathological process is endothelial dysfunction, and this has been shown to be modified by chronic PDE5 inhibitors in many studies. Rosano *et al.* demonstrated significant improvement in endothelial function after 2 weeks, that persisted 2 weeks after treatment cessation.[17] Amano *et al.* investigated 81 men with ED and LUTS treated with tadalafil 5 mg daily for 12 months and found significant improvements in IIEF at 3 months (continuing improvement at 12 months), IPSS after 1 month, brachial-ankle pulse wave velocity and ankle-brachial index after 3 months.[18] Ramirez *et al.* demonstrated that daily dosing with sildenafil improved insulin sensitivity in men with pre-diabetes.[19]

Santi *et al.* investigated 54 men with T2DM in a 24-week placebo- controlled study of Vardenafil 10mg BD and found significant improvements in IIEF, Flow mediated dilatation, IL-6, and testosterone levels in the cohort with hypogonadism.[20]

Aversa *et al.* investigated 45 men with diabetes treated with either 5mg tadalafil daily or 20mg on-demand and found that only daily tadalafil improved flow mediated dilatation, insulin sensitivity and lean muscle mass.[21]

Santi *et al.* conducted a meta-analysis of 12 RCTs (N.=476) involving chronic PDE5 inhibitors in T2DM and concluded clear evidence of improved flow mediated dilatation and reduction of IL-6, with hs-CRP improvement just failing to reach statistical significance. Selected studies involved Sildenafil (25-100 mg daily), usually for 12- week maximum duration.[22]

Lee *et al.* conducted a 12-week study of tadalafil 5 mg daily in men with T2DM and found that severity of ED was related to baseline hs-CRP. Response rate, based on SEP 3 was 70% Median hs-CRP levels were 0.31 mg/dL (range, 0.18 to 0.62 mg/dL) in non-responders and 0.14 mg/dL (range, 0.09 to 0.4 mg/dL) in responders, respectively (P=0.028).[23]

Schwartz *et al.* reviewed studies of tadalafil 5mg daily on endothelial function and suggested that these effects might translate into a long- term reduction in cardiovascular risk.[24]

Daily tadalafil effects on testosterone levels

Men with T2DM have low levels of testosterone in up to 40% with associated cardiovascular risk.[1] Many may be candidates for testosterone therapy to improve sexual symptoms, quality of life and cardiovascular risk. Hellstrom *et al.*, in a placebo-controlled study designed to assess sperm parameters in 253 men treated with tadalafil 20 mg daily for 3 months found significant increases in total testosterone v placebo and no significant effect on semen parameters over 3 cycles.[25]

Oscan *et al.* treated 40 men with metabolic syndrome with tadalafil 5mg for 3 months with an increase of testosterone from 11.4 (baseline) to 16.5 nmol/L, and IIEF-5 from 11.3 to 19.[26] The authors recommend tadalafil 5 mg once daily in those men with erectile dysfunction especially low testosterone levels accompanied by metabolic syndrome. Spitzer *et al.* treated 106 men with sildenafil 50-100 mg for 3-7 weeks (mean 2.6 doses on demand per week) and found a mean rise in testosterone of 3.6 nmol/L and corresponding fall in LH. The authors suggested that the beneficial effect on androgen levels might be related to a direct effect of testicular blood flow rather than increased sexual activity.[27] These studies suggest a possible beneficial effect on androgen levels if there is a wish for testosterone therapy to be avoided or as an adjunct to testosterone treatment.

Clinical studies involving tadalafil 5 mg daily

Unfortunately, PDE5 inhibitors do not have a licence to treat the process of endothelial dysfunction and are only licensed to treat ED and pulmonary hypertension (sildenafil and tadalafil). Tadalafil 5 mg daily is also licensed to treat LUTS/BPH and is the only PDE5 inhibitor licensed for daily use. Many "preference studies" were set up to address this question, with conflicting findings largely due to selection bias, and different dosing advice for different preparations. In the most recent meta-analysis of 16 head-to-head trials of sildenafil and tadalafil, Gong *et al.* concluded "tadalafil shares similar efficacy and safety with sildenafil and significantly improves patients' sexual confidence and quality of life according to multiple outcome measures.[11] Furthermore, patients and their partners prefer tadalafil to sildenafil. Hence, tadalafil may be a better choice for ED treatment".[11] Park *et al.* assigned

118 men with T2DM and ED to either tadalafil 20mg on demand or tadalafil 5mg daily and followed them up for 2 years. The IIEF score improved progressively with daily 5 mg, but not on demand tadalafil 20 mg, 7.2 *vs.* 2.4 (P<0.0001).[28]

The BSSM guidelines 2018, report that on-demand PDE5 inhibitors are only successful in 50% of men with T2DM[17] despite unlimited medication in clinical trials. Buvat *et al.* reported that 89% of patients switched to daily tadalafil were still taking medication after 12 months.[29] The BSSM guidelines suggest that up to 50% of patients who fail with on-demand therapy respond to daily dosing.[17]

Five meta-analyses have compared on-demand *versus* daily tadalafil (Table 13.II). Brock *et al.* compared only RCTs and concluded equal efficacy but these trials were usually of 12- week duration where on-demand patients were provided with unlimited medication such that the total dose level was usually significantly higher than 5 mg daily dosing for tadalafil.[30] Bansal *et al.* reviewed 6 head- to- head studies of up to 12 weeks and concluded that daily tadalafil resulted in a 1.82 higher ED score than unrestricted doses of 20 mg on-demand (2-3 per week taken).[31] This effect is

Table 13.II. Meta-analyses of daily (OAD) tadalafil *vs.* on-demand tadalafil.

Authors	Head-to-Head RCTs Reviewed	Conclusions
Brock *et al.* 2016.[39]	17 prn *vs.* placebo, 4 OAD *vs.* placebo, N.=4345 m Usually, maximum 14 week duration.	Efficacy for both treatments across a wide range of pathologies – No significant difference between regimes.
Peng *et al.* 2017.[40]	6 RCTS OAD *vs.* on *vs.* demand 8 weeks to 9- month duration. N.=1534.	Efficacy for both treatments across wide range of pathologies. Superiority for OAD in terms of SEP 2 and SEP3, especially in prostate cancer. Patient preference for OAD.
Bansal *et al.* 2018.[41]	6 RCTs, 2 open label. 12-week duration. N=672.	Efficacy across a wide range. EF score 1.82 greater for OAD. SEP 2 and 3 not assessed.
Zhongbao *et al.* 2019.[42]	4 RCTs. Greater than 24 weeks N.=1035. SEP 2 and 3 was main outcome.	SEP 2 MD 10.08 (P<0.00001) SEP3 MD 8.09 (P<0.009) tadalafil provided greater efficacy and lower side effect profile in RCTs >24 weeks
Prasetyo *et al.* 2019.[43]	4 RCTS under 14 weeks duration. N.=1200.	Both treatments efficacious but SEP 2 and SEP 3 superior for OAD tadalafil.

RCT-Randomised clinical trial. IIEF. International Index of erectile function. SEP-2. Sexual Encounter Profile Question 2. "Were you able to achieve an erection suitable for penetration?".
SEP-3 Sexual Encounter Profile Question 3. "Were you able an erection suitable for the completion of sexual activity?".

likely to be even greater when on-demand tadalafil is restricted to once per week by legislation.

Peng *et al.* included studies of up to 26 weeks and found superiority for tadalafil 5mg daily especially in men with diabetes and post radical prostatectomy.[32] Prasetyo also concluded greater efficacy for OAD v on-demand tadalafil based on diary data.[33] Most recently Zhou *et al.* conducted a meta-analysis of 4 studies longer than 26 weeks and concluded that daily tadalafil was associated with greater therapeutic benefit and lower side effect profile and that efficacy increased with duration of therapy.[34] This is an important finding, bearing in mind the progressive deterioration in endothelial function and autonomic neuropathy associated with diabetes and suggests that therapy needs to address the pathological damage and not purely a symptom. Shafic *et al.* demonstrated that loss of morning erections was associated with atrophy of the tunica albuginea and veno-occlusive dysfunction and chronic dosing with PDE5 inhibitors is more likely to prevent this progressive decline.[35]

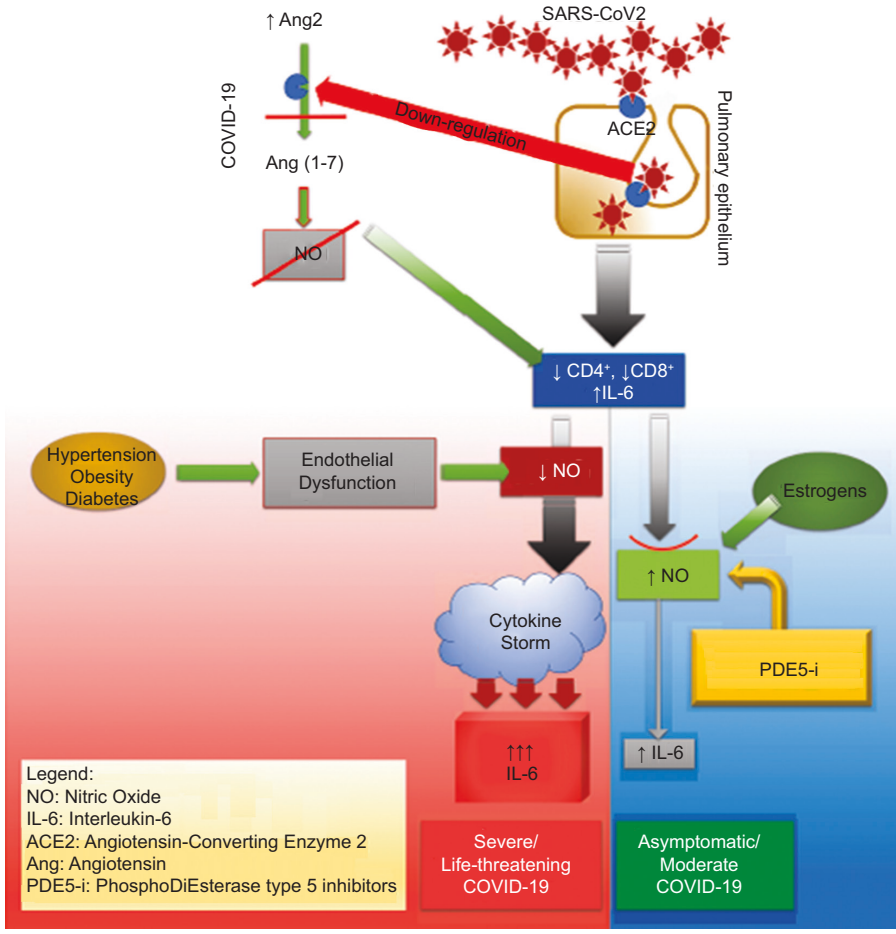
The impact of tadalafil daily dosing on female partners' satisfaction with sexual activity has also been a topic of recent interest and research. A partner preference study of on-demand Sildenafil *vs.* tadalafil indicated that 79% of female partners preferred tadalafil, citing a more relaxed approach to sexual intimacy and greater flexibility with respect to timing of intercourse.[35]

The case for early intervention or prevention of ED

Several studies suggest that the prevalence of ED in T2DM as assessed by IIEF-5 score of 21 or less is 75-77% and is related to duration of diabetes, number of complications and quality of glycaemic control.[4] If current approaches to therapy result in 50% of patients failing to respond to oral therapy, then large numbers are progressing to second- and third-line therapies that, in an age of generic oral drugs results in exponentially higher costs. There are now sound economic reasons for earlier intervention with therapy targeted towards the chronic disease progress, or even prevention, as, it could be argued that ED progression is virtually inevitable as very few men with T2DM have completely normal EF scores and ED often pre-dates the diagnosis of T2DM.[36]

As ED is a recognised risk factor that confers an increased cardiovascular risk of 50%, then logically ED should be prevented as would be the case with any other risk factor.[14] Regular erections and sexual activity have been shown to protect against ED.[37] It might be reasonable to present these facts to the patient and his partner and allow them to be involved in the discussion as to which stage he would wish daily therapy or even early prevention. Clearly lifestyle advice in relation to ED should always be given at the same time. Such interventions should have been implemented as part of standard diabetes care.[3]

Figure 13.1. Scheme of the role of Nitric Oxide in the asymptomatic/Moderate *versus* Severe COVID-19.



Economic issues

Generic tadalafil 5 mg costs approximately £5 per month at NHS tariff. Every patient in the 45% who subsequently fail with the PDE5I will cost £12-15 for each dose of second line therapy, such as Alprostadil or MUSE, meaning £48-60 per month for sexual activity once per week or £96-120 per month for activity twice per week.[36] Each case will usually require secondary care referral at £120 with an average of three follow-up visits at £80 to teach the injection process. Costs will be greater under healthcare systems where the patient pays privately. Involving the patient in discussions about the finances of long- term treatment might result in a positive decision towards early therapy or even prevention.

The case for tadalafil for symptomatic LUTS/BPH in men with T2DM

Over 50% of men over 50 have symptomatic BPH/LUTS and men with T2DM have a 32% increased risk.[38] Among the mechanisms involved in the pathology of BPH/LUTS, the Nitric oxide (NO)/cGMP pathway has an important functional role, and all key enzymes of this pathway, nitric oxide synthase, PKG-1 and phosphodiesterase 5 (PDE5) are expressed in the prostatic tissue. NO exerts a general inhibitory effect of muscle tone on the lower urinary tract. A decrease in NO-mediated relaxation of smooth prostatic muscle contributes to BPH/ LUTS pathology. Latest evidence on the pathophysiology of LUTS/BPE has provided the rationale for use of PDE5-Is: improvement of LUT oxygenation, smooth muscle relaxation, negative regulation of proliferation and trans-differentiation of LUT stroma, reduction of bladder afferent nerve activity, and down-regulation of prostate inflammation are the proven mechanisms of action of PDE5-Inhibitors.[39]

Head-to-head trials show equal efficacy for tadalafil compared with Tamsulosin, with clinically significant reduction of 5-6 IPSS points but greater patient preference for tadalafil, irrespective of improvements in ED.[40] A review by Hantzimouratidis *et al.* pointed out that head-to-head comparison in 12-week studies were unhelpful due to the different mechanism of action of the drugs, improvement of co-morbid ED and that long- term studies of disease progression are more relevant.[41] Donatucci showed that IPSS scores with tadalafil were still improving after 1 year of open label use and that improvements in erectile function were maintained.[42]

One RCT showed significant improvement with tadalafil 5mg daily in the bothersome symptom of post-micturition dribble, whereas Tamsulosin has proved ineffective.[43] Studies suggest a synergistic effect with tadalafil in combination with Tamsulosin, and, for larger prostates with Finasteride. A recent UK DATA-LINK study has suggested a possible 30% increased risk of T2DM in men taking 5-ARIs, and caution in their use in men with metabolic syndrome.[44]

A recent meta-analysis supported the rationale for tadalafil/finasteride in terms of preservation in sexual function in comparison with Tamsulosin/Finasteride, an effect likely to be more important in men with BPH and T2DM where multiple co-morbidities for ED are likely to be present.[45]

The case for tadalafil to prevent BPH progression in T2DM

Over two-thirds of men over 50 with T2DM suffer from some degree of LUTS as assessed by IPSS.[4] Alpha-blockers, as first line BPH therapy for decades, are more

likely to be associated with drug interactions and hypotension in men with T2DM, especially those on multiple anti-hypertensive regimes or those subject to hypoglycaemia. Older, frail men treated with alpha-blockers may be at greater risk of falls, especially related to increased micturition at night.[46]

Alpha-blockers have consistently shown to have no effect of BPH progression as sole therapy.[47] BPH progression has considerable health economic consequence that add to the huge economic burden of T2DM.[47] Long term data on prostate size and disease progression with tadalafil are required to address this question but a review by Gacci *et al.* suggests that improved endothelial dysfunction and reduction of inflammatory markers suggest optimism that daily tadalafil might reduce BPH progression.[48] As 75% of men with T2DM are likely to suffer ED and ejaculatory problems due to vascular and neuropathic complications of T2DM, studies reporting "volunteered" sexual complications are highly likely to underestimate the true impact of both alpha-blockers and finasteride. A recent Canadian meta-analysis of 175,000 men treated for BPH showed 22% increased risk of heart failure in men taking alpha-blockers, especially non-selective drugs. This may be more important in men with T2DM and existing cardiovascular risk factors.[49] The MSAM-7 study clearly showed the close association of LUTS severity with lower IIEF scores.[38]

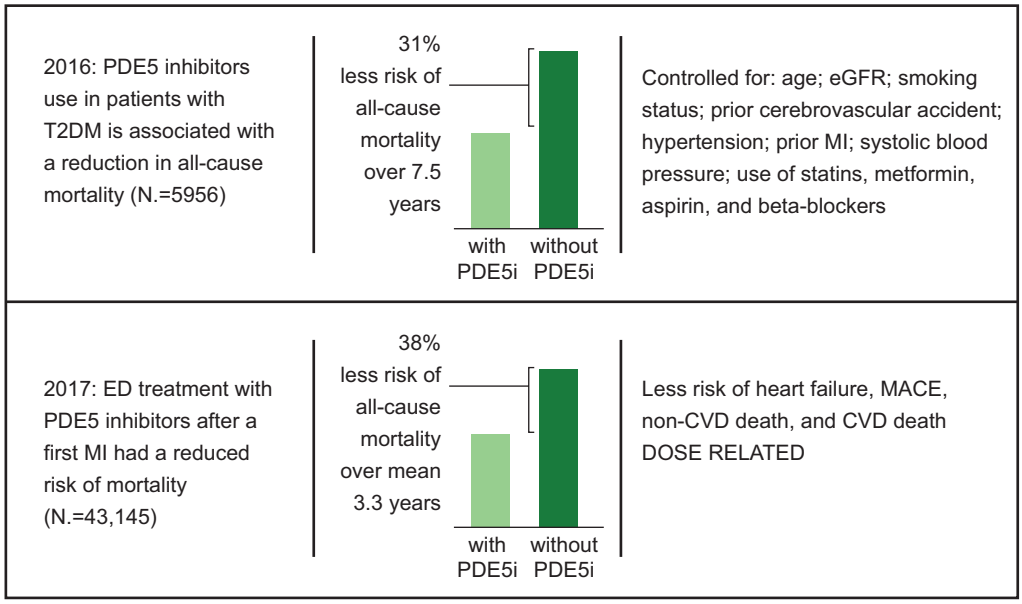
Evidence that PDE5 inhibitors might reduce diabetes related morbidity and mortality

PDE5 inhibitors were developed as daily therapy to treat cardiovascular disease and improvements in ED were an incidental finding during clinical trials.[3] Sildenafil and tadalafil are licensed to treat Pulmonary Hypertension through their beneficial effects on endothelial dysfunction.[3]

Several Cardiology reviews have highlighted the beneficial cardiovascular effects that would potentially reduce cardiovascular events in high-risk populations such as men with type 2 diabetes.[50] Beneficial mechanisms include improved endothelial function, enhanced cGMP and cAMP activity to counterbalance hypertrophic and pro-apoptotic signalling and enhanced post-ischaemic reperfusion. In vitro experiments suggest likely benefits in patients with heart failure.[51] These mechanisms are highlighted in Figure 13.2. Currently tadalafil 5 mg is the only PDE5 inhibitor suitably licensed for daily use.

Anderson *et al.* who followed a UK primary care population of 5956 UK men with T2DM over 6.9 years. A 31% reduction in all-cause mortality and 26% reduction in MI were reported in men taking PDE5 inhibitors. Only 22.8% of men with T2DM had been prescribed a PDE5I, with patients usually restricted to once per week.[52] These findings were supported by Hackett *et al.* in a prospective RCT of testosterone therapy in T2DM. The 175 men taking PDE5 inhibitors showed a significant and independent reduction in all- cause mortality.[53]

Figure 13.2. PDE5 inhibitors and reduced CV events.



MACE = major adverts cardiac events.

Andersson *et al.* reported data from a Swedish database of 43,415 men after first myocardial infarction for 5 years and found significant reduction in all-cause and cardiovascular mortality and 30% reduction in new diagnosis in heart failure and related admissions, in men prescribed PDE5 inhibitors. The benefits were greater in men on more frequent dosing of PDE5 inhibitors and were not seen with other ED therapies.[54]

Scranton *et al.* carried out a complex health care review and concluded that diagnosis and successful treatment of concomitant ED may promote improved adherence and management of comorbid diseases. Concomitant ED management may improve treatment outcome, decrease healthcare costs, and possibly prevent or even improve deterioration in medical conditions comorbid with ED.[55]

Prevention of diabetic peripheral neuropathy

Diabetic peripheral neuropathy (DPN) occurs in approximately 30% of men with T2DM and currently the mainstay of prevention is through tight glycaemic control. There are numerous case reports of improvement in neuropathic pain and paraesthesia with PDE5Is. Nitric oxide is the major neurotransmitter of the vasa nervorum, suggesting an important preventive role in microvascular complications. Currently, drugs used to treat established DPN effectively block pain pathways and frequently aggravate ED. There is huge potential for savings by the prevention of complications of DPN.[56, 57]

Diabetic nephropathy

The multiple potential benefits of PDEIs in renal disease are summarised in Figure 13.3 taken from Afsar *et al.*[58]

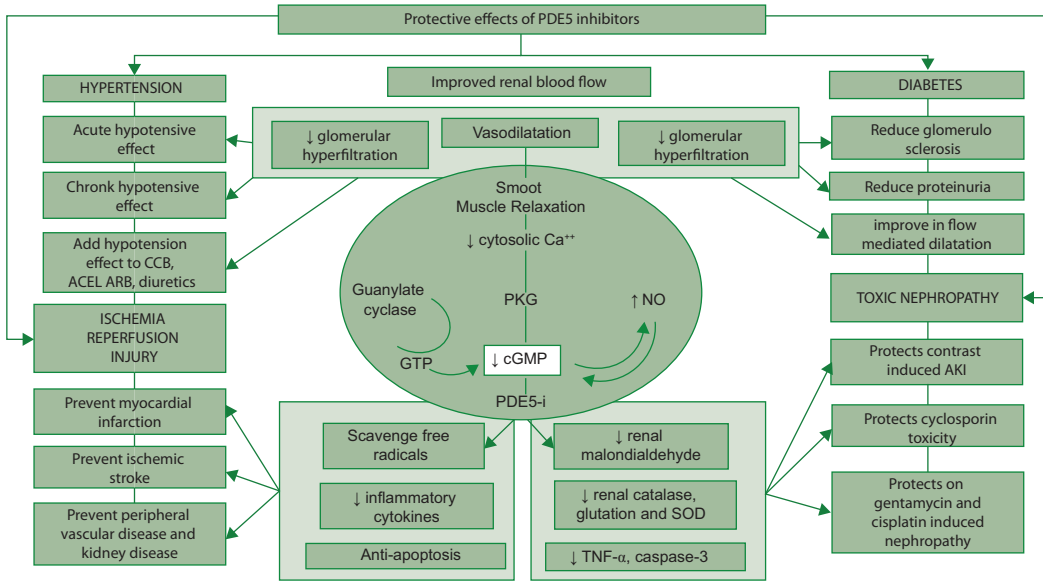
Cancer prevention

T2DM is associated with increased risk of many cancers, particularly colon. Review articles suggested a role for PDE5 inhibitors in cancer prevention.[59] In a Swedish national database study, Huang *et al.* studied a total of 4823 patients prescribed PDE5 inhibitors during the study period; the incidence rate of CRC was 2.64 per 1000 person-years for men prescribed PDE5 inhibitors compared with 4.46 per 1000 person-years for men without a prescription. They found a significant negative association between PDE5 inhibitor use and risk of colorectal cancer (adjusted HR, 0.65; 95% CI, 0.49-0.85); the decreased risk was associated with an increased cumulative dose of PDE5 inhibitors (P=0.003).

Cognitive improvement

Choi *et al.* treated 30 men with ED and mild cognitive impairment with tadalafil 5mg for 8 weeks. Mean baseline IIEF and Montreal Cognitive Assessment (MoCA) scores were 7.52±4.84 and 18.92±1.78. After the eight-week treatment, mean IIEF

Figure 13.3. Multiple potential benefits of PDE5 inhibitors in renal disease.



and MoCA scores were increased to 12.92±7.27 (P<0.05) and 21.8±1.71 (P<0.05), respectively. Patients showed increased relative regional CBF in the postcentral gyrus, precuneus, and brainstem after tadalafil administration *versus* at baseline (P<0.001).[60]

Depression

Depression is twice as common in men with T2DM with prevalence of 25%[61] and sexual disfunction is the complication of T2DM most closely linked with depression. Both ED and LUTS are linked with depression.[62] Nurnberg *et al.*[63] showed that depression score improved when men with ED and moderate depression were treated with sildenafil *versus* placebo. Shim *et al.*[64] conducted a 2-month placebo controlled study in 60 men with ED and with no known cognitive impairment.The changes in the PHQ-9 and PHQ-15 were 2.04±3.14 and 2.17±2.87 with the PDE5 inhibitor, udenafil given daily, and 1.20±1.63 and 0.56±2.48 in the placebo group (both, P<0.001 for udenafil). The authors concluded that daily dosing with a PDE5 inhibitor seems to improve cognitive function, depression and somatization, as well as erectile function, in patients with ED.

COVID-19 infection

Recently there has been interest that endothelial dysfunction is an important factor in the inflammatory process involved in acute COVID-19 infection. It is suggested that increasing nitric oxide by using tadalafil 5mg daily, might mitigate this process, especially in the respiratory tissues (Figure 13.1).[65]

Premature ejaculation

Two studies have produced positive responses in PE, with a 3- fold increase in intra-vaginal ejaculatory time, improving over 3 months.[66, 67] On-demand drugs have produced variable results. As PE often occurs in conjunction with ED, and LUTS, this may be a useful clinical effect, as SSRIs are often used but are associated with unwanted sexual side-effects. A large RCT on tadalafil in PE is due to report soon.

Conclusions

Prescribing policies for T2DM are largely decided by diabetes specialists, with priorities given to glycaemic control. Co-morbid conditions such as ED and BPH are usually managed by urologists. The recognition of ED as an independent risk factor for CHD justifies inclusion in routine risk reduction strategies. Guidelines often suggest "consider measuring testosterone if clinically indicated", especially when sexual histories are still not taken in routine diabetes practice. As ED affects over 75% and hypogonadism over 40%, accurate assessment of both these issues should

be mandatory in routine diabetes care. Evidence that PDE5 inhibitors produce benefits through multiple mechanisms, including a rise in testosterone level (with daily medication) now supports routine prescribing in diabetes care, not least because the patients will potentially experience symptomatic improvements that will increase compliance with other prescribed interventions.

Even despite being licensed for both BPH and ED and recommended as first line therapy for both conditions by the European Urology association, NICE in the UK chooses to classify daily tadalafil as "a drug of limited clinical value"!!!

References

1. American Diabetes Association. Statistics about Diabetes. [Internet]. Available from: http://www.diabetes.org/ diabetes-basics/statistics/ [cited 2018, April 1].
2. Stratton IR, Adler AI, Neil HAW, *et al.* Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405.
3. NICE. Type 2 diabetes in adults: management. 2015 [Internet]. Available from: https://www.nice.org.uk/guidance/ng28 (accessed 30/03/21)
4. Kirby M, Chapple C, Jackson G, *et al.* Erectile dysfunction and lower urinary tract symptoms: a consensus on the importance of co-diagnoses. Int J Clin Pract 2013;67:606-18.
5. Guidelines live. Diagnosis, management, and referral of men with lower urinary tract symptoms due to benign prostatic hyperplasia. [Internet]. Available from: https://www.guidelines.co.uk/mens-health/management-of-bph-with-luts-guideline/453746.article
6. Kloner RA, Jackson G, Emmick JT, *et al.* Interaction between the phosphodiesterase 5 inhibitor, tadalafil and 2 alpha-blockers, doxazosin and tamsulosin in healthy normotensive men. J Urol. 2004 Nov;172(5 Pt 1):1935-40.
7. Lusty A, D. Siemens DR, Tohidi M. Cardiac Failure Associated with Medical Therapy of Benign Prostatic Hyperplasia: A Population Based Study THE JOURNAL OF UROLOGY0022-5347/21/2055-1430/0 Vol. 205, 1430-1437, May 2021
8. European Association of Urology. Management of Non-neurogenic Male LUTS. [Internet]. Available from: www.EAU-Guidelines-on-the-Management-of-Non-Neurogenic-Male-LUTS-2019.pdf [accessed 2019, December 14].
9. Hackett G, Kirby M, Wylie K, *et al.* The BSSM Guidelines on Erectile Dysfunction. J Sex Med 2018;15:430-57.
10. Mulhall JP, Trost LW, Brannigan RE, *et al.* Evaluation and Management of Testosterone Deficiency: AUA Guideline. J Urol 2018;200:423-32.
11. American Diabetes Association. Low Testosterone. [Internet]. Available from: https://www.diabetes.org/resources/men/low-testosterone
12. The American Association of Clinical Endocrinologists and American College of Endocrinology. Guidelines on the care of patients with Obesity. 2017 [Internet]. Available from: https://www.aace.com/publications/guidelines (Accessed 2019, February 17).

13. Hackett G, Kirby M, Edwards D, *et al.* British Society for Sexual Medicine Guidelines on Adult Testosterone Deficiency, With Statements for UK Practice. J Sex Med 2017;14:1504-23.
14. Hippisley-Cox J, Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. BMJ 2017;357:j2099.
15. Welcome to the QRISK®3-2018 risk calculator. [Internet]. Available from: https://qrisk.org/three
16. Yusuf S, Hawken S, Ounpuu S, *et al.* Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937-52.
17. Rosano GM, Aversa A, Vitalea C, *et al.* Chronic treatment with tadalafil improves endothelial function in men with increased cardiovascular risk. Eur Urol 2005;47;214-22.
18. Amano T, Earle C, Imao T, *et al.* Administration of daily 5 mg tadalafil improves endothelial function in patients with benign prostatic hyperplasia. Aging Male 2018;21:77-82.
19. Ramirez CE, Nian H, Yu C, *et al.* Treatment with sildenafil improves insulin sensitivity in prediabetes: a randomized, controlled trial. J Clin Endocrinol Metab 2015;100:4533-40.
20. Santi D, Granata AR, Guidi A. Six months of daily treatment with vardenafil improves parameters of endothelial inflammation and of hypogonadism in male patients with type 2 diabetes and erectile dysfunction: a randomized, double-blind, prospective trial. Eur J Endocrinol 2016;174:513-22.
21. Aversa A, Greco E, Bruzziches R, *et al.* Relationship between chronic tadalafil administration and improvement of endothelial function in men with erectile dysfunction: a pilot study. Int J Impot Res 2007;19:200-7.
22. Santi D, Giannetta E, Isidori A, *et al.* Effects of chronic use of phosphodiesterase inhibitors on endothelial markers in type 2 diabetes mellitus: a meta-analysis. Eur J Endocrinol 2015;172:R103-14.
23. Lee JW, Park HJ, Park NC. Serum High-Sensitivity C-Reactive Protein Levels and Response to 5 mg Tadalafil Once Daily in Patients with Erectile Dysfunction and Diabetes. Korean J Urol 2013;54:858-64
24. Schwartz BG, Jackson G, Stecher VJ, *et al.* Phosphodiesterase Type 5 Inhibitors Improve Endothelial Function and May Benefit Cardiovascular Conditions. Am J Med 2013;126:192-9.
25. Hellstrom W, Gittelman M, Jarow J, *et al.* An evaluation of semen characteristics in men 45 years of age or older after daily dosing with tadalafil 20mg: results of a multicenter, randomized, double-blind, placebo-controlled, 9-month study. Eur Urol 2008;53:1058-65.
26. Oscan L, Polat EC, Kocaaslan R, *et al.* Effects of taking tadalafil 5 mg once daily on erectile function and total testosterone levels in patients with metabolic syndrome. Andrologia 2017;49.
27. Spitzer M, Basaria S, Travison TG, *et al.* Effect of testosterone replacement on response to sildenafil citrate in men with erectile dysfunction: a parallel, randomized trial. Ann Intern Med 2012;157:681-91.

28. Park HJ, Hyan JS, Park NC, A comparison of baseline erectile dysfunction after Tadalafil 20mg v Tadalafil 5mg in men with erectile dysfunction and type 2 diabetes: a 2-year observational study. Eur Urol Supp 2014:13;e602.

29. Buvat J, Hatzichristou D, Boess FG, *et al.* Continuation and effectiveness of tadalafil once daily during a 6-month observational study in erectile dysfunction: the EDATE study Int J Clin Pract 2014;68:1087-99.

30. Brock G, Ni X, Oelke M, *et al.* Efficacy of continual dosing with tadalafil once daily version on-demand in clinical subgroups of men with erectile dysfunction; A descriptive comparison using integrated tadalafil databases. J Sex Med 2016;13:860-75.

31. Bansal UK, Jones C, Fuller TW, *et al.* The Efficacy of Tadalafil Daily vs on Demand in the Treatment of Erectile Dysfunction: A Systematic Review and Meta-analysis. Urology 2018;112:6-11.

32. Peng Z, Yang L, Dong Q, *et al.* Efficacy and Safety of Tadalafil Once-a-Day *versus* Tadalafil On-Demand in Patients with Erectile Dysfunction: A Systematic Review and Meta-Analyses. Urol Int 2017;99:343-52.

33. Prasetyo DT, Raharja PAR, Mantiri BJ, *et al.* Tadalafil Once a Day for Men with Erectile Dysfunction: Is It Superior than On-Demand Administration? Acta Med Indones 2019;51:275-81.

34. Zhou Z, Chen H, Wu J, *et al.* Meta-analysis of the long-term efficacy and tolerance of tadalafil daily compared with tadalafil on-demand in the treatment of men with erectile dysfunction. Sex Med 2019;7:282-91.

35. Shafik A, Shafik I, El Sibai O, *et al.* On the pathogenesis of penile venous leakage: role of the tunica albuginea. BMC Urol 2007;7:14.

36. North Yorkshire County Council's (NYCC) Smoking Cessation Preferred Formulary. Available from: http://gmmmg.nhs.uk/docs/cost_comparison_charts.pdf [accessed 2019, December 23].

37. Koskimäki J, Shiri R, Tammela T, *et al.* Regular intercourse protects against erectile dysfunction: Tampere Aging Male Urologic Study. Am J Med 2008;121:592-6.

38. Rosen R, Altwein J, Boyle P, *et al.* Lower urinary tract symptoms and male sexual dysfunction: the multinational survey of the aging male (MSAM-7). Eur Urol 2003;44:637-49.

39. Andersson KE. PDE5 inhibitors – pharmacology and clinical applications 20 years after sildenafil discovery. Br J Pharmacol 2018;175:2554-65.

40. Pogula VR, Kadiyala LS, Gouru VR, *et al.* Tadalafil vs. tamsulosin in the treatment of lower urinary tract symptoms secondary to benign prostatic hyperplasia: a prospective, randomized study. Cent European J Urol 2019;72:44-50.

41. Hatzimouratidis K. A review of the use of tadalafil in the treatment of benign prostatic hyperplasia in men with and without erectile dysfunction Ther Adv Urol 2014;6:135-47.

42. Roehrborn CG, Chapple C, Oelke M, *et al.* Effects of Tadalafil Once Daily on Maximum Urinary Flow Rate in Men with Lower Urinary Tract Symptoms Suggestive of Benign Prostatic Hyperplasia. J Urol 2014;191:1045-50.

43. Yang DY, Jeong HC, Ko K, *et al.* Effect of tadalafil 5 mg on post-micturition dribble in men with lower urinary tract symptoms: a multicentre, double-blind, randomized, placebo-controlled trial. BJU Int 2019;124:862-9.

44. Oelke M, Giuliano F, Mirone V, *et al.* Monotherapy with tadalafil or tamsulosin similarly improved lower urinary tract symptoms suggestive of benign prostatic hyperplasia in an international, randomised, parallel, placebo-controlled clinical trial. Eur Urol 2012;61:917-25.

45. Olesovsky C, Kapoor A. Evidence for the efficacy and safety of tadalafil and finasteride in combination for the treatment of lower urinary tract symptoms and erectile dysfunction in men with benign prostatic hyperplasia Ther Adv Urol 2016;8:257-71.

46. Schimke L, Schinike J. Urological Implications of Falls in the Elderly: Lower Urinary Tract Symptoms and Alpha-Blocker Medications. Urol Nurs 2014;34:223-9.

47. Marberger M. The MTOPS Study: New Findings, New Insights, and Clinical Implications for the Management of BPH. European Urology Supplements 2006;5:628-33.

48. Gacci M, Andersson KE, Chapple C, *et al.* Latest Evidence on the Use of Phosphodiesterase Type 5 Inhibitors for the Treatment of Lower Urinary Tract Symptoms Secondary to Benign Prostatic Hyperplasia. Eur Urol 2016;70:124-33.

49. Lusty A, Siemens DR, Tohidi M, *et al.* Cardiac failure associated with medical therapy for benign prostatic hypertrophy – a population-based study. J Urol 2021;205:1430-7.

50. Pofi R, Gianfrilli D, Badagliacca R, *et al.* Everything you ever wanted to know about phosphodiesterase 5 inhibitors and the heart (but never dared ask): How do they work? J Endocrinol Invest 2016;39:131-42.

51. Cai Z, Zhang J, Li H. Two Birds with One Stone: Regular Use of PDE5 Inhibitors for Treating Male Patients with Erectile Dysfunction and Cardiovascular Diseases. Cardiovasc Drugs Ther 2019;33:119-28.

52. Anderson S, Hutchings DC, Woodward M, *et al.* Phosphodiesterase type-5 inhibitor use in type 2 diabetes is associated with a reduction in all-cause mortality. Heart 2016;102:1750-6.

53. Hackett G. Heald AH, Sinclair A, *et al.* Serum Testosterone, Testosterone Replacement Therapy and All- cause Mortality in men with Type 3 Diabetes: Retrospective Consideration of the impact of PDE5 Inhibitors and Statins. Int J Clin Pract 2016;70:244-53.

54. Andersson DP, Lagerros YT, Grotta A, *et al.* Association between treatment for erectile dysfunction and death or cardiovascular outcomes after myocardial infarction. Heart 2017;103:1264-70.

55. Scranton RE, Goldstein I, Stecher VJ. Erectile dysfunction diagnosis and treatment to improve medication adherence and optimize comorbidity management. J Sex Med 2013;10:551-61.

56. Hackett G. PDE5 inhibitors in diabetic peripheral neuropathy. Int J Clin Pract 2006;60:1123-6.

57. Sairam K, McNicholas T. Sildenafil in diabetic peripheral neuropathy. Br J Diabetes Vasc Dis 2002;2:304.

58. Afsar B, Ortiz A, Covic A, Phosphodiesterase type 5 inhibitors and kidney disease. Int Urol Nephrol 2015; 47:1521-8.

59. Huang W, Sundquist J, Sundquist K, *et al.* Use of Phosphodiesterase 5 Inhibitors Is Associated with Lower Risk of Colorectal Cancer in Men with Benign Colorectal Neoplasms. Gastroenterology 2019;157:672-81.

60. Choi JB, Cho KJ, Kim JC, *et al.* The Effect of Daily Low Dose Tadalafil on Cerebral Perfusion and Cognition in Patients with Erectile Dysfunction and Mild Cognitive Impairment. Clin Psychopharmacol Neurosci 2019;17:432-7.

61. Urios A, Ordoño F, García-García R, *et al.* Tadalafil Treatment Improves Inflammation, Cognitive Function, And Mismatch Negativity of Patients with Low Urinary Tract Symptoms and Erectile Dysfunction. Sci Rep 2019;9:17119.

62. Corona G, Giorda CB, Cucinotta D, *et al.* Sexual dysfunction at the onset of type 2 diabetes: the interplay of depression, hormonal and cardiovascular factors. J Sex Med 2014;11:2065-73.

63. Nurnberg HG, Seidman SN, Gelenberg AJ, *et al.* Depression, antidepressant therapies, and erectile dysfunction: clinical trials of sildenafil citrate (Viagra) in treated and untreated patients with depression. Urology 2002;60(2 Suppl 2):58-66.

64. Shim YS, Pau C-U, Kim SW, Effects of repeated dosing with Udenafil (Zydena) on cognition, somatization and erection in patients with erectile dysfunction: a pilot study Int J Impot Res 2011;23:109-14.

65. Dal Moro F, Vandramin I, Livi U. The war against SARS Cov2, better to fight it or mitigate it? Med Hypothese 2020;14:110129.

66. Abu El-Hamd M. Efficacy and safety of daily use of tadalafil in treatment of patients with premature ejaculation: A randomised placebo-controlled clinical trial. Andrologia 2018;50:e13005.

67. Ozcan L, Polat EC, Onen E, *et al.* Effects of Tadalafil 5 mg Dosed Once Daily in Men with Premature Ejaculation. Urol Int 2017;98:210-4.

14

Hypogonadism and fertility: alternative therapies

Rowland W. Rees

The two key roles of the testes are to produce testosterone and spermatogenesis, and not infrequently men of reproductive age with hypogonadism may have impaired spermatogenesis, reduced semen parameters and difficulty conceiving.

Male factor infertility contributes to up to 50% of couples failing to conceive, and the majority are due to impairment of spermatogenesis. This can be due to a primary testicular problem, or alternatively an impairment of the Hypothalamic-Pituitary-Gonadal (HPG) endocrine axis. Thus, a proportion of men with subfertility may have an associated low testosterone level, and vice versa. Causes of central hypogonadism include Kallmann syndrome, disease or injury to the pituitary gland, drug induced (*e.g.* opioids, glucocorticoids or anabolic androgenic steroids), as well as obesity, ageing and the metabolic syndrome.

Given that symptoms of low testosterone include reduced libido, erectile dysfunction, fatigue, and depression, it follows that some sub-fertile men wishing to conceive may also require optimisation of their testosterone levels.

Although testosterone replacement therapy is the standard treatment for hypogonadism, it has the disadvantage of supressing spermatogenesis, and thus should not be used in patients who wish to have children in the foreseeable future.[1] This chapter discusses the alternative methods of increasing endogenous testosterone production without the requirement to administer exogeneous testosterone by means of testosterone replacement therapy.

Conservative treatment

Gonadal function can be optimised with changes in lifestyle. In particular, there may be an opportunity to increase testosterone levels and improve symptoms without the need for pharmacological treatment. There is a well-established association between body mass index (BMI) and both total serum testosterone levels and semen parameters. The Massachusetts Male Ageing Study reported that an increase in BMI of 4 kg/m^2 was associated with a similar decline in testosterone levels to that seen after 10 years of ageing,[2] and conversely the European Male Ageing Study showed a

significant increase in total (but not free) testosterone levels with only 5% weight loss.[3] In a meta-analysis by Corona *et al.*, both bariatric surgery and a low-calorie diet were associated with significant increases in testosterone levels, with bariatric surgery showing the greatest increases.[4]

Hypogonadism is also associated with a number of drug therapies - including opioids, glucocorticoids and oestrogens, via their actions on the HPG axis. It is therefore important to review and minimise the dosing of these medications, in order to optimise testosterone and semen parameters.

Hypogonadism is also associated with hyperprolactinaemia, Type 2 diabetes, and other chronic diseases. It is important to treat underlying medical problems to optimise testosterone levels. Hyperprolactinaemia can result in suppression of GnRH secretion, and patients with central hypogonadism and low LH levels should have prolactin levels measured, and if raised, investigated for a pituitary prolactinoma. Elevated prolactin levels suppress the pulsatile secretion of GnRH, thus causing hypogonadism and infertility. The infertility can therefore be treated by a dopamine agonist such as Cabergoline, which can reduce the size of prolactin secreting tumours and normalise prolactin levels. De Rosa *et al.*[5] demonstrated normalisation of semen parameters using Cabergoline in this setting.

Selective estrogen receptor modulators (SERM's)

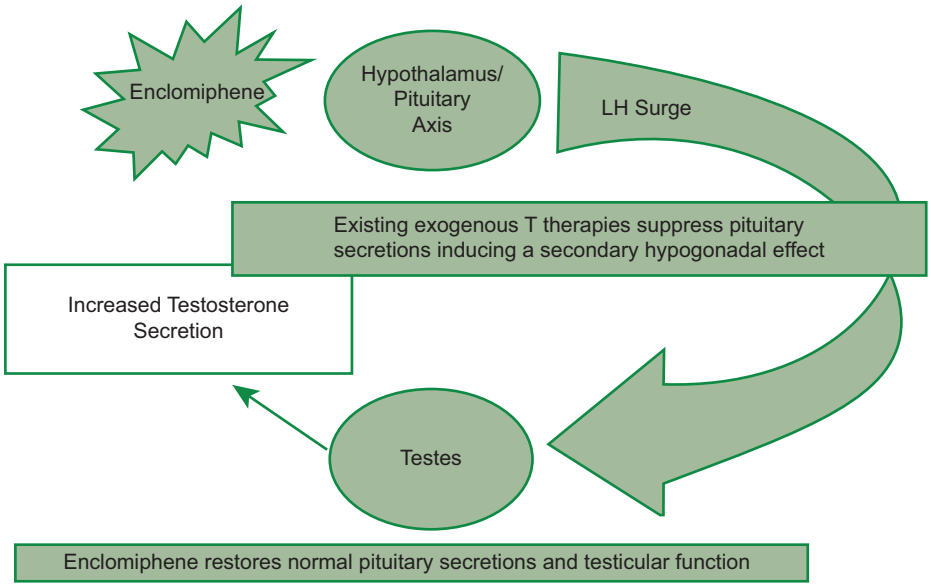
SERMs are a class of drug that act as antagonists to the oestrogen receptors in the central nervous system, acting at the level of the hypothalamus and pituitary. They prevent circulating oestrogen hormones from exerting negative feedback on the HPG axis. This results in an increased secretion of Luteinising Hormone (LH) and Follicle Stimulating Hormone (FSH) from the anterior pituitary, resulting in an increased production of endogenous testosterone.[6]

Clomiphene citrate is a SERM that has been used for ovulation induction therapy in infertile women since the 1960's, but is also used 'off-label' for male infertility at a dose of 25-50 mg daily, and is a simple, low-cost treatment with minimal side-effects. Tamoxifen, which is used extensively in the setting of breast cancer, is another 'off label' drug commonly used in this setting with a dose of 20-30 mg once daily. Enclomiphene, an isomer, is under development as a potential licensed product (Figure 14.1).

Chua *et al.*[7] and other studies demonstrate significant increases in testosterone levels with Clomiphene citrate therapy, but the effects on semen parameters and hypogonadal symptoms is less clear. A meta-analysis revealed a significant improvement in fertility rates with Clomiphene citrate.[8]

In terms of symptoms, a randomised control trial comparing the use of Clomiphene citrate to placebo in 78 obese patients showed no significant changes in the ADAM questionnaire, but this was a short study of only 12 weeks.[9] Whereas a retrospective age-matched comparison by Ramasamy *et al.*[10] showed that men on

Figure 14.1. How clomiphene and enclomiphene work.



Clomiphene citrate achieve similar testosterone levels to those on testosterone gels, along with similar satisfaction levels.[11]

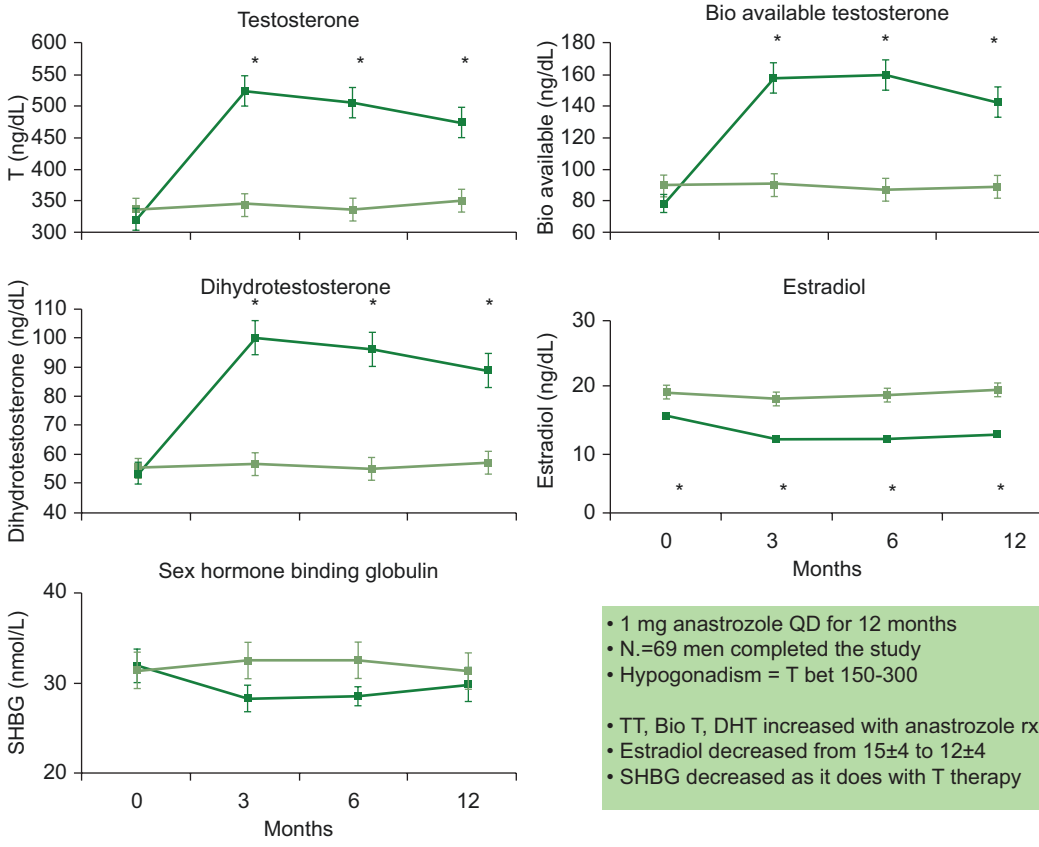
Gonadotrophins

For men with hypogonadotropic hypogonadism (secondary hypogonadism), treatment with exogenous gonadotrophins is an option for increasing testosterone levels.

Human chorionic gonadotrophin (hCG) is an analogue of LH, and therefore stimulates testosterone production from the testes through activity at the LH receptor. It can be given as an intramuscular or subcutaneous injection at doses of between 1000 IU and 3000 IU, 2 or 3 times per week. hCG therapy has been shown to significantly increase testosterone levels and improve hypogonadal symptoms,[11] and is also sufficient to induce spermatogenesis.[12] However, it may be necessary to supplement FSH (or human menopausal hormone (HMG), which contains both FSH and LH), which has a superior effect on sperm concentration.[13]

Pulsatile Gonadotrophin Releasing Hormone (GnRH) therapy requires the use of a continuous infusion pump to titrate the GnRH levels to mimic the pulsatile release of GnRH from the hypothalamus. Therefore, although first published as a therapy in 1979,[14] and the fact that it can induce spermatogenesis in up to 80% of men – the impracticality and expense of this treatment means it is not routinely used in clinical practice.

Figure 14.2. Effects of aromatase inhibition on bone mineral density and bone turnover in older men with low testosterone levels (from: Burnett-Bowie *et al.* J Clin Endo Metab 2009;94:4785-92).



Aromatase inhibitors

The aromatase inhibitors (AI) include Anastrazole, Testolactone and Letrozole, which increase endogenous testosterone levels by inhibiting the peripheral conversion of testosterone to oestradiol by the enzyme aromatase. This also reduces the negative feedback of oestrogens on the HPG axis, thereby increasing gonadotrophin levels.[15]

Aromatase inhibitors have been shown to raise testosterone levels in hypogonadal men and are orally administered. Dias *et al.*[16] showed significant increases in testosterone levels but noticed a lower bone mineral density in men treated with aromatase inhibitors, as compared to testosterone therapy. Burnett-Bowie *et al.* treated 69 men for 1 year with anastrazole 1mg daily for 12 months and found improvement in total, bioavailable and free testosterone with decrease in oestradiol and

SHBG. Once again there was a slight decrease in BMD.[17] This is presumably an effect of the lowering oestrogen levels. Further questions remain as to the effectiveness of these treatments on physiological parameters and symptoms, and no clear improvement in sexual symptoms, erectile function, body composition or muscle strength, has been reported.[18] Furthermore, in the female population where aromatase inhibitors are used for breast cancer, there has been a slight increase in venous thromboembolic events, and although no such association has been demonstrated in men, it is recommended that these medications are avoided in men with a history of venous thromboembolism. Due to the negative effects on bone mineral density the 'off label' use of aromatase inhibitors in male hypogonadism is currently limited.

Varicocele repair

Varicoceles have long been associated with male subfertility,[19] and varicocele treatment is associated with improvements in abnormal semen parameters.[20] Furthermore, both experimental and clinical studies have shown evidence for varicocele-related hypogonadism, and pathophysiological theories include hyperthermia or Leydig cell dysfunction.[21, 22] However, there is a lack of evidence to show that varicocele treatment in hypogonadal men translates into improvements in symptoms, and therefore varicocele treatment is only recommended for infertile men with a clinical varicocele and abnormal semen parameters. However, if such men were to have a lower testosterone level, they may see an improvement in testosterone levels following varicocele repair.

Case history

Richard (28) was previously, a fit athletic young man, who was treated twice, aged 20-22, with six-month courses of Roaccutane for severe acne. Within a few months of his second course, he complained to his GP of lack of energy and motivation, plus loss of libido and poor, almost absent erections. His work performance had suffered, and he had virtually no social life, with no girlfriend for 18 months. His GP diagnosed depression and treated with a course of Mirtazapine followed by a course of sertraline. He was offered 4 sildenafil tablets per month but did not see that as helpful. On arrival at the clinic, he was withdrawn and reluctant to communicate but physical examination was normal. He denied any use of anabolic steroids. Blood tests showed the following:

Total Testosterone 7.8 nmol/L, (8 nmol/L on rechecking), LH 1.8 IU/L. SHBG 32.

He was desperate for treatment as "he could not go on like this". He was warned about the possible effect on fertility and acne. He was treated with Testosterone Undecanoate (TU) 1000 mg every 10 weeks plus tadalafil 5 mg daily. Eight weeks later, TT was 25.8 and LH 0.4 IU/L. He was dramatically improved, but still lacked

the confidence for a relationship. He was commenced on hCG injections 1000 units twice per weekly in an attempt to maintain fertility. Four months later, he complained that although he felt much better, his libido had dipped, and his oestradiol was found to be raised at 269 pmol/L, so he was commenced on anastrozole 1 mg alternate days with his oestradiol falling to 68 pmol/L and his libido improving significantly.

Three years later, he remains on TU, hCG, anastrozole and tadalafil. He has met Sally, aged 28, who has 3 young children from a previous relationship, and they have been living together for over 12 months. He has been promoted at work and is lively and outgoing, working out 4 times per week at the gym. He has lost 4 cm from his waist and 14 pounds in weight. He feels "a different person" from his original visit. All his bloods, including LH and FSH are in the normal range. With 3 children under 10, he feels fertility as a minor issue. He is adamant that he wants to stay on this regime as his life has been transformed.

He is told that the use of hCG and anastrozole for these indications is "off label" and that his fertility and bone mineral density need to be reviewed on a regular basis.

Conclusions

Testosterone deficiency and subfertility often co-exist, and given the negative impact of exogenous testosterone on sperm production, there is often a need to look at alternative therapeutic approaches for men wishing to both start a family and see an improvement in their hypogonadal symptoms. There often co-exists negative lifestyle factors which can be modified in a safe and cost-free way, and therefore this should be the starting point. It is also important to manage underlying contributary co-morbidities and minimise the use of drugs that may interfere with the HPG axis.

Gonadotrophins such as hCG or FSH are effective in increasing testosterone levels and semen parameters, but are costly and require administration via injection. Pulsatile GnRH is a less attractive option due to both cost and the impracticality of a continuous infusion. The SERMs such as Clomiphene citrate have been shown to increase testosterone levels, though there is a lack of data regarding their impact on hypogonadal symptoms. The aromatase inhibitors may also raise testosterone levels, but are associated with reduced bone mineral density, and their use is therefore not recommended.

Further studies of Clomiphene and Aromatase inhibitors in conjunction with testosterone therapy are required to confirm whether these agents can be used in a synergistic way. This might mitigate the risk of adverse events of TRT in terms of reduced fertility and symptoms associated with increased oestradiol levels.

Although varicocele repair appears to improve testosterone levels in hypogonadism, further studies are required to clarify whether there is a corresponding improvement in symptoms.

References

1. Contraceptive efficacy of testosterone-induced azoospermia in normal men. World Health Organization Task Force on methods for the regulation of male fertility. Lancet 1990;336:955-9.
2. Travison TG, Araujo AB, Kupelian V, *et al.* The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. J Clin Endocrinol Metab 2007;92:549-55.
3. Camacho EM, Huhtaniemi IT, O'Neill TW, *et al.* Age-associated changes in hypothalamic-pituitary-testicular function in middle-aged and older men are modified by weight change and lifestyle factors: longitudinal results from the European Male Ageing Study. Eur J Endocrinol 2013;168:445-55.
4. Corona G, Rastrelli G, Monami M, *et al.* Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: a systematic review and meta-analysis. Eur J Endocrinol 2013;168:829-43.
5. De Rosa M, Ciccarelli A, Zarrilli S, *et al.* The treatment with cabergoline for 24 month normalizes the quality of seminal fluid in hyperprolactinaemic males. Clin Endocrinol (Oxf) 2006;64:307-13.
6. Surampudi P, Swerdloff RS, Wang C. An update on male hypogonadism therapy. Expert Opin Pharmacother 2014;15:1247-64.
7. Chua ME, Escusa KG, Luna S, *et al.* Revisiting oestrogen antagonists (clomiphene or tamoxifen) as medical empiric therapy for idiopathic male infertility: a meta-analysis. Andrology 2013;1:749-57.
8. Wheeler KM, Sharma D, Kavoussi PK, *et al.* Clomiphene Citrate for the Treatment of Hypogonadism. Sex Med Rev 2019;7:272-6.
9. Soares AH, Horie NC, Chiang LAP, *et al.* Effects of clomiphene citrate on male obesity-associated hypogonadism: a randomized, double-blind, placebo-controlled study. Int J Obes (Lond) 2018;42:953-63.
10. Ramasamy R, Scovell JM, Kovac JR, *et al.* Testosterone supplementation *versus* clomiphene citrate for hypogonadism: an age matched comparison of satisfaction and efficacy. J Urol 2014;192:875-9.
11. Liu PY, Wishart SM, Handelsman DJ. A double-blind, placebo-controlled, randomized clinical trial of recombinant human chorionic gonadotropin on muscle strength and physical function and activity in older men with partial age-related androgen deficiency. J Clin Endocrinol Metab 2002;87:3125-35.
12. Dwyer AA, Raivio T, Pitteloud N. Gonadotrophin replacement for induction of fertility in hypogonadal men. Best Pract Res Clin Endocrinol Metab 2015;29:91-103.
13. Warne DW, Decosterd G, Okada H, *et al.* A combined analysis of data to identify predictive factors for spermatogenesis in men with hypogonadotropic hypogonadism treated with recombinant human follicle-stimulating hormone and human chorionic gonadotropin. Fertil Steril 2009;92:594-604.
14. Happ J, Ditscheid W, Krause U. Pulsatile gonadotropin-releasing hormone therapy in male patients with Kallmann's syndrome or constitutional delay of puberty. Fertil Steril 1985;43:599-608.
15. Raman JD, Schlegel PN. Aromatase inhibitors for male infertility. J Urol 2002;167:624-9.

16. Dias JP, Shardell MD, Carlson OD, *et al.* Testosterone vs. aromatase inhibitor in older men with low testosterone: effects on cardiometabolic parameters. Andrology 2017;5:31-40.

17. Burnett-Bowie S, McKay EA. Effects of Aromatase Inhibition on bone Mineral Density and Bone Turnover in Older Men with Low Testosterone Levels. J Clin Endo Metab 2009;94:4785-92.

18. Helo S, Ellen J, Mechlin C, *et al.* A Randomized Prospective Double-Blind Comparison Trial of Clomiphene Citrate and Anastrozole in Raising Testosterone in Hypogonadal Infertile Men. J Sex Med 2015;12:1761-9.

19. The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. World Health Organization. Fertil Steril 1992;57:1289-93.

20. Agarwal A, Deepinder F, Cocuzza M, *et al.* Efficacy of varicocelectomy in improving semen parameters: new meta-analytical approach. Urology 2007;70:532-8.

21. Rajfer J, Turner TT, Rivera F, *et al.* Inhibition of testicular testosterone biosynthesis following experimental varicocele in rats. Biol Reprod 1987;36:933-7.

22. Andò S, Giacchetto C, Colpi G, *et al.* Physiopathologic aspects of Leydig cell function in varicocele patients. J Androl 1984;5:163-70.

15

Testosterone and the prostate

Rowland W. Rees

The relationship between testosterone and both the physiology and pathophysiology of the prostate gland is a complex one and is something that has been poorly understood for decades. Testosterone has several genomic and non-genomic routes of action, both directly via the androgen receptor, but also indirectly via dihydroxytestosterone (DHT) and oestradiol. On binding to DHT, the androgen receptor translocates to the nucleus, binds to its target genes and regulates their expressions. The androgen receptor can also be transactivated in the absence, or in very low levels, of DHT. Activating signals arise from several, non-mutually exclusive mechanisms, including extracellular peptides, such as insulin-like growth factor, epidermal growth factor and interleukin 6.

The molecular basis for Benign Prostatic Hyperplasia (BPH) and prostate cancer have not yet been fully clarified, and although it would appear that androgens are necessary for BPH, they are not necessarily causative. The development of the prostate gland is dependent on testicular androgens, and the exponential growth in prostate volume during puberty corresponds to a rise in serum testosterone to adult levels. However, after the human prostate reaches its normal adult size at around 18 years of age, its growth then halts despite a sustained circulating level of androgens in the adult range.

The transition from prostatic growth to a steady state phase after puberty is a result of a balance of cell proliferation and cell death and is controlled by androgen receptor signalling in the stromal and epithelial cells. Mean prostatic weight stabilises and remains fairly constant until the end of the fifth decade of life, at which point it begins to rise slowly, corresponding to the increase in BPH seen in the aging male.[1, 2]

Circulating androgens are primarily bound by proteins in the serum, but it is only androgens which are free of associations with proteins that are capable of diffusing into the cells of target tissue, allowing for a cellular response to circulating hormones.[3] However, sex hormones that are bound to sex hormone binding globulin (SHBG) have an increased half-life, allowing increases in androgen levels without necessarily causing hypertrophy of the reproductive organs.[4] Circulating

testosterone levels have been shown to reduce with age, while SHBG levels increase - meaning that free testosterone becomes less available. In parallel with this is the age-related increase in both BPH and prostate cancer.

When androgens reach the prostate tissue, three 5-alpha reductase isoenzymes convert testosterone to dihydrotestosterone (DHT). 5-alpha reductase (Type 2) is the most predominant in the prostate. There is evidence that DHT is required for complete development of the adult prostate, which is also dependent on androgen receptor expression and 5-alpha reductase.[2] The androgen receptors in the adult prostate are primarily expressed in the prostatic luminal epithelial cells, rather than stromal cells. *In vivo* modelling suggests that involution of prostate results from loss of androgen action on stromal rather than on epithelial cells.[5]

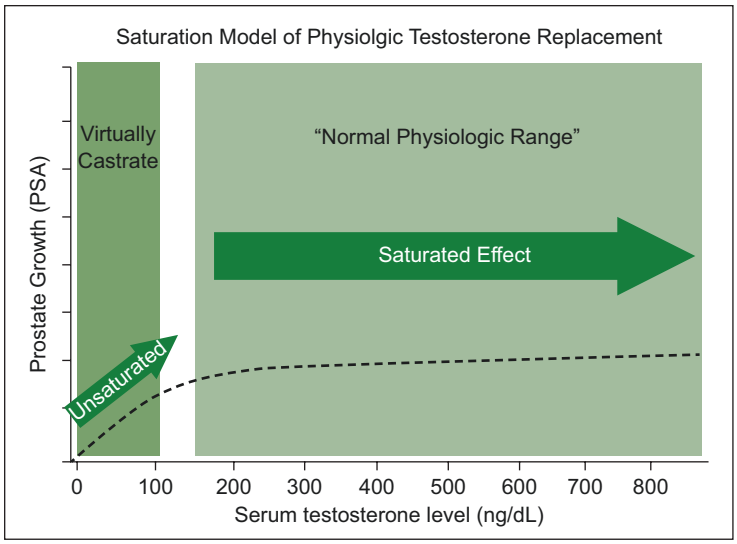
Testosterone regulates the nitric oxide-cyclic Guanosine Mono Phosphate (cGMP) pathway,[6] and testosterone deficiency is known to induce endothelial dysfunction, particularly during ageing. Vascular ageing is a chronic vascular inflammatory disease, associated with oxidative stress, reduced production of nitric oxide, and endothelial dysfunction.[7] Inflammation has been shown to correlate with prostate cancer aggressiveness and symptomatic benign prostatic hyperplasia, leading to the inflammatory theory for BPH.[8-11]

The effects of testosterone therapy on prostate tissue

Marks *et al.*[12] examined the effects of testosterone therapy (TT) *versus* placebo on 44 randomised hypogonadal men who had biopsies at baseline and at 6 months. Although serum testosterone rose to the mid-normal range in the treatment group, median testosterone and DHT levels did not change significantly. Furthermore, there was no treatment-related change in prostate histology, tissue markers, gene expression or cancer incidence. Furthermore, Cooper *et al.*[13] took 31 healthy volunteers and gave them up to 500 mg of testosterone weekly for 15 weeks, and although recorded an expected increase in serum testosterone levels, there was no significant change in prostate volume or serum PSA levels. A further study looking at supra-physiological doses of testosterone in a study designed to look at muscle mass and strength in normal men where 600 mg of testosterone was given weekly for 10 weeks *versus* placebo, there was again no statistically significant increase in neither PSA nor prostate volume.[14] In the BLAST study, Hackett *et al.*[15] randomised 200 diabetic hypogonadal men to receive testosterone or placebo over 30 weeks and detected only very minor increases in PSA of up to 0.5 ug/L.

Morgantaler promulgated the prostate saturation model based on a review of the evidence suggesting that although prostate growth is sensitive to variations in androgen concentration at very low levels, the prostate becomes insensitive to androgen concentration at higher levels – suggesting that the androgen receptors become saturated at serum testosterone levels well below the physiological range (Figure 15.1).[16] Rastrelli *et al.* further examined the relationship between PSA and

Figure 15.1. Prostate saturation model. (from: Morgentaler A, *et al*. Eur Urol 2008;55:310-20).[16]



testosterone levels, and found a saturation point at around 8nmol/L of total testosterone where the PSA levels reached a plateau. Furthermore, they suggested that a low PSA may be a predictor of hypogonadism.[17]

The effect of testosterone therapy on BPH-LUTS (Lower Urinary Tract Symptoms)

A number of studies have examined the effects of testosterone therapy on BPH-related symptoms, the International Prostate Symptom Score (IPSS), urodynamic parameters and prostate volume. Shigehara *et al.*[18] randomised men with BPH-LUTS into receiving either 250 mg of testosterone 4 weekly or no treatment and found a statistically significant improvement in symptom scores in those men treated with testosterone as compared to the control group. There was no difference in PSA values between the two groups, and although small differences were seen for urodynamic parameters, these were not statistically significant. Importantly there was no negative impact of TT on urinary symptoms. In a further study of 95 hypogonadal men treated with testosterone undecanoate for 12 months, there were no significant changes in prostate volume, but an improvement in symptom scores and post-void residual volumes.[19] The same group followed 656 men treated with TT for 8 years, and while the control group showed a progressive increase in urinary symptoms throughout the study period, the treatment group showed a sustained improvement in the IPSS score.[20] In summary, treating hypogonadism with TT is

not detrimental to prostate growth or urinary symptoms, and may produce relief from LUTS.

Testosterone levels and prostate cancer risk

Testosterone deficiency, or hypogonadism, and prostate cancer are both prevalent in the ageing male, and both have major health and Quality of life implications. The widespread use of PSA testing has increased the detection rate and numbers of men being treated for prostate cancer.

The work of Huggins and Hodges in the 1940's in men with metastatic prostate cancer established the androgen hypothesis that prostate cancer development and growth is directly related to a degree of androgenic activity in the body.[21] Their conclusion at the time was that prostate cancer is activated by androgens, and that raising serum androgens promotes malignant cell growth and disease progression. More recent evidence refutes this belief, but despite this there remains concern amongst medical professionals that TT may encourage the development of prostate cancer, even after cure.[22]

A number of studies have looked at the association between testosterone levels in men and their risk of developing prostate cancer. A study by the Endogenous Hormones and Prostate Cancer Collaborative group reviewed 18 prospective studies that included 3886 men with incident prostate cancer and 6438 control subjects and stratified their risk of developing prostate cancer according to their testosterone levels. They found no association between serum testosterone concentration, free testosterone, DHT levels or oestradiol and the risk of prostate cancer.[23] The European Prospective Investigation into Cancer and nutrition (EPIC) in 2007 found no difference in the odds ratios of prostate cancer according to men's baseline testosterone levels.

Some large prospective studies have associated an increased prostate cancer risk with low testosterone levels.[24-26] Furthermore, a low testosterone appears to be a risk factor for higher grade and stage of prostate cancer,[27-29] and a low free testosterone appears to predict an increased risk of reclassification to active treatment amongst men undergoing active surveillance for prostate cancer[30] (Table 15.I). Data from the laboratory appear to corroborate these findings, with studies of prostate cancer cell lines (LNCaP) treated with varying concentrations of testosterone shows that normal levels of androgen were required to inhibit proliferation, and very low levels of androgen are required for their initial growth.[31]

Testosterone therapy and prostate cancer risk

A number of studies have looked at whether the administration of TT alters the risk of developing prostate cancer. The SEER database was analysed, and of 52,579 men, 574 had received testosterone therapy and the remainder had not. No increased risk of prostate cancer was observed in the treatment group, and those who developed

Table 15.I. Low free testosterone levels predict disease reclassification in men with prostate cancer undergoing active surveillance (adapted from: San Francisco IF, *et al.*; 2014).[30]

- 154 men were followed with AS for prostate cancer
- 54 (35%) progressed to active treatment
- Men who progressed had significantly lower free testosterone levels than those who remained on AS (0.75 vs. 1.02 ng/dL, P=0.03)
- Free testosterone levels <0.45 ng/dL were associated with a seven-fold increase in the risk of disease progression (OR 4.3, 95% CI 1.25-14.73)
- Multivariate analysis demonstrated that free testosterone and family history of PCa were independent predictors of disease progression

prostate cancer were not at risk of higher-grade disease.[32] The European RHYME registry of 999 hypogonadal men, where 750 men received TT and 249 did not, showed a prostate cancer incidence of 2.4% in the treatment group and 2.8% in the non-treatment group over 3 years. There was no increased incidence of BPH or any differences in IPSS score either.[33]

In a review of the Swedish National Prostate Cancer Registry there were 1662 men receiving testosterone replacement therapy, but there is no association between therapy and overall prostate cancer risk, and men on testosterone therapy had a lower risk of high-grade prostate cancer (OR 0.5).[34] Lastly in a meta-analysis of 19 randomised control trials, including over 1000 patients comparing testosterone therapy *versus* placebo, no statistically significant difference was seen in the rate of PSA rise, prostate biopsy rates or prostate cancer incidence.[35]

Testosterone therapy in men with treated prostate cancer

Given the high incidence of hypogonadism in men presenting with and treated for prostate cancer, this raises the question as to whether it is safe to treat men who have been successfully cured of their prostate cancer with TT to alleviate their symptoms of hypogonadism. Also given the association between a low testosterone level and the development of prostate cancer, as well as the association between low testosterone levels and tumour grade, this also raises the question as to what effect would testosterone therapy have on the recurrence rates and survival of men who have been treated for prostate cancer.

In 2013, Pastuzak reviewed 103 hypogonadal patients following radical prostatectomy that had been treated with testosterone therapy, in comparison to 49 control subjects (Table 15.II).[36] The biochemical recurrence rate in the testosterone therapy group was 4%, *versus* 15% in the control group, and this despite a higher rate of high-risk disease in the eugonadal control group in the first place.

Table 15.II. Low T increases prostate cancer risk.

References	Number of patients	Study type	Endogenous TTh level	CaP outcomes
Morgentaler *et al.*	77	Retrospective	T<300 ng/dl or free T <1.6 ng/dl	CaP incidence of 14% (11/77)
Mearini *et al.*	206	Prospective	≤2.4 ng/mL ≤0.5 ng/mL	14.2% of patients had clinically locally advanced or metastatic CaP, and 57.1% have a pathological locally advanced CaP
Shin *et al.*	568	Prospective	<3.85 ng/mL	CaP incidence 38.0% (*vs.* 29.5% high testosterone group)
Karamanolakis *et al.*	718	Prospective	<3.0 ng/mL	CaP incidence 30% (29/97)
Morgantaler *et al.*	345	Retrospective	<250 ng/dL	CaP incidence 21% (*vs.* 12% in men with T>250 ng/dL)
Hoffman *et al.*	117	Retrospective	T<300 ng/dL or free T<1.5 ng/dL	CaP incidence 43% (*vs.* 22%)
Garcia-Cruz *et al.*	137	Prospective	<346 ng/dL	Tumor burden 53% (*vs.* 32% in men with T>346 ng/dL); tumor bilaterality 50% (*vs.* 25.5% in men with T>346 ng/dL)
Isom-Batz *et al.*	326	Retrospective	<385 ng/dL	Associated with advanced pathological stage (OR 2.3, 95% CI 1.1-5.0; P=0.03)
Lane *et al.*	455	Prospective	<220 ng/dL	Higher frequency of Gleason 4-5 disease (OR 2.4, 95% CI 1.01-5.7; P=0.48)
Botto *et al.*	431	Prospective	<3 ng/mL	Higher frequency of Gleason 4 disease (47% *vs.* 28%)
Salonia *et al.*	673	Prospective	Total T <1 ng/mL	Higher incidence of seminal vesicle invasion (OR 3.11)
Teloken *et al.*	64	Retrospective	<2.7 ng/mL	Increased positive surgical margins (P=0.026)

Pastuszak AW, Rodriguez KM, Nguyen TM, *et al.* Transl Androl Urol 2016.

In a more recent study by Ahlering *et al.*,[37] 850 patients undergoing radical prostatectomy had their testosterone levels measured pre-operatively. 152 (18%) had a low free testosterone level, and were placed on TT, and matched with 419 control patients with similar stage and grade disease. At 3.5 years, 7.2 % of the treated group had reached biochemical recurrence, compared to 12.6% in the control group. This led the authors to conclude that TT reduces biochemical recurrence after radical prostatectomy, though clearly randomised controlled trials are required to investigate this further.

The same investigators reviewed 98 men following radiotherapy treatment for prostate cancer, all of whom received testosterone therapy. There were very minor changes in PSA, and 6.1% of the cohort developed biochemical recurrence at 41 months - a

lower biochemical recurrence rate than previously reported for radiation therapy.[38] In a more recent systematic review of 36 articles and 2459 testosterone-treated patients, the quality of the overall evidence was found to be poor, but in conclusion TT-treated patients were not found to have increased risk of disease progression compared to untreated patients, though TT may be harmful in men with advanced disease.[39]

The evidence for use of TT during active surveillance of men known to have prostate cancer is more controversial, and of the 6 non-randomised studies reviewed in the above meta-analysis, 2 of the studies imply that testosterone might have harmful effects on the prognosis of patients with active surveillance.

A recent study investigated whether bipolar androgen therapy (BAT), involving rapid cyclic administration of high-dose testosterone, as a novel treatment for metastatic castration-resistant prostate cancer (mCRPC) promotes improvements in body composition and associated improvements in lipid profiles and quality of life.[40] This involved men from two completed trials with computed tomography imaging at baseline and after three cycles of BAT were included. Cross-sectional areas of psoas muscle, visceral and subcutaneous fat were measured at the L3 vertebral level. Functional Assessment of Chronic Illness Therapy - Fatigue questionnaire (FACIT) and 36-item short-form health survey were used to assess quality of life. The 60 included patients lost a mean (sd) of 7.8 (8.2)% of subcutaneous fat, 9.8 (18.2)% of visceral fat, and gained 12.2 (6.7)% muscle mass. Changes in subcutaneous and visceral fat were positively correlated with each other independent of the effects of age, body mass index, and duration of androgen-deprivation therapy. Energy, physical function, and measures of limitations due to physical health were all significantly improved at 3 months. The improvements in body composition were not correlated with decreases in lipid levels or observed improvements in quality of life. The authors concluded that BAT was associated with significant improvements in body composition, lipid parameters, and quality of life. This has promising implications for the long-term health of men with mCRPC.

Conclusions

This chapter reviews the effects of Testosterone therapy on prostate tissue and explains the saturation effect, which is around 8nmol/L of total testosterone. The ''saturation hypothesis'' in 2009 was a step forward in that it explained why TRT was unlikely to be detrimental and reassured prescribers, increasing confidence, and, enabling more men who had curative treatment for prostate cancer to be treated with testosterone, and subsequently also men with biochemical recurrence and metastatic prostate cancer (unfortunately, the 2018 American Urological Association guidelines on TRT makes no recommendation for the use of TRT in hypogonadal men with prostate cancer).

The beneficial effect of Testosterone therapy on BPH-LUTS (Lower Urinary Tract Symptoms) is explained, and the data suggests that treating hypogonadism with TT

is not detrimental to prostate growth or urinary symptoms and may produce relief from LUTS.

There is reassuring data on testosterone levels and prostate cancer risk, not surprisingly, a low testosterone appears to be a risk factor for higher grade and stage of prostate cancer, and a low free testosterone appears to predict an increased risk of reclassification to active treatment amongst men undergoing active surveillance for prostate cancer. There appears to be no association between therapy and overall prostate cancer risk, and men on testosterone therapy actually have a lower risk of high-grade prostate cancer.

With increasing numbers of men with cured prostate cancer the literature suggests that TTh-treated patients do not have increased risk of disease progression compared to untreated patients.

Recently we have seen the use of Testosterone Therapy in Men with treated Prostate Cancer and bipolar androgen therapy (BAT), involving rapid cyclic administration of high-dose testosterone, as a novel treatment for metastatic castration-resistant prostate cancer (mCRPC) which promotes improvements in body composition and associated improvements in lipid profiles and quality of life.

References

1. Berry SJ, Coffey DS, Walsh PC, Ewing LL. The development of human benign prostatic hyperplasia with age. J Urol 1984;132:474-9.
2. Imperato-McGinley J, Guerrero L, Gautier T, *et al.* Steroid 5alpha-reductase deficiency in man: an inherited form of male pseudo hermaphroditism. Science 1974;186:1213.
3. Mendel CM. The free hormone hypothesis: a physiologically based mathematical model. Endocr Rev 1989;10:232-74.
4. Laurent MR, Hammond GL, Blokland M, *et al.* Sex hormone-binding globulin regulation of androgen bioactivity in vivo: validation of the free hormone hypothesis. Sci Rep 2016;6:35539.
5. Kurita T, Wang YZ, Donjacour AA, *et al.* Paracrine regulation of apoptosis by steroid hormones in the male and female reproductive sys- tem. Cell Death Differ 2001;8:192e200.
6. Hotta Y, Kataoka T, Kimura K. Testosterone Deficiency and Endothelial Dysfunction: Nitric Oxide, Asymmetric Dimethylarginine, and Endothelial Progenitor Cells. Sex Med Rev 2019;7:661-8.
7. Moreau KL. Modulatory influence of sex hormones on vascular aging. Am J Physiol Heart Circ Physiol 2019;316:H522-H526.
8. Adekoya TO, Richardson RM. Cytokines and Chemokines as Mediators of Prostate Cancer Metastasis. Int J Mol Sci 2020;21:4449.
9. Maynard JP, Ertunc O, Kulac I, *et al.* IL8 Expression Is Associated with Prostate Cancer Aggressiveness and Androgen Receptor Loss in Primary and Metastatic Prostate Cancer. Mol Cancer Res 2020;18:153-65.
10. Cakir SS, Polat EC, Ozcan, L, *et al.* The effect of prostatic inflammation on clinical outcomes in patients with benign prostate hyperplasia. Prostate Int 2018;6:71-4.

11. Wu D, Shi ZE, Xu D, *et al.* Serum interleukin 6 and acute urinary retention in elderly men with benign prostatic hyperplasia in China: A cross-sectional study. Transl Androl Urol 2021;10:455-65.
12. Marks LS, Mazer NA, Mostaghel E, *et al.* Effect of testosterone replacement therapy on prostate tissue in men with late-onset hypogonadism: a randomized controlled trial. JAMA 2006;296:2351-61.
13. Cooper CS, Perry PJ, Sparks AE, *et al.* Effect of exogenous testosterone on prostate volume, serum and semen prostate specific antigen levels in healthy young men. J Urol 1998;159.
14. Bhasin S, Storer TW, Berman N, *et al.* The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 1996;335:1-7.
15. Hackett G, Cole N, Bhartia M, *et al.* The response to testosterone undecanoate in men with type 2 diabetes is dependent on achieving threshold serum levels (the BLAST study). Int J Clin Pract 2014;68:203-15.
16. Morgentaler A, Traish AM. Shifting the paradigm of testosterone and prostate cancer: the saturation model and the limits of androgen-dependent growth. Eur Urol 2009;55:310-20.
17. Rastrelli G, Corona G, Vignozzi L, *et al.* Serum PSA as a predictor of testosterone deficiency. J Sex Med 2013;10:2518-28.
18. Shigehara K, Sugimoto K, Konaka H, *et al.* Androgen replacement therapy contributes to improving lower urinary tract symptoms in patients with hypogonadism and benign prostate hypertrophy: a randomised controlled study. Aging Male 2011;14:53-8.
19. Haider A, Gooren LJ, Padungtod P, *et al.* Concurrent improvement of the metabolic syndrome and lower urinary tract symptoms upon normalisation of plasma testosterone levels in hypogonadal elderly men. Andrologia 2009;41:7-13.
20. Haider A *et al.*, Eur Urol Suppl. 15(3)
21. Huggins C, Hodges CV. The effect of castration, of estrogen and of androgen injection on serum phosphatase on metastatic carcinoma of the prostate. Cancer Res 1941;1:293-7.
22. Gooren LJ, Behre HM. Diagnosing and treating testosterone deficiency in different parts of the world: changes between 2006 and 2010. Aging Male 2012;15:22-7.
23. Endogenous Hormones and Prostate Cancer Collaborative Group, Roddam AW, Allen NE, *et al.* Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. J Natl Cancer Inst 2008;100:170-83.
24. Shin BS, Hwang EC, Im CM, *et al.* Is a decreased serum testosterone level a risk factor for prostate cancer? A cohort study of korean men. Korean J Urol 2010;51:819-23.
25. Morgentaler A, Rhoden EL. Prevalence of prostate cancer among hypogonadal men with prostate-specific antigen levels of 4.0 ng/mL or less. Urology 2006;68:1263-7.
26. Hoffman MA, DeWolf WC, Morgentaler A. Is low serum free testosterone a marker for high grade prostate cancer? J Urol 2000;163:824-7.
27. Isom-Batz G, Bianco FJ Jr, Kattan MW, *et al.* Testosterone as a predictor of pathological stage in clinically localized prostate cancer. J Urol 2005;173:1935-7.
28. Lane BR, Stephenson AJ, Magi-Galluzzi C, *et al.* Low testosterone and risk of biochemical recurrence and poorly differentiated prostate cancer at radical prostatectomy. Urology 2008;72:1240-5.

29. Ferro M, Lucarelli G, de Cobelli O, *et al.* Circulating preoperative testosterone level predicts unfavourable disease at radical prostatectomy in men with International Society of Urological Pathology Grade Group 1 prostate cancer diagnosed with systematic biopsies. World J Urol 2021;39:1861-7.

30. San Francisco IF, Rojas PA, DeWolf WC, *et al.* Low free testosterone levels predict disease reclassification in men with prostate cancer undergoing active surveillance. BJU Int 2014;114:229-35.

31. Song W, Khera M. Physiological normal levels of androgen inhibit proliferation of prostate cancer cells in vitro. Asian J Androl 2014;16:864-8.

32. Baillargeon J, Kuo YF, Fang X, *et al.* Long-term Exposure to Testosterone Therapy and the Risk of High-Grade Prostate Cancer. J Urol 2015;194:1612-6.

33. Debruyne FM, Behre HM, Roehrborn CG, *et al.* Testosterone treatment is not associated with increased risk of prostate cancer or worsening of lower urinary tract symptoms: prostate health outcomes in the Registry of Hypogonadism in Men. BJU Int 2017;119:216-24.

34. Loeb S, Folkvaljon Y, Damber JE, *et al.* Testosterone Replacement Therapy and Risk of Favorable and Aggressive Prostate Cancer. J Clin Oncol 2017;35:1430-6.

35. Calof OM, Singh AB, Lee ML, *et al.* Adverse events associated with testosterone replacement in middle-aged and older men: a meta-analysis of randomized, placebo-controlled trials. J Gerontol A Biol Sci Med Sci 2005;60:1451-7.

36. Pastuszak AW, Pearlman AM, Lai WS, *et al.* Testosterone replacement therapy in patients with prostate cancer after radical prostatectomy. J Urol 2013;190:639-44.

37. Ahlering TE, My Huynh L, Towe M, *et al.* Testosterone replacement therapy reduces biochemical recurrence after radical prostatectomy. BJU Int 2020;126:91-6.

38. Pastuszak AW, Khanna A, Badhiwala N, *et al.* Testosterone Therapy after Radiation Therapy for Low, Intermediate and High-Risk Prostate Cancer. J Urol 2015;194:1271-6.

39. Kim M, Byun SS, Hong SK. Testosterone Replacement Therapy in Men with Untreated or Treated Prostate Cancer: Do We Have Enough Evidences? World J Mens Health 2021;39:705-23.

40. Marshall CH, Tunacao J, Danda V, *et al.* Reversing the effects of androgen-deprivation therapy in men with metastatic castration-resistant prostate cancer. BJU Int 2021;128:366-73.

16

Testosterone, frailty, mood and quality of life

Adrian Heald

Introduction

As we go forward through the 21st Century, life expectancy for men and women in many countries continues to increase. Longevity for some is associated with frailty, with attendant consequences for activity levels and for quality of life. In this chapter we review the evidence concerning how the hormone testosterone in men and its circulating level plays a key role in modulating health trajectory in relation to frailty, activity level, mood and quality of life.

Frailty

Testosterone, the most important androgen produced by the testes, plays a pivotal role in men's health.[1] Androgens act at multiple tissue sites across the body. Frailty in older men and women describes a state of reduced homeostatic reserve and diminished resistance to external and internal stressors, which is associated with adverse outcomes such as disability, falls and death.[2, 3] With rising life expectancy across the globe, frailty is increasingly recognized as a critical healthcare issue. The pathophysiology of frailty is poorly understood, but it has been linked with disruptions in several body systems, including metabolic and inflammatory pathways.[4, 5] Both aging and frailty share common features in relation to changes in body composition, muscle strength and physical function, which are accompanied by a parallel decline in androgen levels. Several studies have investigated associations of circulating testosterone level with parameters of muscle function and physical performance[6, 7] but relatively few, predominantly cross-sectional, studies have focused on the association between androgens and frailty[8, 9] with conflicting results. Moreover, to date, frailty per se (as opposed to muscle strength and physical performance) has not been studied as a clinical outcome of interventional trials of testosterone replacement in older men.

It is well established that testosterone levels decline with age and that a significant proportion of men older than 70 years are androgen deficient. Reduced testosterone levels are associated with frailty.[10, 11] Low testosterone levels in frail older men

with limited mobility have been related to dependency in activities of daily living (ADL) and to increased hip fractures. In the Massachusetts Male Aging Study, the probability of frailty increased with reduced circulating total testosterone.[6] Also, it has been reported that men with low free testosterone levels have a 57% higher odds of reporting limitation of mobility and 68% higher odds of worsening limitation of mobility.[8] In a study of 3,616 community-dwelling men aged 70-88 years, fatigue, difficulty climbing a flight of stairs, difficulty walking >100 metres, the presence of >5 illnesses, or weight loss >5% were all associated with low testosterone levels.[12]

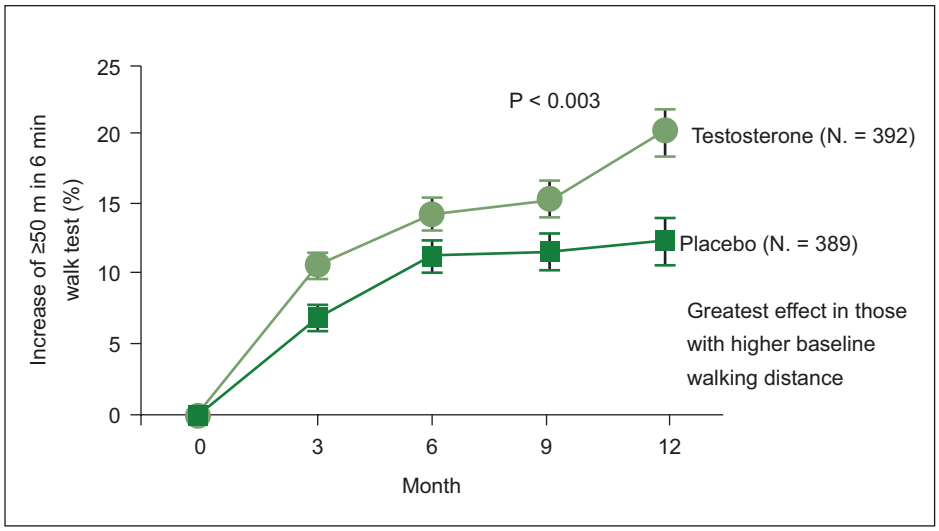
In the European Male Ageing Study (EMAS) a higher free testosterone level was associated with a lower risk of worsening frailty status,[13] and higher androgen levels (free testosterone, total testosterone and dihydrotestosterone (DHT)) remained significantly associated with improving frailty status. The Health in Men Study also reported statistically significant associations between baseline levels of testosterone and calculated free testosterone and frailty measured by the FRAIL scale in 1586 men aged 70 to 88 years followed for 6 years.[8] By contrast in the Concord Health and Ageing in Men Project[14] in which 1166 men >70 years of age were followed for 2.1 years, the authors found no association between baseline circulating levels of testosterone, free testosterone, DHT and worsening frailty.

Decline in muscle mass and function is thought to be central to the development of frailty, and a large body of evidence strongly supports the important anabolic role of testosterone on skeletal muscle. Testosterone stimulates muscle fibre hypertrophy through its action on muscle protein synthesis and inhibition of degradation pathways.[15] However, the associations between circulating testosterone level and measurements of muscle strength and physical performance remain inconsistent.[16, 17] In addition, having a low circulating testosterone level is thought to be pro-inflammatory, which has been linked to frailty development.[18]

Although the circulating level of oestradiol is thought to be related to adiposity rather than muscle function in men,[19] negative associations between oestradiol and muscle mass and strength have also been reported.[20, 21] Oestradiol might therefore be linked to frailty through obesity or sarcopenic obesity. It is relevant to point out here that the effects of oestradiol on frailty in the EMAS study[13] were independent of testosterone level.

In relation to testosterone supplementation and frailty, considerable evidence exists suggesting that testosterone treatment improves some components contributing to frailty and physical decline, such as muscle mass, muscle strength, and physical function.[22, 23] A number of interventional and observational studies have demonstrated consistently that testosterone treatment improves body composition and contributes to increased lean body mass and reduced fat mass.[22, 24, 25] Page *et al.*[25] demonstrated that testosterone treatment alone or with finasteride, a 5α-reductase inhibitor that blocks conversion of testosterone to dihydrotestosterone, improves body composition. In this study, in older men with a mean age of 71 years and total testosterone levels were <12.1 nmol/L, a significant improvement in lean body mass

Figure 16.1. 6-minute walking distance increased with testosterone therapy – the T trial. (from: Snyder P, *et al.* N Engl J MEd 2016;374:611-24).



with testosterone or testosterone + finasteride was noted compared to placebo at up to 36 months follow-up, while fat mass decreased. In a relevant study, Srinivas-Shankar *et al.*[26] reported that in 274 community-dwelling intermediate-frail and frail older men ≥65 years of age with a total testosterone level ≤12 nmol/L or a free testosterone level ≤250 pmol/L who were randomized to transdermal testosterone (50 mg/day) or placebo gel for 6 months, testosterone treatment significantly increased lean body mass and reduced fat mass compared to placebo. A similar effect was also seen in an earlier study of Snyder *et al.*[27] in the T trial.

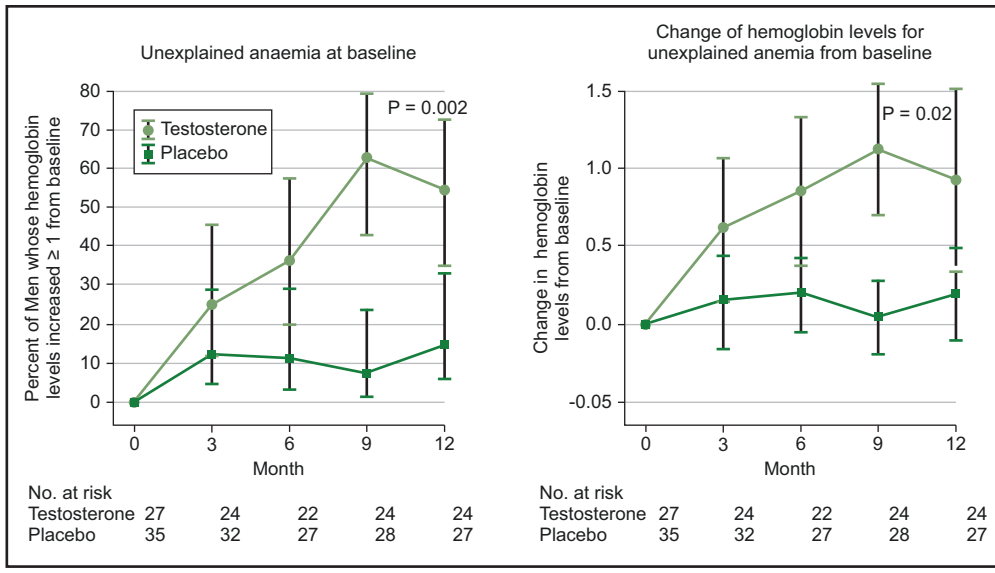
The T trial found a significant improvement in 6-minute walking test with T therapy with the greatest benefit seen in those with better baseline performance, suggesting an advantage in earlier intervention (Figure 16.1). There was also a correction of anaemia in 20% of patients, whether or not there was a previous clinical diagnosis (Figure 16.2) along with significant improvement in bone mineral density, most marked in the spinal trabecular bone.

O'Connell *et al.*[22] reviewed a large number of observational and interventional studies available and showed that testosterone treatment was associated with significantly increased lean body mass and reduced fat mass.

Mood and quality of life

The association between low testosterone levels and depressed mood in older men is well documented, with an inverse relation between testosterone levels and

Figure 16.2. Association of testosterone *vs.* placebo treatment for 12 months with hemoglobin concentrations in participants in the anemia trial.



severity of depression in healthy older men. Ucak *et al.*[28] assessed elements of the comprehensive geriatric assessment such as activities of daily living (ADL), the Mini-Mental State Examination (MMSE), the Mini Nutritional Assessment (MNA), and the Geriatric Depression Scale (GDS) in 250 older men with hypogonadism and in 250 older men who were biochemically eugonadal. Measures of ADL, MMSE and the MNA scores were significantly lower in the compensated hypogonadism group when compared with the normal testosterone group, independent of age and BMI, suggesting that testosterone-deficient older men exhibited significantly less good cognitive function, nutritional status and mood compared with healthy controls.

Concerning depression itself, the European Male Aging Study (EMAS) demonstrated that depression was related to hypogonadism in community-dwelling older men, with a potentially mutual causal relationship.[29] Such a relation was also shown in a clinical sample of 296 older men, in whom symptoms of dysthymia, fatigue, inertia and listlessness and major depression were related both to serum testosterone concentrations and to the genetic modulation of androgen effects by the CAG repeat polymorphism within the androgen receptor gene.[30, 31] This was substantiated by a longitudinal investigation in 748 older men, in whom the 2-year incidence of a newly diagnosed depressive illness was 18.5% in men with previous hypogonadism vs. 10.4% in controls. A 14-year follow-up of 423 men (mean age 59) screened in 2002, showed that men with 21 CAG repeats on the baseline sample had a 48%

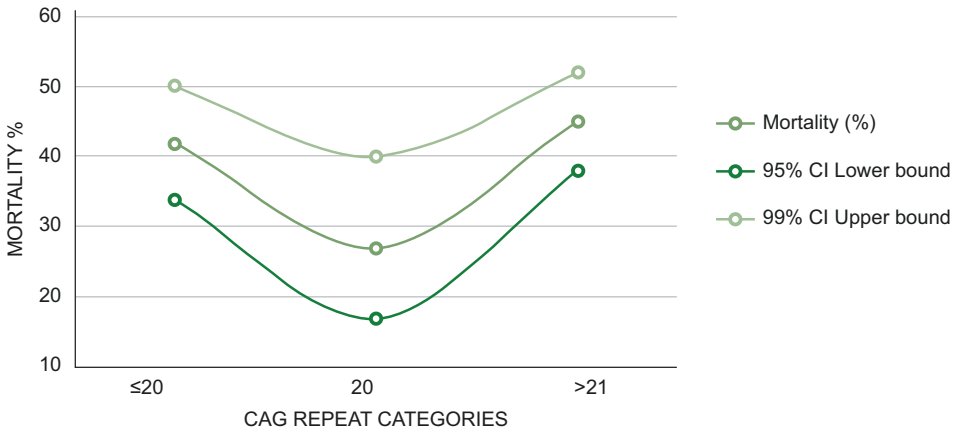
lower mortality than those with higher or lower numbers after adjusting for other variables (Figure 16.3).

Shores *et al.*[32] found that low testosterone levels predicted incident depressive illness in older men. Regarding therapeutic potential, a randomized, double-blind, placebo-controlled study in 33 hypogonadal men with a concomitant subthreshold depression (dysthymia or minor depression) investigated the effects of either testosterone gel or placebo gel. Men on testosterone supplementation had a greater reduction in scores of the Hamilton Rating Scale for Depression and a higher remission rate of subthreshold depression.[33] A randomized, placebo-controlled, double-blind trial in 184 hypogonadal men receiving intramuscular testosterone undecanoate vs placebo demonstrated a marked decrease in the Beck Depression Inventory scores in the men receiving testosterone replacement.[34]

A recent random-effects meta-analysis of 27 randomized controlled trials including 1890 men demonstrated that TRT is associated with a significant reduction in depressive symptoms compared with placebo, showing an odds ratio of efficacy =2.30 (95% CI:1.30-4.06, P=0.004).[35] These effects exceed the efficacy thresholds for pharmacologic agents for depression therapy proposed by the British Psychological Society for treatment-resistant depression.[36, 37]

The association between testosterone and erectile function is well-known, and likewise, the association between mood disorders (including depression and anxiety) and sexual dysfunction is supported by solid evidence. However, it is also true that sexual dysfunction might cause depression because of the negative psychological burden associated with it. Hence, testosterone treatment in hypogonadal

Figure 16.3. Mortality proportion with 95% CI by CAG repeat.



Relation between CAG repeat number and mortality rate. Adjusted logistics regression indicated that the presence of 21 CAG repeats reduced the risk of death by 45% compared with <21 CAG repeats [odds ratio 0.55 (95% CI 0.28–0.92), P = 0.02]; and that >21 repeats increased the risk of death by 48% [odds ratio 1.48 (95% CI 1.08–2.37), P = 0.015]. CI, confidence interval.

patients might be beneficial to psychological symptoms both directly and indirectly by improving erectile function.

For depression, still preferential is the use of established anti-depressant therapies, cognitive behavioral approaches, and psychiatric consultation in patients with first-line depressive symptoms or in those with diagnosed major depression.[36] Nevertheless, additional testosterone supplementation can improve the outcome of such therapies if the patient is hypogonadal. For this to happen, we need to consider hypogonadism in such patients as NICE guidance on frailty does not even consider hypogonadism or suggest testosterone measurement.[37] Paradoxically, a question on sexual function (often omitted from assessments in the elderly) would result in a near-certain positive response for ED, with testosterone measurement being deemed mandatory in current guidelines, irrespective of age.[38]

Quality of Life (QoL) is a summation of psychological variables, which contribute to the subjective perception that life is worthwhile.[39] QoL is principally the degree to which a person enjoys the possibilities of life, based on the categories 'being', 'belonging' and 'becoming', respectively, who one is, how one is connected to one's environment and whether one achieves one's personal goals, hopes and aspirations. Thus, QoL is a multi-dimensional construct comprising the individual's physical, mental, and social well-being, each including both cognitive and emotional components.[40] Items contributing to the impression of QoL are the ability to perceive enjoyment of life in balance with perceived stress, a general motivation in life as well as overall work performance, self-confidence, and the ability to focus, the subjective energy in life and the abilities to cope.[40] This also includes, in most men, parameters related to sexual function. Early use of PDE5 inhibitors, especially daily tadalafil are likely to lead to a more rapid improvement in sexual symptoms, especially when used in conjunction with testosterone therapy.[38]

Positive effects of testosterone replacement treatment on QoL have been seen in larger cohorts of hypogonadal men of up to more than 1000 patients in uncontrolled 'real-life' settings or registries.[41, 42] Importantly in one meta-analysis,[41] the effect was not statistically significant in eugonadal men and in addition, the effect size was larger in subthreshold depression compared with major depression. Oral testosterone compared with oral dehydroepiandrosterone, testosterone gel, and intramuscular testosterone did not show a significant result.[41]

It is evident that hypogonadism can have a negative impact on quality of life and mood. Testosterone substitution is seen to improve these parameters in older frail patients.

Low testosterone and dementia/Alzheimer's disease

In 159,411 community-dwelling men[43] (median age 61, followed for 7 years), 826 developed dementia, including 288 from AD. Lower total testosterone was associated with a higher incidence of dementia (overall trend: P=0.001, lowest *vs.* highest

quintile: hazard ratio [HR] =1.43, 95% confidence interval [CI] =1.13-1.81), and AD (P=0.017, HR=1.80, CI=1.21-2.66). Lower SHBG was associated with a lower incidence of dementia (P<0.001, HR=0.66, CI=0.51-0.85) and AD (P=0.012, HR=0.53, CI=0.34-0.84) Lower total testosterone and higher SHBG are independently associated with incident dementia and AD in older men. Additional research is clearly needed to determine causality. The impact of therapy would clearly require a large long-term study.

Conclusions

Frailty is a clinical syndrome related to changes in metabolism concomitant with sarcopenia, a decline in muscle mass and strength, bone loss, and reduced physical function with aging. Testosterone is implicated in many metabolic functions. Reduced testosterone levels may contribute to the changes noted in body composition and to sarcopenic obesity. Although a direct relation between testosterone deficiency and frailty is not established (due to the multiple factors that contribute to the pathophysiology of this syndrome), many studies have suggested that testosterone treatment in frail older men with low testosterone levels can improve body composition, QoL and physical and sexual function. Furthermore, there is a developing evidence base of benefits in treating low mood in the context of confirmed hypogonadism.

The evidence currently available suggests that the reported increase in disability, comorbidities, and death rates among frail older individuals with testosterone deficiency warrants providing testosterone treatment to attenuate the impact of sarcopenia, to reduce disability, and to increase functional independence.

Before concluding, it should be noted that in many studies, the findings may be confounded by the short duration of treatment, as well as by inaccuracies in the methods of assessment of body composition or physical function. It should also be pointed out that long-term testosterone treatment provides the most impressive gains in lean body mass and reductions in fat mass, and therefore may contribute to improved muscle strength and physical performance. These long-term studies also provide considerable evidence of safety for testosterone therapy in elderly frail men.

Even modest improvements in muscle mass and strength and gains in physical function in response to testosterone treatment may be of great importance for attenuating the progression of muscle and physical decline in older men. The challenge remains how to differentiate frail older men who may benefit from testosterone treatment from those who may not, in relation to impact on physical function, mood and cognition.

In summary, the data available today suggest a strong relation between testosterone deficiency and frailty and such findings warrant further investigation research into the effects of testosterone treatment in attenuating and preventing the incidence or progression of sarcopenia and frailty, in reducing hospitalization,

institutionalization, and disability, and in improving physical functional independence, mood and quality of life in older men.

It is the opinion of many experts and professional bodies, that health prospects in older hypogonadal men can be positively influenced by checking testosterone levels in frail patients and prescribing appropriate testosterone substitution after a careful evaluation by a qualified health care professional.

References

1. Mooradian AD, Morley JE, Korenman SG. Biological actions of androgens. Endocr Rev 1987;8:1-2.
2. Zaslavsky O, Cochrane BB, Thompson HJ, *et al.* Frailty: a review of the first decade of research. Biol Res Nurs 2013;15:422-32.
3. Fried LP, TangenCM, Walston J, *et al.* Frailty in older adults: evidence for a phenotype. J Gerontol A Biol SciMed Sci 2001;56:M146-M156.
4. Reiner AP, Aragaki AK, Gray SL, *et al.* Inflammation and thrombosis biomarkers and incident frailty in postmenopausal women. Am J Med 2009;122:947-54.
5. Barzilay JI, Blaum C, Moore T, *et al.* Insulin resistance and inflammation as precursors of frailty: the Cardiovascular Health Study. Arch Intern Med 2014;167:635-41.
6. O'Donnell AB, Travison TG, Harris SS, *et al.* Testosterone, dehydroepiandrosterone, and physical performance in older men: results from the Massachusetts Male Aging Study. J Clin Endocrinol Metab 2006;91:425-31.
7. Krasnoff JB, Basaria S, Pencina MJ, *et al.* Free testosterone levels are associated with mobility limitation and physical performance in community dwelling men: the Framingham Offspring Study. J Clin Endocrinol Metab 2010;95:2790-9.
8. Hyde Z, Flicker L, Almeida OP, *et al.* Low free testosterone predicts frailty in older men: The Health in Men Study. J Clin Endocrinol Metab 2010;95:3165-72.
9. Cawthon PM, Ensrud KE, Laughlin GA, *et al.* Sex hormones and frailty in older men: the osteoporotic fractures in men (MrOS) study. J Clin Endocrinol Metab 2009;94:3806-15.
10. Baillargeon J, Deer RR, Kuo YF, *et al.* Androgen therapy and rehospitalization in older men with testosterone deficiency. Mayo Clin Proc 2016;91:587-95.
11. Morley JE, von Haehling S, Anker SD, *et al.* From sarcopenia to frailty: a road less traveled. J Cachexia Sarcopenia Muscle 2014;5:5-8.
12. Krasnoff JB, Basaria S, Pencina MJ, *et al.* Free testosterone levels are associated with mobility limitation and physical performance in community-dwelling men: the Framingham Offspring Study. J Clin Endocrinol Metab 2010;95:2790-9.
13. Lee DM, Tajar A, Ravindrarajah R, *et al.* Frailty and sexual health in older European men. J Gerontol A Biol Sci Med Sci 2013;68:837-44.
14. Travison TG, Nguyen A-H, Naganathan V, *et al.* Changes in reproductive hormone concentrations predict the prevalence and progression of the frailty syndrome in older men: The Concord Health and Ageing in Men Project. J Clin Endocrinol Metab 2011;96:2464-74.
15. Sinha-Hikim I, Artaza J, Woodhouse L, *et al.* Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. Am J Physiol Endocrinol Metab 2002;283:E154-E164.

16. Auyeung TW, Lee JSW, Kwok T, *et al.* Testosterone but not estradiol level is positively related to muscle strength and physical performance independent of muscle mass: a cross-sectional study in 1489 older men. Eur J Endocrinol 2011;164:811-7.
17. Baumgartner RN, Waters DL, Gallagher D, *et al.* Predictors of skeletal muscle mass in elderly men and women. Mech Ageing Dev 1999;107:123-36.
18. Barzilay JI, Blaum C, Moore T, *et al.* Insulin resistance and inflammation as precursors of frailty: the Cardiovascular Health Study. Arch Intern Med 2007;167:635-41.
19. Finkelstein JS, Yu EW, Burnett-Bowie SA. Gonadal steroids and body composition, strength, and sexual function in men. N Engl J Med 2013;369:1011-22.
20. Schaap LA, Pluijm SMF, Smit JH, *et al.* The association of sex hormone levels with poor mobility, low muscle strength and incidence of falls among older men and women. Clin Endocrinol (Oxf) 2005;63:152-60.
21. Gielen E, O'Neill TW, Pye SR, *et al.* Endocrine determinants of incident sarcopenia in middle-aged and elderly European men. J Cachexia Sarcopenia Muscle 2015;6:242-25.
22. O'Connell MD, Tajar A, Roberts SA, *et al.* Do androgens play any role in the physical frailty of ageing men? Int J Androl 2011;34:195-211.
23. Saad F. The relationship between testosterone deficiency and frailty in elderly men. Horm Mol Biol Clin Investig 2010;4:529-38.
24. Svartberg J, Agledahl I, Figenschau Y, *et al.* Testosterone treatment in elderly men with subnormal testosterone levels improves body composition and BMD in the hip. Int J Impot Res 2008;20:378-87.
25. Page ST, Amory JK, Bowman FD, *et al.* Exogenous testosterone (T) alone or with finasteride increases physical performance, grip strength, and lean body mass in older men with low serum T. J Clin Endocrinol Metab 2005;90:1502-10.
26. Srinivas-Shankar U, Roberts SA, Connolly MJ, *et al.* Effects of testosterone on muscle strength, physical function, body composition, and quality of life in intermediate-frail and frail elderly men: a randomized, doubleblind, placebo-controlled study. J Clin Endocrinol Metab 2010;95:639-50.
27. Snyder PJ, Peachey H, Hannoush P, *et al.* Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 1999;84:2647-53.
28. Ucak S, Basat O, Karatemiz G. Functional and nutritional state in elderly men with compensated hypogonadism. J Am Med Dir Assoc 2013;14:433-6.
29. Wu FC, Tajar A, Beynon JM, *et al.* Identification of late-onset hypogonadism in middle-aged and elderly men. New Engl J Med 2010;363:123-35.
30. Schneider G, Nienhaus K, Gromoll J, *et al.* Aging males' symptoms in relation to the genetically determined androgen receptor CAG polymorphism, sex hormone levels and sample membership. Psychoneuroendocrinology 2010;35:578-58.
31. Heald A, Yadegar far G, Livingston M, *et al.* Androgen receptor-reduced sensitivity is associated with increased mortality and poorer glycaemia in men with type 2 diabetes mellitus: a prospective cohort study. Cardiovasc Endocrinol Metab 2020;10:37-44.
32. Shores MM, Moceri VM, Sloan KL, *et al.* Low testosterone levels predict incident depressive illness in older men: effects of age and medical morbidity. J Clin Psychiatry 2005;66:7-14.

33. Shores MM, Kivlahan DR, Sadak TI, *et al.* Arandomized, double-blind, placebo-controlled study of testosterone treatment in hypogonadal older men with subthreshold depression (dysthymia or minor depression). J Clin Psychiatry 2009;70:1009-16.

34. Giltay EJ, Tishova YA, Mskhalaya GJ, *et al.* Effects of testosterone supplementation on depressive symptoms and sexual dysfunction in hypogonadal men with the metabolic syndrome. J Sex Med 2010;7:2572-82.

35. Walther A, Breidenstein J, Miller R. Association of testosterone treatment with alleviation of depressive symptoms in men: a systematic review and meta-analysis. JAMA Psychiatry 2019;76:31-40.

36. National Collaborating Centre for Mental Health UK. Depression: The Treatment and Management of Depression in Adults (Updated Edition). Leicester, UK: British Psychological Society; 2010.

37. NICE. Improving care and support for people with frailty. Available from: https://stp-support.nice.org.uk/frailty/index.html (accessed 2021, October 15).

38. Hackett G, Kirby M, Edwards D. British Society for Sexual Medicine Guidelines on Adult Testosterone Deficiency, With Statements for UK Practice. J Sex Med 2017;14:1504-23.

39. Button KS, Kounali D, Thomas L, *et al.* Minimal clinically important difference on the Beck Depression Inventory–II according to the patient's perspective. Psychol Med 2015;45:3269-79.

40. Testa MA, Simonson DC. Assessment of quality-of-life outcomes. N Engl J Med 1996;334:835-40.

41. Amanatkar HR, Chibnall JT, Seo BW, *et al.* Impact of exogenous testosterone on mood: a systematic review and meta-analysis of randomized placebo-controlled trials. Ann Clin Psychiatry 2014;26:19-32.

42. Wu F, Zitzmann M, Heiselman D, *et al.* Demographic and clinical correlates of patient-reported improvement in sex drive, erectile function, and energy with testosterone solution 2%. J Sex Med 2016;13:1212-9.

43. Marriott RJ, Murray K, Flicker L, *et al.* Lower serum testosterone concentrations are associated with a higher incidence of dementia in men: The UK Biobank prospective cohort study. Alzheimers Dement 2022 Jan 3. [Epub ahead of print].

17

Osteoporosis and serum testosterone levels: the impact of testosterone therapy

Michael Kirby, Geoffrey I. Hackett

Introduction

Osteoporosis, as described by the World Health Organization (WHO) since 1994, is a condition characterized by low bone mass and microarchitectural bone deterioration that leads to bone fragility and fracture susceptibility.[1]

Hypogonadism in adults is a cause of overall bone loss and a contributor to the development of secondary osteoporosis. Male hypogonadism is correlated with losses in bone quality, the connection is not simply dependent on testosterone levels, but also oestrogen levels which contribute to bone health in men, the relative bioavailable oestrogen levels have the strongest correlation with maintenance of bone density. Testosterone contributes to indirect effects on bone through its conversion via aromatase to oestrogen but has direct effects on bone quality via the androgen receptor as well as indirect effects via conversion to oestrogen by aromatase.

Testosterone has direct effects via the androgen receptor on osteoblasts by promoting trabecular bone formation and on osteocytes by preventing age-related resorption of trabecular bone.[2–4]

Low levels of oestrogen and high SHBG, together with a low bioavailable testosterone, are likely to contribute to low BMD in men. In terms of fracture risk, the important role for testosterone in risk of fracture compared to BMD may be related to the role of testosterone in muscle strength and physical performance. Muscle weakness predisposes to an increased rate of falls potentially leading to fracture.[5]

Low testosterone and osteoporosis – pivotal studies

The osteoporotic fractures in men study (MrOS), followed thousands of men over the age of 65 in Sweden, the United States, and Hong Kong for an average of 4.5 years.

The initial results of the Sweden cohort of MrOS found that free testosterone levels were positively correlated with BMD in the hip, femur, and arm but not the lumbar spine. Lower levels of free testosterone were also correlated with increased fracture risk. Oestrogen levels were positively correlated with BMD in all locations including the lumbar spine.[6]

Further evidence of the important role of testosterone in the increased fracture risk of older men, is the data from men with prostate cancer treated with androgen deprivation therapy (ADT). In a study from New Zealand the researchers found that androgen deprivation therapy with gonadotropin-releasing hormone agonist ADT was significantly associated with an increased risk of any fracture and hip fracture requiring hospitalisation. Among patients receiving ADT, 10.8% had a fracture compared to 3.2 % of those not receiving ADT (P<0.0001). After controlling for age and ethnicity, the use of ADT was associated with a significantly increased risk of any fracture (OR=2.83; 95% CI 2.52-3.17) and of hip fracture requiring hospitalisation (OR=1.82; 95% CI 1.44-2.30). Those who received combined androgen blockade (OR=3.48; 95% CI 3.07-3.96) and bilateral orchiectomy with pharmacologic ADT (OR=4.32; 95% CI 3.34-5.58) had the greatest risk of fracture.[7]

The excess risk was partly driven by pathologic fractures and spinal cord compression which are associated with decreased survival in ADT users. Identification of those at higher risk of fracture and close monitoring of bone health while on ADT is an important factor to consider. Monitoring of bone density and bone marker profiles in these men is advisable.

Men and women with a 10-year probability of a major osteoporotic fracture derived from FRAX, above the upper assessment threshold, should be considered for treatment. Men and women with a 10-year probability between the upper and lower assessment threshold should be referred for bone mineral density measurement and their fracture probability reassessed if their 10-year fracture probability is above the intervention threshold after reassessment.

The traditional diagnostic category of BMD T-score SD≤-2.5 and between ≤1.0 and ≥2.5 is universally used to define osteoporosis and osteopenia in males aged ≥50, respectively. For individuals under age 50, the BMD Z-score, which uses an age- and a gender-specific reference, is used, with Z-scores of -2.0 or lower defined as either "low bone mineral density for chronological age" or "below the expected range for age" and those above -2.0 being "within the expected range for age".[8]

The prevalence of osteoporosis amongst males ≥50 is significantly lower than the female population, but male osteoporosis and osteopenia leading to fractures and disability is very significant. Men may sustain osteoporotic fractures up to 10 years later in life than women, however, the mortality and morbidity associated with male hip fractures are higher than that of women, and men with known fragility fractures are less likely to receive treatment as compared to women.[9]

The rate of identified secondary male osteoporosis (all causes) or osteoporosis-related fracture having an attributable secondary cause for their osteoporosis is

about 50%. Those few studies that reported the secondary causes for male osteoporosis have found rates ranging from 16 to 30% for hypogonadism as the attributable cause. These small studies illustrate the high degree of identifiable causes of secondary male osteoporosis and the significant amount of hypogonadal-related male osteoporosis and osteoporosis-related fractures.[11-13]

Table 17.I. All cause prevalence of osteoporosis by age in men and women.[10]

Age	50-60	60-70	70-80	80-90	>90 years
Male	0.6%	1.7%	4.3%	10.4%	22.6%
Female	3.4%	8.5%	19.2%	37.3%	61.3%

Indications for therapy for osteoporosis in men

The 2012 Endocrine Society Osteoporosis in Men guideline recommends the use of testosterone therapy in men with symptomatic low testosterone who are at high risk of fracture, though this should be done in combination with a medication with a proven antifracture effect such as a bisphosphonate.[14]

Hypogonadal males with spine or hip fragility fracture, osteoporosis, or osteopenia with a high calculated fracture risk have indication for testosterone treatment by virtue of their hypogonadal symptoms and have indication for bisphosphonates by virtue of their BMD and fracture risk.

Alternatives include denosumab or teriparatide therapy. There have been some studies showing the drugs that can be prescribed to prevent fragility fractures include bisphosphonates (alendronate, ibandronate, risedronate and zoledronic acid) and non-bisphosphonates (raloxifene, denosumab, teriparatide, calcitriol and hormone replacement therapy).

Alendronate and risedronate are first line treatments in men. Where these are contraindicated or not tolerated, zoledronic acid or denosumab provide the most appropriate alternatives, with teriparatide as an additional option.

For estimation of fracture probability, femoral neck BMD T-scores in men should be based on the NHANES female reference database. When using the online version of FRAX for the estimation of fracture probability, femoral neck BMD values (g/cm^2) should be entered, and the manufacturer of the densitometer specified.[15]

Recent studies on the role of testosterone replacement therapy (TRT) in men with osteoporosis

Several small studies have shown positive results for TRT in osteoporosis in men, but they have usually been under-powered and of insufficient duration to establish positive endpoints.[16-18] There have also been ethical issues around treating men with

severe osteoporosis with placebo over many years. In one placebo-controlled study, testosterone therapy did not improve spine BMD overall.[19] Another study demonstrated significantly greater effect on spine and hip BMD with TRT, but supraphysiological doses of testosterone were used.[20] We will therefore focus on 2 recent large scale RCTs that have clarified the issues.

The T trial (2017)

The US "T trial" sub study on bone health involved 211 men over 65 with TT of 275 ng/dl or less recruited from 9 US academic centres between 2011 and 2014.[21] Men were randomised to either testosterone gel or placebo and treated to a target level of normal testosterone levels for younger men over a 1-year period. Men in both groups took 2 Calcium and Vitamin D3 tablets daily.

Spine and hip vBMD (volumetric bone density) were measured at baseline at 12 months, using quantitative computerised tomography (Figures 17.1, 17.2). Areal BMD was assessed by dual energy and x-ray absorptiometry. TRT was associated with a significant increase in vBMD (7.5% *vs.* 0.8% P<0.001). The magnitude of change in vBMD in the spine was closely related to change in total testosterone and oestradiol levels. Similar increases in vBMD were seen in the distal tibia. A 200ng/dl increase in TT was associated with a 6.1% increase in vBMD. A

Figure 17.1. Effect of testosterone on volumetric bone mineral density (adapted from: Snyder PJ, *et al*. Lessons From the Testosterone Trials. Endocr Rev 2018;39:369-86).

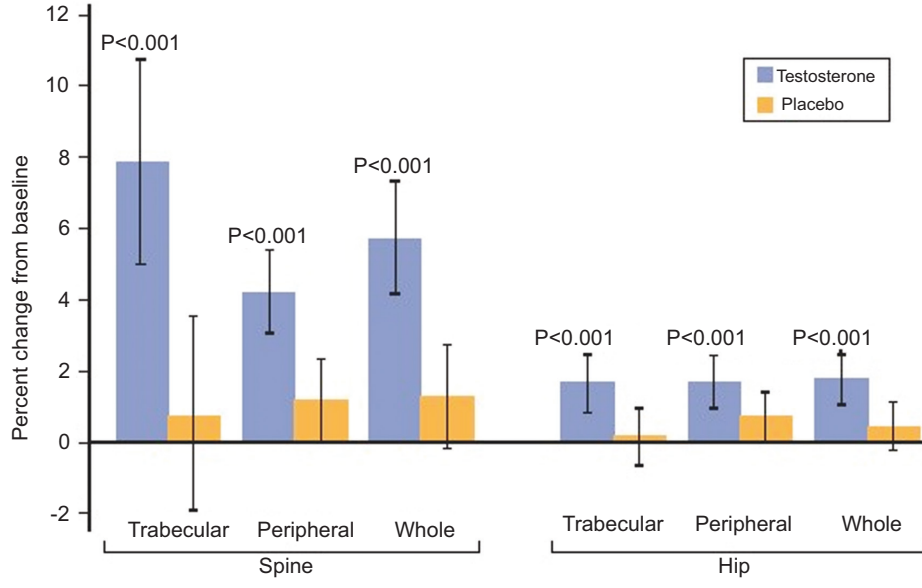
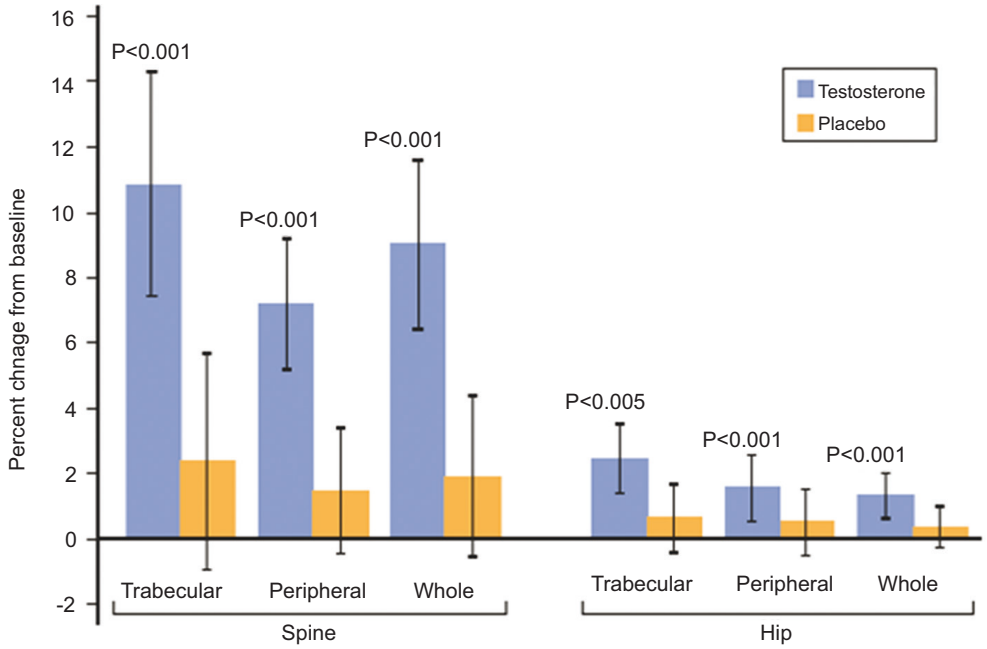


Figure 17.2. Effect of testosterone on estimated bone strength (adapted from: Snyder PJ, *et al.* Lessons From the Testosterone Trials. Endocr Rev 2018;39:369-86).



15 pmol/L increase in oestrone was associated with a 6.3% increase. Volumetric bone density was chosen at it is not affected by osteophyte formation or aortic calcification.

The estimated increase in peripheral bone strength was greatest in trabecular than peripheral bone and effect was greatest in the spine than the hip. Of course, the authors suggested that a longer study would be required to assess the impact on fracture rate. During the treatment year, 6 fractures were confirmed in each group. In a second observational year, there were 3 fractures in the active and 4 in the placebo group.

The T4DM Bone Study - T4Bone

The Australian T4DM bone sub-study involved 1007 men randomised to either Testosterone Undecanoate 1000 mg or matching placebo every 12 weeks for 2 years. The mean baseline in this study was 13.6 nmol/L *versus* 8.2 in the T trial, due to the primary end point of T4DM being prevention of T2DM.[22]

Greatest effect was seen in trabecular spine (6.8% and total hip 1.3%, both P< 0.001), with lesser effect on peripheral spine. In T4DM there were significant treatment effects in hip (1.9%) and femoral neck (1.7%). In a larger cohort of 601 men in this study,

the lumbar spine treatment effect was 3.3% at 2 years *vs.* 1.2% at 1 year in the T Trial. The authors suggest that this might be due to 2 years *vs.* 1 year duration and a possible greater effect of the depot injection *vs.* gel. In the T4DM bone study the effects on cortical bone were consistent at all levels whereas effects on trabecular bone were variable, in line with findings from earlier studies. It is important to note that T4DM included obese men at high risk of diabetes.

The cut off for inclusion was a TT of 14 nmol/L, far higher than 9.4 nmol/L in the T trial and earlier studies. This higher cut off would include around 50% of obese men over 50 with T2DM or pre-diabetes. Fractures were not evaluated in the T4DM bone study. The standardised effects from this study of 3.1-1.3% are in line with the effects of resorptive therapy, such as alendronate, in postmenopausal women over 2 years (0.3-3.8%) denosumab and zoledronic acid. T4DM also found a 40% reduction in progression from pre-diabetes to confirmed T2DM over 2 years in men treated with Testosterone Undecanoate. There are estimated to be over 4 million men with pre-diabetes at risk and the majority of these will have testosterone levels below 14 nmol/L.

In Recent medicine reviews (2020) Dos Santos and Bhasin[23] concluded

"Testosterone therapy for older men with confirmed testosterone therapy improves sexual activity, sexual desire, and erectile dysfunction: areal and volumetric bone density, as well as estimated bone strength in the spine and hip corrects unexplained anaemia of ageing increases skeletal muscle mass, strength and power: self-reported mobility, and some forms of physical function and modestly improves depressive symptoms".

Curiously, they go on to state

"The Endocrine Society recommends against testosterone therapy for all older men with low testosterone levels but suggests consideration of therapy on an individualised basis in men who have consistently low levels of testosterone and symptoms or conditions suggestive of testosterone deficiency".

It is unlikely that men who have fought their way to a specialist would not have multiple symptoms on that list. When presented with the beneficial effects of treatment, it is difficult to imagine that they would not opt to be treated.

Conclusions

There is conclusive evidence that osteoporosis in obese men with T2DM is associated with increased risk of fracture and increased mortality with age. There is also conclusive evidence that TRT in hypogonadal men improves osteoporosis, but studies have been of too short duration to confirm reduction in fractures. Improvements are in line with those seen with resorptive therapies, but it is likely that such TRT

will be used in conjunction with resorptive therapies in the medium term. The multiple additional benefits of TRT are likely to make this more attractive for long term compliance in older men.

References

1. World Health Organization. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organization Technical Report Series 1994;843:1-129.
2. Golds G, Houdek D, Arnason T. Male Hypogonadism and Osteoporosis: The Effects, Clinical Consequences, and Treatment of Testosterone Deficiency in Bone Health. Int J Endocrinol 2017;2017:4602129.
3. Vanderschueren D, Laurent MR, Claessens F, *et al.* Sex steroid actions in male bone. Endocr Rev 2014;35:906-60.
4. Marcus R, Leary D, Schneider DL, *et al.* The contribution of testosterone to skeletal development and maintenance: lessons from the androgen insensitivity syndrome. J Clin Endocrinol Metab 2000;85:1032-7.
5. Auyeung TW, Lee JS, Kwok T, *et al.* Testosterone but not estradiol level is positively related to muscle strength and physical performance independent of muscle mass: a cross-sectional study in 1489 older men. Eur J Endocrinol 2011;164:811-7.
6. Mellström D, Johnell O, Ljunggren O, *et al.* Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden. J Bone Miner Res 2006;21:529-35.
7. Wang A, Obertová Z, Brown C, *et al.* Risk of fracture in men with prostate cancer on androgen deprivation therapy: a population-based cohort study in New Zealand. BMC Cancer 2015;15:837.
8. Cosman F, de Beur SJ, LeBoff MS, *et al.* Clinician's guide to prevention and treatment of osteoporosis. Osteoporosis Int 2014;25:2359-81.
9. Kaufman JM, Reginster JY, Boonen S, *et al.* Treatment of osteoporosis in men. Bone 2013;53:134-44.
10. Kanis JA. Assessment of osteoporosis at the primary health-care level. Sheffield, UK: WHO Collaborating Centre, University of Sheffield; 2007.
11. Pye SR, Adams KR, Halsey JP, *et al.* Frequency and causes of osteoporosis in men. Rheumatology (Oxford) 2003;42:811-2.
12. Ryan CS, Petkov VI, Adler RA. Osteoporosis in men: the value of laboratory testing. Osteoporos Int 2011;22:1845-53.
13. Lambert JK, Zaidi M, Mechanick JI. Male osteoporosis: epidemiology and the pathogenesis of aging bones. Curr Osteoporos Rep 2011;9:229-36.
14. Watts NB, Adler RA, Bilezikian JP, *et al.* Osteoporosis in men: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2012;97:1802-22.
15. NOGG 2017: Clinical guideline for the prevention and treatment of osteoporosis, updated 2018. Available from: https://www.sheffield.ac.uk/NOGG/NOGG%20Guideline%202017.pdf
16. Ebling PR. Osteoporosis in men. NEJM 2008;358;1474-82.

17. Kenny AM, Kleppinger A, Annis K, *et al.* Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels, low bone mass, and physical frailty. J Am Geriatr Soc 2010;58:1134-43.

18. Permpongkosol S, Khupulsup K, Leelaphiwat S, *et al.* Effects of 8-year treatment of long-acting testosterone undecanoate on metabolic parameters, urinary symptoms, bone mineral density, and sexual function in men with late-onset hypogonadism. J Sex Med 2016;13:1199-211.

19. Konak H, Sugimoto K, Orikasa H, *et al.* Effects of long-term androgen replacement therapy on the physical and mental statuses of aging males with late-onset hypogonadism: A multicenter randomized controlled trial in Japan (EARTH Study). Asian J Androl 2016;18:25-34.

20. Aversa A, Bruzziches R, Francomano D, *et al.* Effects of long-acting testosterone undecanoate on bone mineral density in middle-aged men with late-onset hypogonadism and metabolic syndrome: Results from a 36- months controlled study. Aging Male 2012;15:96-102.

21. Snyder P, Koppendahl DL, Stephens-Sheilds AL, *et al.* Effect of Testosterone Treatment on Volumetric Bone Density and Strength in Older Men with Low Testosterone: A Controlled Clinical Trial. JAMA Intern Med 2017;177:471-9.

22. Ng Tang Fui M, Mark Ng Tang Fui, Hoermann R, Bracken K, Effect of Testosterone treatment on bone microarchitecture and bone mineral density in men: a two-year RCT. J Clin Endocrinol Metab 2021;106:e3143-e3158.

23. Rodrigues Dos Santos M, Bhasin S. Benefits and Risks of Testosterone Treatment in Men with Age-Related Decline in Testosterone. Annu Rev Med 2021;72:75-91.

18

Testosterone, diabetes and COVID-19

Michael Kirby

As the COVID-19 pandemic spreads across the globe, the UK has been particularly hard hit. The death toll in March 2021 stood at 127,000 across Great Britain, of which two thirds were men.[1] This disparity in male mortality has been noted elsewhere.[2]

A large-scale global statistical analysis showed that whilst males and females are at equivalent risk of infection, male sex is associated with the development of severe disease as measured by ITU admission (OR=2.84; 95% CI=2.06, 3.92; P=1.86×10-10) and death (OR=1.39; 95% CI=1.31, 1.47; P=5.00×10-30).[3] Surprisingly, despite this notable feature of the pandemic, sex is still not routinely reported in all available regional data, particularly as the sex difference was also noted by Karlberg *et al,* in the American Journal of Epidemiology in 2004 and by Channappanavar *et al.*, in the Journal of Epidemiology in 2017, during the two previous significant outbreaks of coronavirus: the 2003 Severe Acute Respiratory Syndrome [SARS-CoV], and more recently by Ahmadzadeh *et al.*, during the 2012 Middle East Respiratory Syndrome [MERS].[4]

Kalberg *et al.* noted that during the SARS-CoV outbreak in particular, men had a higher case fatality rate of 21.9% as compared to 13.2% for females, and twice as many male-to-female deaths in the 0-44-year age range. Published mortality data consistently shows that two-thirds of nearly 45 000 UK COVID-19 deaths are in men, with rates in patients aged <85 years of 50.6/100 000 for men in the UK, *versus* 25.5/100 000 in women.

According to a report published online by the UK Office for National Statistics on the 25th January 2021 (ons.gov.uk), 7,961 deaths involving the coronavirus (COVID-19) in the working age population (those aged 20 to 64 years) of England and Wales were registered between 9 March and 28 December 2020.

Nearly two-thirds of these deaths (5,128) were among men, with the age-standardised mortality rate of death involving COVID-19 being statistically significantly higher in men, at 31.4 deaths per 100,000 men aged 20 to 64 years *versus* 16.8 deaths per 100,000 women (2,833 deaths).

When considering broad groups of occupations, men who worked in elementary occupations (consisting mainly of simple and routine tasks which mainly require the use of hand-held tools and often some physical effort or caring,

leisure and other service occupations) had the highest rates of death involving COVID-19.

Over the age of 85 years, men account for only around 30% of the population, meaning that female deaths predominate in this group.

Comorbidities such as age, obesity, type 2 diabetes (T2D), chronic kidney disease (CKD), hypertension, heart failure and COPD all increase the risk, and are more common in men. The fact that COVID-19 is associated with a fall in testosterone levels[5] may compound the risk of mortality because several age-related conditions are associated with increased rates of hypogonadism; notably, type 2 diabetes mellitus (T2DM), obesity, coronary heart disease (CHD), heart failure, CKD, COPD, HIV, and men on long-term opiates. Within these groups, secondary hypogonadism has been shown to be associated with an increased all-cause mortality.

Men who contract coronavirus disease 2019 (COVID-19) appear to have worse clinical outcomes compared with women which raises the possibility of androgen-dependent effects.

Why more of a problem for men?

The angiotensin converting enzyme 2 (ACE2) is the main route the virus takes to get into cells, and this is more highly expressed in males.[6]

ACE2 is present in the lungs; the blood vessels, the renal tubular cells; the stomach and intestines; endothelial and smooth muscle cells in the human brain; and the Leydig and Sertoli cells in the seminiferous ducts in the testis. Monteiro *et al.*, noted in PlosONE in 2020, that it is more highly expressed in smokers and in obese patients, which might partly explain their increased risk of infection and need for ventilation.

The ACE2 receptor is part of the Renin Angiotensin Aldosterone System (RAAS), converting Angiotensin II into Angiotensin *via* its binding to the Mas receptor. Angiotensin II is vasoconstrictive, pro-inflammatory and pro-coagulation, as well having a role in increasing blood pressure. The interaction between the virus and ACE2 leaves it depleted through receptor endocytosis and, therefore, leaves the damaging Angiotensin II unopposed, meaning that the body loses the positive effects of Angiotensin. The loss of the beneficial metabolic effect of angiotensin may also explain why both obese patients and those with diabetes are at greater risk. A link has also been made with the metabolic syndrome, which is more common in males, and the severity of the disease.[7, 8]

The beneficial effects of angiotensin include its vasodilatory and anti-inflammatory properties, and has a role in glucose homeostasis, lipid metabolism, and energy balance. It is both cardio-protective and neuro-protective and has a positive effect in reducing lung injury and kidney pathology.[9]

The XX hypothesis

As described by Channappanavar *et al.*, in the *Journal of Epidemiology* in 2017, ACE2 is produced by the X-chromosome, females have two X-chromosomes and have twice the capacity to form the enzyme and create two types of the ACE2. As males only have one X-chromosome, they also have only one form of ACE2. This means that if the virus can unlock the single form of male ACE2 it has access to every cell in which the enzyme is present, while in women the virus has to unlock both of the two forms of ACE2 (one from each X-chromosome) to have the same impact. The effect of this on males is two-fold: it means that the higher ACE2 levels in males may make it easier to get the infection. Once men become infected, they may have less ACE2 and therefore less angiotensin available to help counter the damaging effects of Angiotensin II, as described above. For females there may be less virus entry into the cells, and also more remaining unaffected cells and angiotensin to tackle subsequent lung injury.

The highest levels of ACE2 are found in the younger age patients, which would appear counterintuitive in relation to infection rates and severity of the disease in older age patients. The young are less likely to have chronic diseases and comorbidities and they may be more able to use its protective function to fight the disease. In older age patients, a reduced ACE2 may mean the enzyme is more quickly exhausted, leading the risk of more severe disease.

The ACE2 is also highly expressed within the testis and the prostate, with orchitis, infertility and testicular tumour identified in the earlier SARS-CoV outbreak.[6]

As a result, there may be a long-term impact on fertility of this ACE2 prevalence in male-specific organs and the virus may be transmissible in seminal fluid. Therefore, precautions are needed during intercourse until further research clarifies the situation.

Endothelial dysfunction

The 2017 BSSM ED guideline points out that endothelial dysfunction is an important risk factor for cardiovascular disease (CVD), and is a feature in men with erectile dysfunction and Type 2 diabetes.

The SARS-CoV-2 infection induces endothelitis in multiple organs leading to apoptosis which plays an important role in endothelial cell injury in these patients. From the practical viewpoint, using drugs that improve endothelial function, such as PDE5i's, ACE inhibitors and statins, could be very important in these patients, although many of the vulnerable patients will already be on these drugs because of pre-existing endothelial dysfunction and its known association with male sex and vascular risk factors.

A large body of experimental evidences have shown that the combination of T replacement therapy (TRT) and phosphodiesterase type 5 inhibitors (PDE5i) is, usually, effective in restoring erectile function in patients with LOH and ED who have not responded to monotherapy for sexual disturbances. In the situation of a

significant SARS-CoV-2 infection, a combination may be helpful, PDE5is potentiate the action of nitric oxide (NO) produced by endothelial cells, resulting in a vasodilator effect, while T facilitates PDE5i effects by increasing the expression of PDE5. Meta-analytic data have identified a protective role of PDE5i on the cardiovascular health in patients with decreased left ventricular ejection fraction. In addition, several studies have shown pleiotropic beneficial effects of these drugs throughout the body (*i.e.*, on bones, urogenital tract and cerebral, metabolic, and cardiovascular levels). TRT itself is able to decrease endothelial dysfunction, oxidative stress and inflammation, thus lowering the cardiovascular risk.[10]

Coagulopathy

In addition, the vascular immunopathology associated with COVID-19 presents as a diffuse pulmonary intravascular coagulopathy, which in its early stages is distinct from disseminated intravascular coagulation. Increased circulating D-dimer concentrations caused by pulmonary vascular bed thrombosis with resultant fibrinolysis and elevated cardiac enzyme concentrations in the face of normal fibrinogen and platelet levels are key early features of severe pulmonary intravascular coagulopathy related to COVID-19. Extensive immunothrombosis over a wide pulmonary vascular territory before the confirmation of early COVID-19 viraemia possibly explains the adverse impact of male sex, hypertension, obesity, and diabetes on the prognosis of patients with COVID-19. The combination of immunomodulatory and anticoagulant strategies in patients with high D-dimer concentrations is a possible approach to reducing morbidity and mortality.[11]

Cytokine proteins

The immune system is supported by the cytokine proteins, which act as a communicator between cells. They are involved in the pro-inflammatory process, with some (CCL2, CCL3, CCL4 and CCL16) having a protective effect and are found more often in women. On the other hand, men to tend to have more of the interleukin cytokines (IL6ST, IL-7, IL-16 and IL-18) that provoke more of an inflammatory response and, with excess stimulation, can lead to the cytokine release syndrome (or cytokine storm) that can rapidly overtake the immune system of the body and result in a catastrophic shock. Men also tend to have more highly expressed TNFSF13b (BAFF), which is associated with an increased risk of inflammation and is associated with the progression of chronic obstructive pulmonary disease (COPD).[6]

FURIN AND TMPRSS2

For the virus to get into the cells they also need two spike proteins (FURIN and TMRPSS2), with FURIN more highly expressed in the lungs of smokers (men being

more prevalent smokers) and the fact that TMPRSS2 is an androgen-responsive gene and more responsive to testosterone and dihydrotestosterone has been suggested to contribute to male predominance of COVID-19 infection. This is because the androgen receptor activates the transcription of TMPRSS2 enhancing the transmissibility of COVID-19 infection and because TMPRSS2 are expressed also at pulmonary level, the use of TMPRSS2 inhibitors, and antiandrogens are being investigated to modulate COVID-19 infections and treat COVID-19 pneumonia.[12]

Despite this link with TMPRSS2, a recent paper has cast doubt on the advisability of using ADT (androgen deprivation therapy) because it upregulates ACE2, hence the advice to avoid ADT in certain groups of prostate cancer patients.[13]

Wider immunity factors

Females also have higher expression of TLR7 and TLR8, both of which are important in immune responses and found (and remain active) on both of the X-chromosome, whereas in men there is just a single copy. As such, females are more likely to activate a successful immune response and have been suggested to be more active with single strand viruses such as SARS-CoV-2.[14]

A hormonal component is also at play, with oestrogen having an immuno-protective function by the regulation of myeloid cells and innate lymphocytes and in dampening the proinflammatory cytokines.[15]

Wu *et al.* noted, in the New England Journal of Medicine in 2010, that oestrogen may also have a more direct effect on the metabolic function of the cell limiting viral replication. It also promotes type 2 repair responses of alveolar macrophages and resolution of the immune response to the virus. Age may therefore have an effect on the viral infection, with oestrogen levels falling in women quickly in the perimenopause whereas testosterone levels remain stable in 75% of men into old age, but this does leave a significant number of men both testosterone and oestrogen deficient.

Female patients are able to achieve viral clearance significantly earlier than males, this may be because the testes were shown to be one of the highest sites of ACE2 expression in 3 independent RNA expression databases (Human Protein Atlas, FAMTOM5 and GETx).

Testosterone

ACE2 was also determined to be highly expressed in testicular cells at the protein levels. In contrast very little expression of ACE2 was seen in ovarian tissue. High expression of ACE2 in testes raises the possibility that testicular viral reservoirs may play a role in viral persistence in males.[16]

Serum testosterone levels may be adversely affected by the testicular involvement and have a significant impact on recovery. Testosterone may also be helpful by downregulating inflammation. Testosterone deficiency (TD) is associated with

increased pro-inflammatory cytokines and testosterone treatment reduces IL-1β, IL-6, and TNF-α, and a pro-inflammatory state and decline in testosterone has been demonstrated in aging men and those with vascular disease.[17]

In theory testosterone may have a role in the events leading to progression of COVID-19 infection and the cytokine storm. Suppression of ACE2 expression by inflammatory cytokines accompanied by the decrease of androgen and oestrogen in some ageing men, may establish a negative correlation between ACE2 expression and COVID-19 mortality.

Testosterone levels should be investigated in these men affected by COVID-19 because of the known adverse impact of testosterone deficiency on cardiovascular mortality and heart failure, and men with a low testosterone may be at high risk if they become infected.

Several publications where non-covid patients have been treated with testosterone replacement therapy (TRT) to restore testosterone levels to normal have shown significant reduction in hospitalisation and all-cause mortality, and reversal and prevention of diabetes, and therefore testosterone may have a role in relevant patients who are hypogonadal, with COVID-19.[18]

What is the impact of covid infection on testosterone levels in the real world?

In a study from Germany involving 45 acute SARS-CoV-2 admissions (35 male, 10 female) 54% of male patients had TT levels below 4.9 nmol/L, with 25.7% below 3 nmol/L on admission. They considered 6.68 nmol/l as normal. Luteinising hormone (LH) levels were raised in 31.4%, and oestradiol was raised in 31.4% and was associated with increased inflammatory markers, especially IL-6. The authors concluded that critically ill male COVID-19 patients suffer from severe testosterone and dihydrotestosterone deficiencies.[5]

In a prospective cohort study[19] which included 262 men aged between 20 and 65 years. The researchers studied 3 groups of patients with COVID-19 (N.=89), cases with non-COVID-19 respiratory tract infection (N.=30), and age-matched controls (N.=143). Correlation analysis revealed significant negative correlation between serum TT levels and hospitalization time of patients with COVID-19 ($r=-0.45$, $P<0.0001$). In addition, a significant positive correlation was observed between SpO_2 and serum TT levels in patients with COVID-19 ($r=0.32$, $P=0.0028$).

This study demonstrates COVID-19 is associated with decreased level of TT and increased level of LH and prolactin. More serious COVID-19 causes a greater reduction in TT levels and prolongs hospitalisation period.

In a case control study,[20] demographic, clinical, and hormonal values were collected for all patients. Hypogonadism was defined as tT ≤9.2 nmol/L. The Charlson Comorbidity Index (CCI) was used to score health-significant comorbidities.

Severe clinical outcomes were defined as patients either transferred to intensive care unit (ICU) or death. Descriptive statistics and multivariable linear and logistic regression models tested the association between clinical and laboratory variables and tT levels. Univariable and multivariable logistic regression models tested the association between tT and severe clinical outcomes.

Overall, a significantly lower levels of LH and tT were found in patients with COVID-19 compared to healthy controls (all P<0.0001); conversely, healthy controls depicted lower values of circulating E2 (P<0.001).

Testosterone levels suggestive for hypogonadism were observed in a large proportion, 257 (89.8%) patients at hospital admission. In as many as 243 (85%) cases, hypogonadism was secondary. SARS-CoV-2 infection status was independently associated with lower tT levels (P<0.0001) and greater risk of hypogonadism (P<0.0001), after accounting for age, BMI, CCI, and IL-6 values. Lower tT levels were associated with higher risk of ICU admission and death outcomes (all P≤0.05), after accounting for clinical and laboratory parameters.

There was an independent association between SARS-CoV-2 infection status and secondary hypogonadism already at hospital admission, with lower testosterone levels predicting the most severe clinical outcomes.

In terms of progression of COVID-19 in men: the question of testosterone as a key factor, was raised by Giagulli *et al.*,[21] who searched PubMed/MEDLINE, Web of Science, EMBASE, Cochrane Library, Google, and institutional websites for medical subject headings terms and free text words referred to "SARS-CoV-2," "COVID-19," "testosterone," "male hypogonadism," "gender" "immune system," "obesity," "thrombosis" until May 19th 2020.

They demonstrated that in comparison to oestrogen, testosterone may predispose men to a widespread COVID-19 infection. Low serum levels of T, which characterises the hormonal milieu in seriously ill individuals, may predispose men, especially elderly men, to poor prognosis or death.

In an Italian study involving 31 male patients, those managed routinely on medical wards had a mean TT level of 8.8 nmol/L, with those on respiratory intensive care units having a mean level of 5 nmol/L, and those on intensive care units (N.=4) having a mean TT level of 1.0 nmol/L, with two out of every four of these men dying. There was a steep increase in both ICU transfer and mortality risk in men with TT<5 nmol/L or cFT<100 pmol/L. A low calculated free testosterone (cFT) on admission predicted a poor prognosis.[22]

A study of 81 admissions of male patients with COVID-19 infection from Wuhan, China, with a mean age of 38 years, also showed reduced TT levels and raised LH, suggestive of a compensated primary hypogonadism.[23]

In this pre-publication study, the time-to-clearance of the SARS-CoV-2 virus in symptomatic patients was investigated by serial oropharyngeal/nasopharyngeal swabs followed by RT-PCR (RdRp gene) test. A total of 68 Indian subjects with median age of 37 years (the range was 3-75 years) were examined and included 48

(71%) males and 20 (29%) females. Female patients were able to achieve viral clearance significantly earlier than males, with a median difference of two days in achieving a negative PCR result (P value =0.038). In order to explain this variance, the researchers studied expression patterns of the ACE2 in tissue specific repositories. The testes were one of the highest sites of ACE2 expression in three independent RNA expression databases (Human Protein Atlas, FAMTOM5 and GETx). ACE2 was also determined to be highly expressed in testicular cells at the protein levels.

In contrast, very little expression of ACE2 was seen in ovarian tissue. The authors pointed out that the high expression of ACE2 in testes raises the possibility that testicular viral reservoirs may play a role in viral persistence in males requiring further research. Serum testosterone levels may also be adversely affected and impact on recovery.

Cayan *et al.*,[24] reported their results in 232 men hospitalised with COVID-19 in Turkey. Serum hormone levels were obtained on the day of hospitalisation. Testosterone deficiency was noted in 51.1%, including severely depressed values (<100 ng/dL) in 26 men, and 25 with testosterone levels of 101-199 ng/dL. The probability of transfer to ICU increased with decreasing serum testosterone. Of the 11 deaths, 10 (90.9%) had serum testosterone <300 ng/dL. Eight of 11 deaths (72.7%) were in men with markedly reduced serum testosterone levels (<200 ng/dL). Only one death occurred in a man with normal serum testosterone level on the first day of hospitalisation.

Counter-intuitively ADT has been suggested as a treatment on the basis of a reported improved survival from COVID-19 in men with prostate cancer.[25]

Five alpha reductase inhibitors (5ARIs) have also been advocated, despite evidence provided by Keeting *et al.*, in the Journal of the National Cancer Institute in 2010 and Wei *et al.*, in the BMJ in 2019, that both ADT and 5ARIs increase the risk of T2DM by up to 30%.

However, both of these strategies appear inadvisable in the light of the recent findings from numerous studies described above, demonstrating extremely low testosterone and di-hydro testosterone (DHT) levels in acutely ill patients.

Rambhatia *et al.*[26] sought to determine if testosterone replacement therapy (TRT) was associated with worse clinical outcomes. Using a retrospective chart review, they identified 32 men diagnosed with COVID-19 and on TRT. These men were propensity score matched to 63 men diagnosed with COVID-19 and not on TRT. Data regarding comorbidities and endpoints such as hospital admission, intensive care unit admission, ventilator utilization, thromboembolic events, and death were extracted. Chi-square and Kruskal-Wallis tests examined differences in categorical and continuous variables, respectively. Logistic regression analysis tested the relationship between TRT status and the study endpoints.

There were no statistically significant differences between the 2 groups, and TRT was not a predictor of any of the endpoints on multivariate analysis. These results suggest that TRT is not associated with a worse clinical outcome in men diagnosed with COVID-19 and appears to be a safe approach.

Conclusions

Multiple studies suggest that men admitted with COVID-19 have significantly lower testosterone levels than with other acute hospital admissions. Furthermore, the virus is associated with a severe primary hypogonadism, occurring in addition to the functional secondary hypogonadism that is associated with comorbid conditions.

By not measuring testosterone, we are missing a trick, because there is an opportunity to treat men acutely with testosterone to boost resistance to the 'cytokine storm' associated with COVID-19 infection. Studies carried out both in animals and humans have shown that hypogonadism is associated with increased pro-inflammatory cytokines and that testosterone treatment reduces IL-1β, IL-6, and TNF-α.[27]

There seems no logic for using oestrogens in the acute phase as victims of COVID-19 already have raised oestradiol levels.[5]

We need to identify the untreated hypogonadal population with comorbidities, who may have survived the current pandemic but who may be at considerable risk from third wave infection, or future viral pandemics. We now have firm evidence that TRT reverses progression to T2DM.[28]

Current evidence-based guidelines from multiple medical disciplines already recommend screening, diagnosing, and treating men with hypogonadism in high-risk groups, such as T2D, BMI >30 kg/m^2 and men with ED, and there have been two recent reviews by Kirby in Eur Cardiol 2019 and Sarkar in Prostate Cancer Prostatic Dis 2020, underscoring the cardiovascular and prostate safety of TRT, together with a recent study demonstrating the safety of TRT.[26]

In seriously ill patients, using dexamethasone, managing thrombotic risk, protecting endothelial function and considering testosterone replacement, if subnormal, in a trial setting should be considered in every male patient.

From the practical viewpoint, using drugs that improve endothelial function, such as daily PDE5 inhibitors, ACE inhibitors/Angiotensin Receptor Blockers (ARBs) and statins, could also be very important in these patients. Anti-thrombotics also have a place because of diffuse microvascular thrombi in multiple organs, mostly in pulmonary micro-vessels. The thrombotic risk seems to be directly related to disease severity and worsens patients' prognosis.[29]

In addition, the WHO has produced a guideline for the use of corticosteroids in patients infected with SARS-CoV-2.[30]

The importance of this chapter in the book is highlighted, and the need to check testosterone levels in men with COVID-19 both during and after hospital admission, by a 2021 publication from Italy. The authors aimed to assess total testosterone levels and the prevalence of total testosterone still suggesting for hypogonadism at 7-month follow-up in a cohort of 121 men who recovered from laboratory-confirmed COVID-19. Hypogonadism was defined as total testosterone ≤9.2 nmol/L. The authors concluded that, although total testosterone levels increased over time after COVID-19, more than 50% of men who recovered from the disease still

had circulating testosterone levels suggestive for a condition of hypogonadism at 7-month follow-up. In as many as 10% of cases, testosterone levels even further decreased. Of clinical relevance, the higher the burden of comorbid conditions at presentation, the lower the probability of testosterone levels recovery over time. This may well be a contributing factor to so called "Long COVID" which appears to be often ignored.[31]

References

1. Coronavirus Disease Statistics. [Internet]. Available from: https://www.google.com/search?q=the+current+death+toll+for+coronavirus&sxsrf=ALeKk01sDeCwM4D7RVsUTXXQwdDn5yJBvQ%3A1618039735035&source=hp&ei=tlNxYNfYPMm7lwTF7JGIBQ&iflsig=AINFCbYAAAAAYHFhxxqkvCEt3VHxFhLOAjDRsP7jJHYj&oq=The+current+death+toll&gs_lcp=Cgdnd3Mtd2l6EAEYADICCAAyBggAEBYQHjIJCAAQyQMQFhAeMgYIABAWEB46BwgjEOoCECdQmBxYmBxgjTpoAXAAeACAAT-IAT-SAQExmAEAoAECoAEBqgEHZ3dzLXdperABCg&sclient=gws-wiz [Accessed 2021, May 5].
2. Márquez EJ, Trowbridge J, Kuchel GA, *et al.* The lethal sex gap: COVID-19. Immun Ageing 2020;17:13.
3. Peckham H, de Gruijter NM, Raine C, *et al.* Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. Nat Commun 2020;11:6317.
4. Ahmadzadeh J, Mobaraki K, Mousavi SJ, *et al.* The risk factors associated with MERS-CoV patient fatality: A global survey. Diagn Microbiol Infect Dis 2020;96:114876.
5. Schroeder M, Tuku B, Jarczak D, *et al.* The majority of male patients with COVID-19 present low testosterone levels on admission to Intensive Care in Hamburg, Germany: a retrospective cohort study. MedRxiv 2020.
6. Wei X, Xiao Y-T, Wang J, *et al.* Sex Differences in Severity and Mortality Among Patients With COVID-19: Evidence from Pooled Literature Analysis and Insights from Integrated Bioinformatic Analysis. ArXiv (Preprint) 2020. Available from: http://arxiv.org/abs/2003.13547
7. Márquez EJ, Trowbridge J, Kuchel GA, *et al.* The lethal sex gap: COVID-19. Immun Ageing 2020;17:13.
8. Marhl M, Grubelnik V, Magdič M, *et al.* Diabetes and metabolic syndrome as risk factors for COVID-19. Diabetes Metab Syndr 2020;14:671-7.
9. Hess DC, Eldahshan W, Rutkowski E. COVID-19-Related Stroke. Transl Stroke Res 2020;11:322-5.
10. Aversa A, Duca Y, Condorelli RA, *et al.* Androgen Deficiency and Phosphodiesterase Type 5 Expression Changes in Aging Male: Therapeutic implications. Front Endocrinol (Lausanne) 2019;10:225.
11. McGonagle D, O'Donnell JS, Sharif K, *et al.* Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia. Lancet Rheumatol 2020;2019:1-9.
12. Hoffmann M, Kleine-Weber H, Schroeder S, *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020;181:271-80.

13. Bhowmick NA, Oft J, Dorff T, *et al.* COVID-19 and androgen targeted therapy for prostate cancer patients. Endocrine-related cancer. Endocr Relat Cancer 2020;27:R281-R292.

14. de Groot NG, Bontrop RE. COVID-19 pandemic: is a gender-defined dosage effect responsible for the high mortality rate among males? Immunogenetics 2020;72:275-7.

15. Kadel S, Kovats S. Sex hormones regulate innate immune cells and promote sex differences in respiratory virus infection. Front Immunol 2018;9:1-15.

16. Shastri A, Wheat J, Agrawal S, *et al.* Delayed clearance of SARS-CoV2 in male compared to female patients: High ACE2 expression in testes suggests possible existence of gender-specific viral reservoirs. MedRxiv (Preprint) 2020.

17. Mohamad NV, Wong SK, Wan Hasan WN, *et al.* The relationship between circulating testosterone and inflammatory cytokines in men. Aging Male 2019;22:129-40.

18. Yassin A, Haider A, Haider K, *et al.* Testosterone Therapy in Men With Hypogonadism Prevents Progression From Prediabetes to Type 2 Diabetes: Eight-Year Data From a Registry Study. Diabetes Care 2019;42:1104-11.

19. Kadihasanoglu M, Aktas S, Yardimci E, *et al.* SARS-CoV-2 Pneumonia Affects Male Reproductive Hormone Levels: A Prospective, Cohort Study. J Sex Med 2021;18:256-64.

20. Salonia A, Pontillo M, Capogrosso P, *et al.* Severely low testosterone in males with COVID-19: A case-control study. Andrology 2021;9:1043-52.

21. Giagulli VA, Guastamacchia E, Magrone T, *et al.* Worse progression of COVID-19 in men: Is testosterone a key factor? Andrology 2021;9:53-64.

22. Rastrelli G, Di Strasi, Inglese F, *et al.* Low testosterone levels predict clinical adverse outcomes in SARS-CoV-2 pneumonia patients. Andrology 2021;9:88-98.

23. Ma L, Xie W, Danyang L, *et al.* Effect of SARS-CoV-2 infection upon male gonadal function: A single center-based study. MedRxiv 2020.

24. Çayan S, Uguz M, Saylam B, *et al.* Effect of serum total testosterone and its relationship with other laboratory parameters on the prognosis of coronavirus disease 2019 (COVID-19) in SARS-CoV-2 infected male patients: a cohort study. Aging Male 2020;3:1-11.

25. Montopoli M, Zumerle S, Vettor R, *et al.* Androgen-deprivation therapies for prostate cancer and risk of infection by SARS-CoV-2: a population-based study (N = 4532). Ann Oncol 2020;31:1040-5.

26. Rambhatla A, Bronkema CJ, Corsi N, *et al.* COVID-19 Infection in Men on Testosterone Replacement Therapy. J Sex Med 2021;18:215-8.

27. Pozzilli P, Lenzi A. Testosterone, a key hormone in the context of COVID-19 pandemic. Metabolism 2020;108:154252.

28. Wittert GA, Robledo KP, Grossman M, *et al.* Effect of Testosterone Treatment on Type 2 Diabetes Incidence in High-Risk Men Enrolled in a Lifestyle Program: A Two-Year Randomized Placebo-Controlled Trial. Diabetes Jun 2020;69 (Suppl 1):274-OR.

29. Carfora V, Spiniello G, Ricciolino R, *et al.* Anticoagulant treatment in COVID-19: a narrative review. J Thromb Thrombolysis 2020;18:1-7.

30. Lamontagne F, Agoritsas T, Macdonald H, *et al.* A living WHO guideline on drugs for covid-19. BMJ 2020;370:m3379.

31. Salonia A, Pontillo M, Capogrosso P, *et al.* Testosterone in males with COVID-19: A 7-month cohort study. Andrology 2022;10:34-41.

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Future developments in hypogonadism and diabetes and the role of androgen receptor sensitivity

T. Hugh Jones

Symptomatic testosterone deficiency is common in men with type 2 diabetes with up to 40% having the condition.[1] The American Diabetes Association (ADA) now recognise in their Standards of Care that testosterone deficiency is a common co-morbidity.[2] In the presence of symptoms of hypogonadism, testosterone replacement therapy can be considered. There is however increasing evidence from clinical trials that testosterone has other therapeutic benefits on key components of the metabolic syndrome, obesity and mortality.

Health problems in men with type 2 diabetes can be complex with the effects on the disease affecting their physical illness being confounded by psychological, emotional and social factors. Erectile dysfunction in diabetes is common with atherosclerosis being the most likely cause, however, in combination with testosterone deficiency a response to PDE-5 inhibitors may be impaired or ineffective. Erectile dysfunction independently reduces quality of life which is further adversely affected by lower testosterone levels. Many people with type 2 diabetes live with one or more common co-morbidities such as cardiovascular disease, chronic obstructive pulmonary disease COPD), kidney failure, osteoarthritis, retinopathy and reduced mobility. Many of these conditions are also associated with testosterone deficiency.[3] These conditions in addition to sexual dysfunction all have an effect on worsening quality of life.

Evidence from several research publications has shown that testosterone deficiency adversely affects glycaemic control which is the corner stone of diabetes complications risk reduction. Hypogonadism is also associated with obesity, increased risk of cardiovascular disease and mortality. Furthermore, low testosterone is associated with an increased risk of infection linked to immune system dysfunction. Recent evidence suggests that a low testosterone state causes an increased severity of COVID infection (see Chapter 18).[4]

Prediabetes and metabolic syndrome

Obesity is part of a triad linked with type 2 diabetes and testosterone deficiency.[5] Waist size and BMI are both negatively associated with total, bioavailable and free testosterone and SHBG but are positively associated with oestradiol.[1] Reduction in weight is well known to be associated with an improvement in diabetes control and an increase in testosterone levels. Weight loss in type 2 diabetes clinical studies can be successful up to 10% reduction with diet and exercise.[6] This effect is not however maintained after 12 months. In clinical practise in the real world only very few people manage a 5% weight loss. An increase of testosterone levels by 2-4 nmol/L requires a weight reduction of 10%. This degree of change is unlikely to make any significant changes in symptoms of testosterone deficiency. Patients with Testosterone deficiency have reduced or lack of motivation which will impair a successful fall in weight.

Bariatric surgery does increase testosterone by approximately 10 nmol/L with marked weight loss.[6] However, the surgery is not available economically or in some not appropriate to the majority of patients with morbid obesity. Lifestyle advice a combination of diet and exercise is important in both obesity and testosterone deficiency in association with obesity.

Pre-diabetes and metabolic syndrome are conditions which are very common in the western world but not all subjects will progress to develop overt type 2 diabetes. Testosterone deficiency is also common in these conditions. There has been a great interest by doctors and governments in the prevention of diabetes and the reduction in stopping or delaying the number of patients progressing from prediabetes to type 2 diabetes. In the USA, the Centre for Disease Control and Prevention has reported that, 1 in 3 people in the population has prediabetes and 10.5% have Type 2 Diabetes. The main areas of research have been in improved diets and physical exercise resulting in weight loss. There is some evidence that metformin reduces the risk of type 2 diabetes developing in those with prediabetes. However, metformin is rarely used to reduce this risk and the mainstay of treatment in the world is diet and physical exercise.[6]

A recent large, randomised placebo-controlled trial of over a thousand men in Australia T4DM with prediabetes and low testosterone (symptoms and total testosterone $\leq$14 nmol) has clearly demonstrated that testosterone replacement with long-acting intra-muscular testosterone undecanoate along with lifestyle intervention demonstrated a 41% relative risk reduction in progression to full diabetes over two years of the trial when compared to lifestyle intervention alone.[7] The primary outcome measured was the oral glucose tolerance test with a 2-hour glucose $\geq$11 mmol/L). Secondary outcomes included improved sexual function, reduction in body fat, waist circumference and increased muscle mass.

Safety analysis did not find any increase in serious adverse events of testosterone therapy over placebo including no increase in cardiovascular events. Increases in

haematocrit as expected in testosterone therapy occurred but had no deleterious clinical effects. It is important that clinicians understand that evidence confirms that a haematocrit level of ≤0.54 is not associated with any increased risk of arterial or venous thrombo-embolism as stated in international guidelines on testosterone therapy management. If the haematocrit exceeds 0.54 then adjustment in testosterone dose or formulation and if necessary venesection can lower the haematocrit to guideline safety levels.[8] There was a small increase in PSA levels which again is expected due to the fact the prostate shrinks in testosterone deficiency and regains its normal size for an individual after replacement.

Type 2 diabetes

The T4DM study is currently the largest published clinical trial of testosterone replacement therapy and the first in prediabetes. There have been a number of RCT trials in type 2 diabetes which have reported an improvement in insulin resistance, reduction in body fat, waist circumference and lipid profile. Some but not all have found a benefit on reduction of HbA1c.[1, 7-10] Some studies have not shown benefits. The reasons between differences in the findings of these studies are likely to be due to a combination of study length and the number of subjects as it is probable that the testosterone induced changes to body composition and pathways of glucose uptake and metabolism require several months and years to have its full effects. In addition, the severity of insulin resistance and longer duration of diabetes may have an impact.

The central biochemical defect in type 2 diabetes is insulin resistance a condition which mediates the development of the components of the metabolic syndrome and type 2 diabetes. These include hyperglycaemia, hyperlipidaemia (hypertriglyceridaemia, low HDL-cholesterol), visceral adiposity and hypertension. Insulin resistance is defined as the reduced ability of the major metabolic tissues (fat, liver and muscle) to uptake and utilise glucose and triglycerides and store fat in the normal physiological depots. Over the last few years, scientific research has shown that testosterone stimulates the expression of the major glucose transporter GLUT4, key regulatory enzymes of glycolysis and stimulates mitochondrial oxidative phosphorylation but suppresses the regulatory enzymes of fatty acid synthesis.[11] In addition, a state of testosterone deficiency reduces the expression of enzymes which take up fatty acids into subcutaneous fat depots. This results in excess saturated fats in the circulation being deposited in visceral fat, the liver, muscle and other ectopic fat depots.[12] This 'overspill' of fat can also involve the development of lipid streaks and atheromatous plaques thus increasing the risk of cardiovascular disease and consequent events. Several epidemiological studies have shown that atherosclerosis is more prevalent in men.

Several animal studies have reported that testosterone deficiency enhances the development of the atherosclerotic plaque whereas testosterone replacement protects against the first stages of atherosclerosis.[13] Further scientific work in the

laboratory is needed to provide more detail on testosterone actions. Future developments in mechanistic and translational science combined with clinical studies will support and verify the actions of testosterone in men.

Evidence from registry studies have reported that the benefits on glycaemic control, body composition and obesity as well as many other parameters improve after one year and continue at least up to 11 years.[14, 15] These studies have also found a significant number of men pass into remission of their diabetes over the years after initiation of testosterone therapy. A high percentage of 34.3% of the testosterone treated compared to none in the control group had remission of their diabetes and 90% of the testosterone treated patients achieved a HbA1c <53 mmol/mol (7%). These studies have also found that there is continual reduction in body weight, BMI and waist circumference. These findings support a role for testosterone replacement in men with type 2 diabetes and warrant further investigation.

Mortality

Testosterone deficiency has been demonstrated in several studies to be associated with an increased risk of earlier death over periods of follow up of between 5.8 and 14 years in men with Type 2 diabetes as well as in community populations and men with specific diseases including cardiovascular and renal diseases.[16-18] Importantly one study has shown that men with hypogonadism treated with testosterone replacement improved survival which was similar to men with type 2 diabetes and normal testosterone.[16]

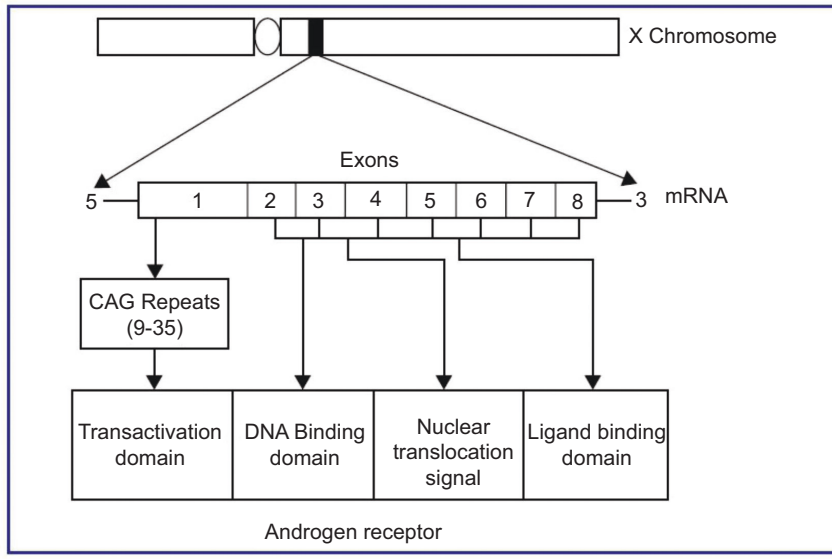
Several registry studies have found that testosterone deficiency is associated with an increased risk of early mortality.[15, 17] Some studies have shown that the major cause of death is due to cardiovascular disease.[19] However low testosterone is also linked to respiratory disorders, cancer and renal disease.[20]

Epidemiological studies and registry studies as described elsewhere found that testosterone replacement improved survival.[15-17, 19]

Androgen receptor sensitivity

Testosterone mediates its effects by either activation of the androgen receptor (AR) or by non-AR mechanisms including non-genomic actions. The majority of the biological actions of testosterone are mediated via the AR.[21, 22] The AR is a nuclear receptor which is present in the cytosol as well as within the nucleus of the cell. Testosterone binds to the AR in the cytosol and then the complex translocates to the nucleus. The activated AR then binds to various genes within the genome to mediate its effects. The AR gene has eight exons (Figure 19.1). Exon 1 encodes for the region where activators bind (*e.g.*, Heat Shock Protein 70) which then through conformational changes in the AR protein enhance the binding of testosterone and other androgens including dihydrotestosterone. There is a CAG repeat polymorphism within Exon

Figure 19.1. Androgen receptor gene.



1 which in men, ranges between 9 to 35 repeats which codes for a polyglutamine amino acid stretch.[23] The greater the number of CAG repeats and hence polyglutamine stretch leads to a more insensitive receptor. In a population the mean number of repeats is 21 with the majority of men having 19-23 repeats.

Men with more sensitive receptors (low CAG repeat numbers) are more likely to have low normal range (<12 mol/L) serum testosterone levels compared to those with less sensitive AR's (high CAG repeats) who have higher normal range testosterone. The men with the less sensitive receptors have been shown to have higher serum Luteinising hormone (LH) and Follicle Stimulating Hormone (FSH) levels. This indicates that the less sensitive AR has a reduced negative feedback of testosterone on the hypothalamic-pituitary axis which leads to stimulation of the testes to secrete more testosterone to compensate for relative AR insensitivity. This finding has been confirmed in men with type 2 diabetes.

The spread of CAG repeats is similar in normal and type 2 diabetes male populations. However, in the diabetes population men with lower androgen sensitivity are more likely to have a higher waist circumference, BMI and serum leptin which correlates with total body fat content.[24] A less sensitive AR was also associated with a higher systolic blood pressure. This study above does support the hypothesis that a more sensitive AR leads to better body anthropomorphology which may reduce the risk of developing type 2 diabetes. It is also fact that a CAG repeat number greater than 35 increases the risk of developing Kennedy syndrome which is a rare neurological degenerative condition which is associated with the onset of diabetes (Table 19.I).[25]

Table 19.I. Effects of Androgen Receptor Sensitivity (CAG Polymorphism) on Biological Parameters in Men with and without Type 2 Diabetes.

Metabolic	Hormonal	Prostate	Mental Health
↑BMI	↑Testosterone	↓Prostate size	↑Risk of depression
↑Waist circumference	↑LH/FSH	↓Risk of BPH	↓Risk of conduct disorders
↑Body fat content	↓Sperm count	↓Prostate cancer risk	↓drug abuse
↑Leptin	↓BMD		↓pathological gambling
↑Fasting insulin		↓Attention deficit disorder	
↑HbA1c			
↑HDL-cholesterol			
Response to TRT			
↑Hb and Hct			
↑LH suppression			
↑Prostate growth			
↑Fasting insulin (trend to improve HOMA-ir)			
↑Triglycerides			
↑Diastolic b lood pressure			

Low AR Sensitivity (High CAG Repeats) compared with High AR Sensitivity (Low CAG Repeats)

Men in general with more sensitive AR's have been reported to have a greater association with the onset of symptoms of loss in vitality, increased risk of benign prostatic hypertrophy and prostate carcinoma, and a low HDL-cholesterol. In men with type 2 diabetes HDL-cholesterol also correlated negatively with AR sensitivity with men with less sensitive AR having a greater HDL-cholesterol.[26] AR sensitivity did not correlate with total or LDL-cholesterol or triglycerides. However, fasting triglycerides were higher in men with lower serum testosterone. Men with type 2 diabetes who have a combination of low testosterone as well as low oestradiol had higher total and LDL-cholesterol but no association with AR sensitivity.

The TIMES2 (Testosterone in men with Metabolic Syndrome and/or type 2 Diabetes) study has shown that testosterone replacement in men with a less sensitive AR have a greater response to improvement in insulin sensitivity reflected in a significant reduction in circulating insulin and a trend to a benefit on reducing the HOMA-ir (Homeostatic Model of Insulin resistance).[27] Furthermore, testosterone treatment had a significant improvement in fasting serum triglyceride levels and diastolic blood pressure in men with less sensitive AR .

A long-term, 14 year follow up cohort study of 423 has found that men with relative AR insensitivity had evidence of poorer glycaemic control with elevated HbA1c.[25] For each increase in CAG number of 1 repeat there was a 0.1% increase in HbA1c. There was a higher mortality in the men with hypogonadism (55.8%) compared with men who did not have testosterone deficiency (36.1%). This study also showed that there was a 'U' shaped curve for mortality with the mean value of CAG repeats (N.=21) had a 50% lower mortality than those men with <21 or >21 CAG repeats. This analysis was independent of testosterone status.

The evidence provided above does clearly support a role of knowledge of the AR receptor sensitivity in the clinical management of patients with both diagnosis and testosterone replacement therapy. The assessment of CAG repeat number is currently not available in normal clinical practise. Current studies are in progress to determine the clinical value of the CAG polymorphism especially in men with type2 diabetes.

Clinical awareness

Testosterone deficiency in men is common but is under-recognised and therefore under-diagnosed in men with type 2 diabetes.[26] The American Diabetes Association (ADA) in their Standards of Care since 2018 have included Testosterone Deficiency as a common co-morbidity in men with Type 2 Diabetes.[2] The presence of a higher prevalence of low testosterone is also recognised in the UK on the website of Diabetes.[28] The association of symptomatic testosterone deficiency is included in many national and international guidelines on the management of this condition.[29] Yet it is a condition that is not considered by many clinicians not only in medicine in general but also those in specialties such as diabetes, endocrinology and urology. Many specialists do not treat testosterone deficiency unless it has a classical cause but not so-called functional hypogonadism due to obesity for example.[30] Testosterone deficiency is a cause of fatigue and tiredness but again is not usually considered in the differential diagnosis of these symptoms. It is even not thought of and measured when a standard baseline screen for causes of fatigue and tiredness do not reveal an aetiology. Many men may not volunteer symptoms of sexual problems due to embarrassment but will report a symptom which they are comfortable with on first meeting with a health professional.

So, for the future recognition and appropriate management of hypogonadism it must be considered by health professionals who are usually in first contact with patients. These are the primary care physicians in general practice and their nursing staff. Erectile dysfunction is recognised and accepted as having a high prevalence in diabetes (>70%) and is caused by atherosclerosis and/or testosterone deficiency in the majority of patients. Erectile dysfunction may be the first presentation of cardiovascular disease. There is a strong correlation of erectile dysfunction with silent coronary heart disease in diabetes. Even though this is well known, health professionals do not always ask the question at the diabetes annual review. In the UK when this

question was part of the Quality Outcome Framework (QOF) which was linked to monetary payments to the general practice there was an increased diagnosis of the condition. Measurement of testosterone is mandatory in a man with erectile dysfunction. Even cardiovascular risk scores now include erectile dysfunction as a scoring point *e.g.*, QRISK3.[31]

The future development of enhanced recognition and diagnosis of hypogonadism in diabetes is primarily dependent on increasing the awareness of the condition which has to include a knowledge of the benefits to patients not only in disease outcome but also in the improvement in quality of life. The clinical awareness including patient benefit does need to be conveyed to doctors and nurses by education, local and national guidelines. As with women's health in primary care where a practice doctor is assigned to this interest area there should equally be a doctor for men's health. Importantly specialists in an area from either endocrinology, diabetes, sexual health and urology should take a lead role for developing a support network for general practitioners. This should include a referral system for patient assessment and review of patients if required, local guidelines, national guidelines and the involvement of medical societies and government.

Diagnostics

There is no doubt that male hypogonadism in many cases is not as easy to diagnose as for example many conditions in medicine e.g., primary hypothyroidism. The core of the problem is that there is no clear cut-off level whereby diagnosis is clear. However, clinicians understand that the diagnosis of endocrine disorders can be a challenge for example with understanding cortisol and growth hormone. Testosterone has a circadian rhythm and is affected by meals so needs to be measured in the morning under fasting condition.[29] It is also measured as a total testosterone and not a free hormone. The normal range for testosterone varies according to which immunoassay is used. There is a lack of knowledge that some patients with symptoms in the lower part of the normal range have hypogonadism which does respond to testosterone replacement.

Furthermore, there needs to be an understanding of the measurement of Sex Hormone Binding Globulin (SHBG) in that testosterone bound to SHBG is considered to be inactive with the biologically active (bioavailable) component being a combination of free testosterone and albumin bound testosterone. Men with high levels of SHBG may have an insufficient levels of circulating bioavailable testosterone and therefore may require testosterone replacement therapy.

In addition, there is a poor understanding on the interpretation of LH and FSH. It is usually clear if LH and FSH are elevated in the presence of a low testosterone this is primary hypogonadism and if LH and FSH are below normal the patient has secondary hypogonadism. The fact that LH and FSH can be inappropriately normal in the presence of a low testosterone can be present in secondary hypogonadism is not widely understood.

It is also not appreciated that factors such as acute illness, surgery and trauma can suppress LH, FSH and testosterone can transiently suppress LH, FSH and testosterone levels. Investigation for testosterone deficiency should take place when the individual has returned to normal after the acute event usually 2 to 3 months later.

In summary, again an important future development in the progression of diagnosing and treated appropriately men with hypogonadism does need an uplift in education, guidelines and specialist support.

In the future, the evidence in the medical literature also demonstrates a role of the AR sensitivity in diagnosis and response to treatment. Knowledge of the CAG repeat length could be used to assess prognosis in individuals and to target higher testosterone replacement to achieve high normal testosterone levels in men with low sensitivity AR's. In addition, those men with either high and low sensitivity may require increased intensity to achieve health targets such as improved glycaemic and blood pressure control and weight reduction.

Cardiovascular benefit or risk

The publication of some studies which raised a potential possibility of an increased cardiovascular risk of testosterone therapy has caused a stumbling block in many clinicians against considering testosterone treatment. These studies have been reviewed by the FDA and been shown to have several flaws in the studies with the conclusion that there is no convincing evidence that normalisation of testosterone levels on replacement increases cardiovascular risk. Several short term albeit small, randomised placebo-controlled trials and longer term epidemiological and registry studies have found no increased cardiovascular risk. In fact, many studies have reported a reduction in Major Adverse Cardiovascular Events (MACE) and including mortality. Based on the current evidence the FDA could not exclude a weak link hence the requirement for a cardiovascular outcome study described below. The European Medicines Agency on their review of the data found no increased cardiovascular risk.

A large RCT study of testosterone *versus* placebo in men who have an increased cardiovascular risk, is underway in the United States called the TRAVERSE trial. This is a Study to Evaluate the Effect of Testosterone Replacement Therapy (TRT) on the Incidence of Major Adverse Cardiovascular Events (MACE) and Efficacy Measures in Hypogonadal Men. The proposed recruitment is of 6000 subjects. The duration of this study which is already in progress will be 4 to 5 years of testosterone therapy against those who are not treated.[32]

Conclusions

The major task of experts and researchers in the role of testosterone in prediabetes and type 2 diabetes is to convince doctors and nurses who manage men with these

conditions to investigate and treat if indicated. This however is a very significant challenge to be able to achieve these goals. Many of us who have performed clinical and scientific research studies and have successfully treated men with hypogonadism and diabetes are concerned that many men will not be diagnosed or treated. There is a substantial peer reviewed medical literature now on testosterone deficiency and its management. Future developments in the recognition, education and actions from health professionals is clearly dependent on several factors. Many of the patients will present to primary care physicians however they will need support from the specialist team in their area and also from national societies and governmental health departments.

In summary future developments include an increased clinical awareness and understanding. Better laboratory diagnostics which include standardisation of the biochemical ranges and widespread use of testosterone by mass spectroscopy. Knowledge that symptomatic men in the lower normal range may respond to testosterone therapy. Clear uncomplicated guidelines from the key national and international medical societies. Importantly recognition of the condition by endocrinologists, diabetologists and urologists that the condition of male hypogonadism from causes other than those described as the 'classical' aetiologies. The T4DM study does demonstrate a clinically relevant and beneficial response to the patient. This study should be large enough and well conducted to reassure clinicians of some of the advantages of testosterone therapy. This study builds on previous studies of greater than 200 participants in diabetes,[27, 17] and the TTrials on testosterone in older men.[33] None of these studies have found any concerns in regard to safety of testosterone replacement. However, it is in the nature of cardiologists and diabetologists that they would require data on efficacy and safety from very large, randomised placebo-controlled trials before being convinced and reassured.

The TRAVERSE trial is powered to determine if testosterone replacement is cardiovascular safe or increase MACE events.[32] This study will include a sub-cohort of men with prediabetes and type 2 diabetes. It is hoped it will provide evidence of cardiovascular safety but may also provide other important findings.

There have been a number of registry studies which have provided positive and reassuring data. Clinicians also usually require real world data studies. The Association of British Clinical Diabetologists (ABCD) are conducting a Worldwide Audit of Testosterone and Type 2 Diabetes. This audit hopes to cumulate data from current clinical practise on benefits and risks of treating men with type 2 diabetes and hypogonadism.

References

1. Kapoor D, Goodwin E, Channer KS, *et al.* Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. Eur J Endocrinol 2006;154:899-906.

2. Garvey WT, Mechanick JI, Brett EM, *et al.* Reviewers of the AACE/ACE Obesity Clinical Practice Guidelines. AACE/ACE guidelines: American Association of Clinical Endocrinologists and American College of Endocrinology clinical practice guidelines for comprehensive medical care of patients with obesity. Endocr Pract 2016;22:842-84.
3. Zarotsky V, Huang MY, Carman W, *et al.* Systematic literature review of the risk factors, comorbidities and consequences of hypogonadism in men. Andrology 2014;2:819-34.
4. Hackett G, Kirby M. Testosterone deficiency in men infected with COVID-19. Trends in Urology 2020;11:7-10.
5. Wang C, Jackson G, Jones TH, *et al.* Low testosterone associated with obesity and the metabolic syndrome contributes to sexual dysfunction and cardiovascular disease risk in men with diabetes. Diabetes Care 2011:34;1669-75.
6. Grossmann M. Low testosterone in men with type 2 diabetes: significance and treatment. JCEM 2011;96:2341-53.
7. Wittert G, Bracken K, Robledo KP, *et al.* Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diab Endocrinol 2021;9:32-45.
8. Salonia AB, Bettocchi C, Boeri L, *et al.* European Association of Urology Guidelines on Sexual and Reproductive Health—2021 Update: Male Sexual Dysfunction. Eur Urol 2021;80:333-57.
9. Hackett G, Cole N, Bhartia M, *et al.* Testosterone replacement therapy improves metabolic parameters in hypogonadal men with type 2 diabetes but not in men with coexisting depression: the BLAST study. J Sex Med 2014;11:840-56.
10. Hackett G, Heald AH, Sinclair A, *et al.* Serum testosterone, testosterone replacement therapy and all- cause mortality in men with type 2 diabetes: retrospective consideration of the impact of PDE5 inhibitors and statins. Int J Clin Pract 2016;70:244-53.
11. Dhindsa S, Ghanim H, Batra M, *et al.* Insulin resistance and inflammation in hypogonadotrophic hypogonadism and their reduction after testosterone replacement in men with type 2 diabetes. Diabetes Care 2016;39:82-91.
12. Kelly DM, Jones TH. Testosterone: a metabolic hormone in health and disease. J Endocrinol 2013;217:R25-45.
13. Fairweather D. Clin Med Insights Cardiol 2015;8(Suppl 3):49-59.
14. Malipatil NS, Yadegarfar G, LuntM, Keevil B, *et al.* Male hypogonadism:14-year prospective outcome in 550 men with type 2 diabetes. Endocrinol Diab Metab 2019;2:e00064.
15. Haider KS, Haider A, Saad F, *et al.* Remission of type 2 diabetes following long-term treatment with injectable testosterone undecanoate in patients with hypogonadism and type 2 diabetes: 11-year data from a real-world registry study. Diabetes Obes Metab 2020;22:2055-68.
16. Muraleedharan V, Marsh H, Kapoor D, *et al.* Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. Eur J Endocrinol 2013;169:725-33.
17. Sharma R, Oni OA, Gupta K, *et al.* Normalization of testosterone level is associated with reduced incidence of myocardial infarction and mortality in men. Eur Heart J 2015;36:2706-15.

18. Kelly DM, Jones TH. Testosterone: a vascular hormone in health and disease. J Endocrinol 2013;217:R47-71.

19. Traish AM, Haider A, Haider KS, *et al.* Longterm testosterone therapy improves cardiometabolic function and reduces risk of cardiovascular disease in men with hypogonadism: A real-life observational registry study setting comparing treated and untreated (control) groups. J Cardiovasc Pharmacol Ther 217;22:414-33.

20. Zhao JV, Schooling C M. The role of testosterone in chronic kidney disease and kidney function in men and women: a bi-directional Mendelian randomization study in the UK Biobank. BMC Med 2020;18:122.

21. Tirabassi G, Cignarelli A, Perrini S, *et al.* Influence of CAG Repeat Polymorphism on the Targets of Testosterone Action. Int J Endocrinol 2015;2015:298107.

22. Alevizaki M, Cimponeriu AT, Garofallaki M, *et al.* The androgen receptor gene CAG polymorphism is associated with the severity of coronary artery disease in men. Clin Endocrinol (Oxf) 2003;59:749-55.

23. Zitzmann M, Nieschlag E. The CAG repeat polymorphism in the androgen receptor gene and maleness. Int J Androl 2003;26:76-83.

24. Stanworth RD, Kapoor D, Channer KS, *et al.* The role of androgen receptor CAG repeat polymorphism and other factors which affect the clinical response to testosterone replacement in metabolic syndrome and type 2 diabetes: TIMES2 sub-study. Eur J Endocrinol 2014;170:193-2014.

25. Heald AH, Yadegar G, Livingston M, *et al.* Androgen receptor-reduced sensitivity is associated with increased mortality and poorer glycaemia in men with type 2 diabetes mellitus: a prospective cohort study. Cardiovasc Endocrinol Metab 2012;10:37-44.

26. Stanworth RD, Kapoor D, Channer KS, *et al.* Androgen receptor CAG repeat polymorphism is associated with serum testosterone levels, obesity and serum leptin in men with type 2 diabetes. Eur J Endocrinol 2008;159:739-46.

27. Jones TH, Arver S, Behre HM, *et al.* Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). Diabetes Care 2011;34:828-37.

28. Low Testosterone and Diabetes. 2019 [Internet]. Available from: https://www.diabetes.co.uk/low-testosterone-and-diabetes.html [accessed 2021, September 24].

29. British Society for Sexual Medicine. British Society for Sexual Medicine guidelines on adult testosterone deficiency, with statements for UK practice. 2018 [Internet]. Available from: https://www.guidelines.co.uk/mens-health/bssm-guideline-on-adult-testosterone-deficiency/453888.article

30. Grossmann M, Jones TH. Functional hypogonadism in middle-aged and older men: testosterone treatment or not? Eur J Endocrinol 2021;183:D1-D9.

31. Welcome to the QRISK®3-2018 risk calculator. Available from: https://www.qrisk.org/three/ [Accessed 2021, September 25].

32. Good Clinical Practice Network. A Study to Evaluate the Effect of Testosterone Replacement Therapy (TRT) on the Incidence of Major Adverse Cardiovascular Events (MACE) and Efficacy Measures in Hypogonadal Men. Available from: https://ichgcp.net/clinical-trials-registry/NCT03518034 [Accessed 2021, September 25].

33. Snyder PJ, Bhasin S, Cunningham GR, *et al.* Effects of testosterone treatment in older men. N Engl J Med 2016;374:611-24.

20

Opioids, testosterone and men's health

Janine David, Michael Kirby

Opioid deaths in the USA, mainly occurring in young people reached over 100,000 in 2021.[1]

This death-toll apparently, equals more than the total of car accidents and gun deaths. Importantly, it is much more common in men than women. Data from the Centres for Disease Control and Prevention shows that overdose deaths rose 28.5% in the 12 months ending April 2021. This partly explains the increase of available organ body parts to Transplant clinics.[2]

The fatalities will have had lasting repercussions on families and friends, because most of them occurred among people aged 25 to 55 years. The rise in deaths were mainly caused by synthetic opioids and aggravated by the widespread use of fentanyl, and may be added surreptitiously to other illegally manufactured drugs to enhance their potency.

In the UK the situation is also very worrying. According to the deaths related to drug poisoning in England and Wales in 2020, there were 4,561 deaths related to drug poisoning registered in England and Wales (equivalent to a rate of 79.5 deaths per million people); this is 3.8% higher than the number of deaths registered in 2019 (4,393 deaths; 76.7 deaths per million). More than twice as many deaths occurred men compared to women.

Among males, there 109.7 drug poisoning deaths registered per million in 2020 (3,108 registered deaths), compared with 49.8 deaths per million among females (1,453 deaths). (https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/bulletins/deathsrelatedtodrugpoisoninginenglandandwales/2020).

This, raises the question as to why more men than women in this scenario, and could suppression of normal testosterone levels be a contributing cause, via increases in anxiety, depression, metabolic changes and difficulties with sexual activity?

Most studies do suggest that lower testosterone levels are associated with depressive symptoms. Furthermore, testosterone replacement therapy (TRT) has been shown to improve depressive symptoms in most men. This could be due to the fact that testosterone is a modulator of GABAA receptors and inhibits 5-HT3 receptors

centrally. Men with depressive symptoms and testosterone deficiency syndrome should be given a trial of testosterone replacement therapy, as TRT alone may improve clinical symptoms of depression. Furthermore, men already on SSRIs may also experience further improvement in depressive symptoms after initiating TRT.[3]

Depression, anxiety and decreased quality of life are the most common psychopathological conditions in young hypogonadal men. Thirty-nine young male patients with congenital hypogonadotropic hypogonadism (CHH) and 40 age-matched healthy males were enrolled in a study at the Family Medicine School, Ankara, Turkey. The impact of testosterone replacement treatment (TRT) on the patients' anxiety and depression levels, sexual function and quality of life were assessed before and after 6 months of treatment using valid and reliable scales, including the Short Form-36 (SF-36), Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), and Arizona Sexual Experiences (ASEX). Patients with CHH had significantly higher scores for BDI, BAI, and ASEX than the control subjects at baseline ($P=0.011$, $P=0.036$, $P<0.001$, respectively). The ASEX and BDI scores significantly improved after the TRT ($P<0.001$ for both). When compared to the control group, treatment naïve hypogonadal patients had more severe symptoms of sexual dysfunction, anxiety, depression, and worse quality of life. After 6 months of TRT, there were observed improvements in the above parameters, suggesting that low endogenous levels of testosterone might be related to the increased incidence of psychological symptoms.[4]

Two-thirds (or 2,996) of registered drug poisoning deaths in the UK in 2020 were related to drug misuse, accounting for 52.3 deaths per million people. Rates of drug-misuse death continue to be elevated among those born in the 1970s, with the highest rate in those aged 45 to 49 years. The North East continues to have the highest rate of deaths relating to drug misuse (104.6 deaths per million people); London had the lowest rate (33.1 deaths per million people). Approximately half of all drug poisoning deaths registered in 2020 involved an opiate (49.6%; 2,263 deaths); 777 deaths involved cocaine, which is 9.7% more than 2019, and more than five times the amount recorded a decade ago (144 deaths in 2010).

Statistics are based on the year of death registration – because of death registration delays, around half of these deaths will have occurred in the previous year (2019), and the majority will have occurred before the coronavirus (COVID-19) pandemic in the UK.[5]

Managing pain in primary care can be very difficult, and achieving an understanding of how pain is affecting a person's life and those around them and knowing what is important to the person is the first step in developing an effective care and support plan. This recognises and treats a person's pain as valid and unique to them. Pain that lasts for more than 3 months is known as chronic or persistent pain. In the UK the prevalence of chronic pain is uncertain, but appears common, affecting perhaps one-third to one-half of the population. Chronic pain that is caused by an underlying condition (for example, osteoarthritis, rheumatoid arthritis, ulcerative

colitis, endometriosis) is known as chronic secondary pain. Where the cause of the pain is unclear it is called chronic primary pain.

Chronic pain is one of the most common GP consultations and up to 50% of adults in the UK have chronic non-cancer pain.[6] In addition, pain affects more Americans than diabetes, heart disease and cancer combined,[7] chronic pain is very costly on both an individual and societal level.[8]

The NICE guideline makes recommendations for treatments that have been shown to be effective in managing chronic primary pain. These include exercise programmes and the psychological therapies CBT and acceptance and commitment therapy (ACT). Acupuncture is also recommended as an option. People with chronic primary pain should not be started on commonly used drugs including paracetamol, non-steroidal anti-inflammatory drugs, benzodiazepines or opioids. This is because there is little or no evidence that they make any difference to people's quality of life, pain or psychological distress, but they can cause harm, including possible addiction.[9]

Opioids are very good analgesics for acute pain and pain at the end of life but there is little evidence that they are helpful for long-term pain. Despite this, they are widely prescribed for this reason – opioid prescribing more than doubled in the period 1998 to 2018. This has been referred to as an opioid epidemic in the UK, similar but not at the same scale as the opioid crisis in the USA.[10]

Most opioids are μMOP agonists and they are classified according to receptor binding and opioid receptors are ubiquitous throughout the body.

Agonists: Morphine, Codeine, Fentanyl, Heroin, Oxycodone.

Partial agonists: Buprenorphine, Tramadol, Tapentadol.[11]

In US, New Zealand and Australia, the illicit use of prescription opioids outpaces that of heroin (Table 20.I).[12]

The endocrine system can be severely affected by chronic opioid treatment, leading to a decrease in total testosterone levels and opioid-induced hypogonadism. Opioids depress the secretion of hormones at different levels of the hypothalamus-pituitary-gonadal axis, and generally increase levels of growth hormone, thyroid-stimulating hormone and prolactin, but there are conflicting reports on the effects of opioids on arginine vasopressin and adrenocorticotropic hormone. In addition, opioids can lead to the development of hypogonadism by directly inhibiting gonadotropin-releasing hormone (GnRH) through the μ-opioid receptor, reducing libido and causing erectile dysfunction, bone loss and/or infertility (Figure 20.1).[17]

The impact of opioid ingestion occurs rapidly - often within 1 week, and the highest risk appears to be among patients receiving significant doses for longer than 1 month. Use of the more potent opioids are more likely to cause a greater risk of hypogonadism, but the effects seem to be reversible after a few days of withdrawal. As one might expect, long-acting opioids have a greater risk compared with short-acting drugs. There is a significant correlation between increased dose and development of OPIAD (Figure 20.2).[17]

Table 20.I. From 1996-2012 Oxycontin sales in the US increased from $48 million to $2.4 billion. From 1999 to 2019, the US lost nearly half a million people to overdoses of prescription and illegal opioids, and according to US federal data. 1) In July 2021 – 4 drug companies agreed to pay $26 billion to resolve opioid lawsuits.[13, 14] Sources: 2019 National Survey on Drug Use and Health, 2020. 2) NCHS Data Brief No. 394, December 2020.[15] NCHS, National Vital Statistics System. Provisional drug overdose death counts https://www.hhs.gov/opioids/sites/default/files/2021-02/opioids-infographic.pdf.[16]

70,630	People died from drug overdose in 2019[2]
1.6 million	People had an opioid use disorder in the past year[1]
754,000	People used heroin in the past year[1]
1.6 million	People misused prescription pain relievers for the first time[1]
48,006	Deaths attributed to overdosing on synthetic opioids other than methadone (in 12-month period ending June 2020)[2]
10.1 million	People misused prescription opioids in the past year[1]
2 million	People used methamphetamine in the past year[1]
50,000	People used heroin for the first time[1]
14,480	Deaths attributed to overdosing on heroin (in 12-month period ending June 2020)[2]

This retrospective US cohort study evaluated data from men aged 26-79 years (N.=81; mean age: 51 years; median/mean BMI: 29/31 kg/m^2) who had a diagnosis of chronic pain (defined as pain lasting for >3 months without any aetiology that allowed for definitive or curative treatment) and were on a stable dose of opioid therapy for ≥3 months, of whom 46 were hypogonadal and 35 were not.

After controlling for daily opioid dose and BMI, men on long-acting opioids* had 4.78 times greater odds of becoming hypogonadal than men on short-acting opioids* (95% CI: 1.51 to 15.07; P=0.008).

In total, 74% (N.=34/46) of men receiving long-acting opioids were hypogonadal compared with 34% (N.=12/35) of men using short-acting opioids exclusively.

After controlling for daily opioid dose and duration of action of opioid, BMI was also found to be significantly associated with hypogonadism; for every unit increase of BMI, patients had an additional 13% higher odds of being hypogonadal (95% CI: 1.03 to 1.24; P=0.006).

The model also showed that for every 10 mg increase in daily opioid dose, patients had an additional 2% greater chance of being hypogonadal, but this was not significant (95% CI: 0.99 to 1.05; P=0.29).[18]

In a retrospective US cohort study, which evaluated data from men aged 18-80 years (N.=1159) who had chronic non-cancer pain and were on a stable regimen of a single opioid for ≥90 days (N.=190 received a long-acting opioid, N.=969 received

Figure 20.1. Pathogenesis of opioid-induced hypogonadism (modified from: Coluzzi F, *et al.*).[17]

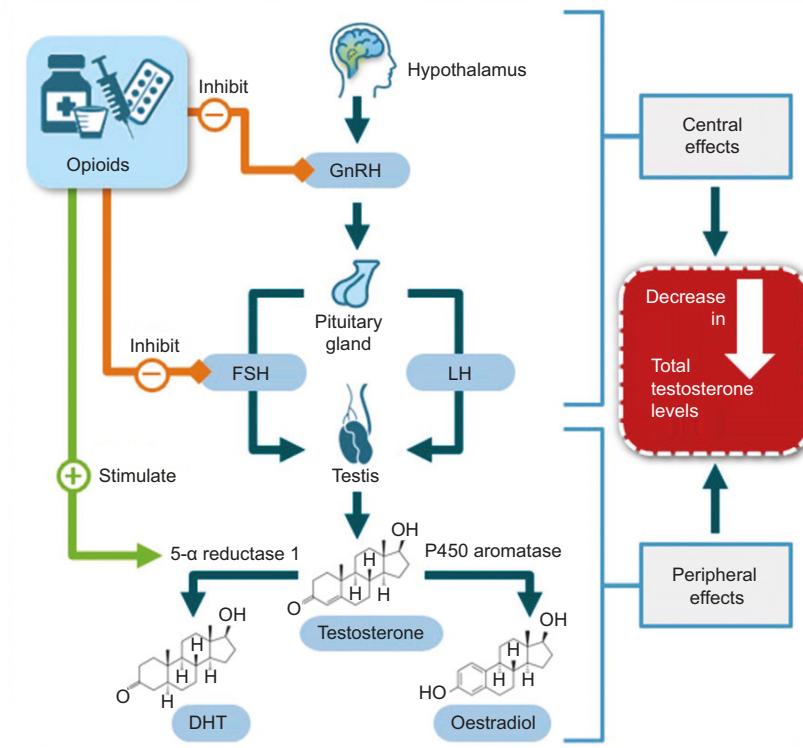
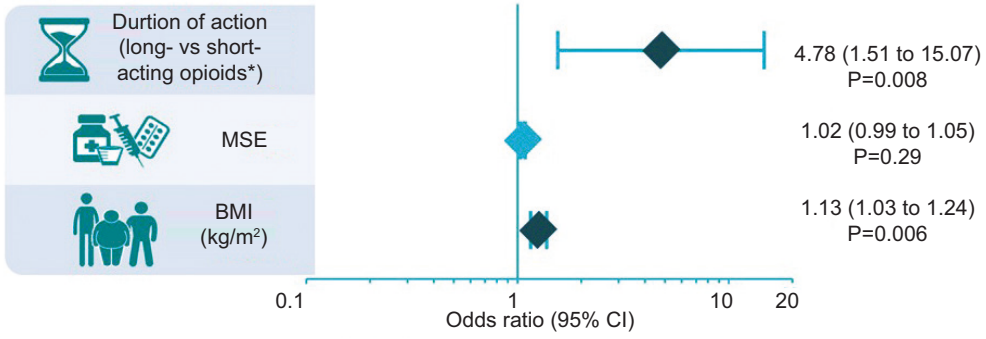


Figure 20.2. Prevalence of hypogonadism among men using long or short-acting opioids. Long-acting opioids: buprenorphine, fentanyl, methadone, controlled-release morphine, standard-release oxycodone; Short-acting opioids: hydrocodone, immediate-release oxycodone; BMI, body mass index; CI, confidence interval; MSE, morphine-standardised equivalent dose (modified from: Rubinstein AL, *et al.*).[18]



a short-acting opioid). Hypogonadism was noted in 69.2%, 60.8%, 52.1%, 50.4%, 42.9%, 35.5% and 34.2% of patients receiving fentanyl, methadone, morphine, oxycodone, hydromorphone, codeine and hydrocodone, respectively. Results for each opioid were analysed with reference to hydrocodone, because the bivariate results indicated that patients using hydrocodone were least likely to be androgen-deficient; moreover, it was the largest group.

Fentanyl [OR 25.73 (95% CI: 2.82 to 234.97)], methadone [OR 7.33 (95% CI: 3.29 to 16.33)] and oxycodone [OR 3.15 (95% CI: 1.87 to 5.33)] were all associated with higher odds of hypogonadism than hydrocodone. (CI, confidence interval; OR, odds ratio).

Morphine was also associated with elevated odds of hypogonadism compared to hydrocodone [OR 2.40 (95% CI: 0.92 to 6.28)]; however, this result was not statistically significant. The highest odds of hypogonadism appeared to be associated with those opioids that maintain very stable serum drug levels. The conclusions drawn from this study suggest that before commencement of opioid therapy or modification of existing opioid therapy, patients should undergo testosterone testing.[19]

Further data came from a retrospective cohort study of men with chronic non-cancer pain managed with a single type of long- (N.=190) or short-acting (N.=969) opioid for ≥90 days: transdermal fentanyl, methadone and oxycodone were associated with higher odds of hypogonadism *versus* hydrocodone, and increased doses of hydrocodone and oxycodone were associated with higher odds of hypogonadism (Figure 20.3).[19]

The prevalence of OPIAD ranges from 19% to 86% with most studies report an overall prevalence higher than 50%, confirming the significant impact of opioids in reducing testosterone levels.[20-22]

A systematic review and meta-analysis of testosterone suppression in opioid users concluded that testosterone level was suppressed in men with regular opioid use regardless of opioid type and they found a mean T difference of 5.7 nmol/L between opioid users and controls. They found that opioids affect testosterone levels differently in men than women, and testosterone was not found to be suppressed in studies examining opioid-using women.[23]

Rubenstein *et al.* studied 1585 men receiving long-acting opioids (LAO) 57% were diagnosed with T <345 ng/dL (12 nmol/L).[24]

Opioids can induce several hypogonadism related signs and symptoms including: sexual dysfunction, mood impairment and fatigue, obesity and CVD, osteoporosis, sexual dysfunction (Figure 20.4).

Chronic pain itself causes sexual dysfunction and can be a non-organic cause of ED, and men with OPIAD have reported poorer pain control and hyperalgesia. Lower testosterone levels lead to ED and loss of libido and sex inertia resets the reproductive axis to a lower level of activity inducing a secondary hypogonadism by reducing LH production.[26]

Figure 20.3. Adjusted odds ratio from hypogonadism among men using opioids for chronic pain (modified from: Rubinstein AL, *et al.*).[19]

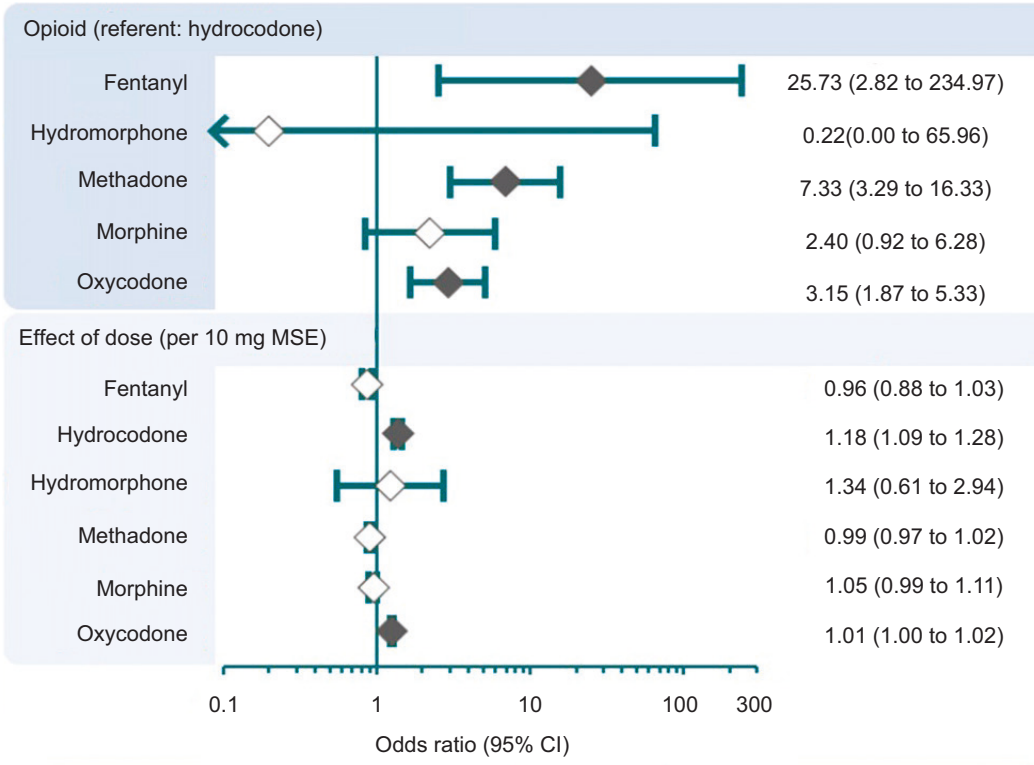
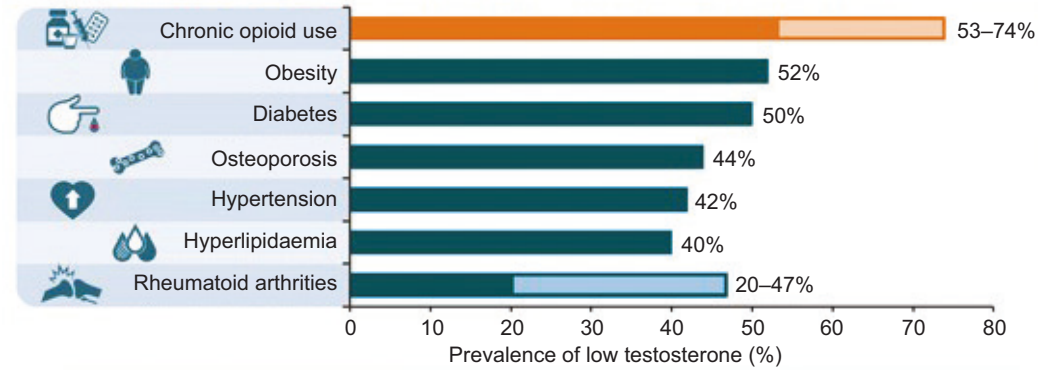


Figure 20.4. Prevalence of low testosterone among chronic opioid users and men with other conditions (modified from Khera M, *et al.*).[25]



Depressive symptoms seem to be dose and length of duration related which can be exacerbated by OPIAD. As well as opioids affecting T levels, T may also be involved in regulation of endogenous opioid activity. A registry study of male opioid users with low T found that sexual function and mood improved significantly over a 12-month course of T gel administration.[27]

Pain, physical limitations, and depression lead to decreased activity and increased eating and opioids increase appetite, the association between a decreased testosterone, obesity and MetS is bidirectional and increases the risk of CVD.

UK GP research database found an increased risk of MI in 1.7 million patients with at least one prescription for an opioid to treat CNCP between 1990 and 2008.[28]

Osteoporosis is a consequence of TD and opioid treatment is associated with a 50-60% increase risk of osteoporotic fractures. The mechanism is due to a direct effect on bone formation by impairing osteoblastic activity. It appears that patients on Tramadol which has less MOP affinity have a lower incidence of osteoporosis. The fall risk is increased due to the CNS effects of opioids such as dizziness.[22]

So, would treating these men with testosterone (TTh) make a difference?

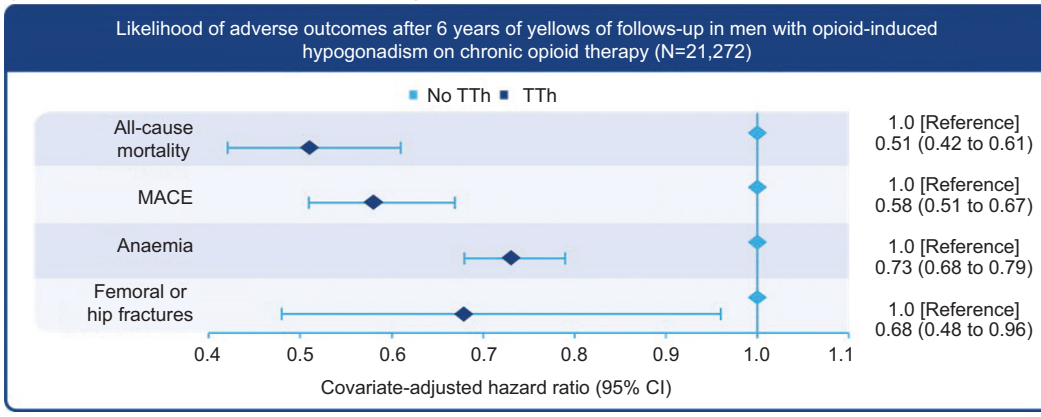
TTh significantly decreases all-cause mortality and other adverse health outcomes in men with opioid-induced hypogonadism *versus* TTh non-use, confirmed in a cohort study which was performed on men using long-term opioid therapy with low testosterone levels [<10.4 nmol/L (<300 ng/dL)] under the care of Veterans Health Administration facilities in the USA from 1 October 2008 to 30 September 2014.[29]

Male patients with HIV infection, gender dysphoria or prostate cancer, or who received TTh in 2008 were excluded. In total, 21,272 long-term opioid users [mean (SD) age: 53 (10) years] were included for analysis, of whom 14,121 (66.4%) received TTh and 7151 (33.6%) did not. After adjusting for covariates*, opioid users who received TTh had significantly lower all-cause mortality [HR 0.51 (95%CI: 0.42 to 0.61)] and incidences of MACE** [HR 0.58 (95% CI: 0.51 to 0.67)], anaemia [HR 0.73 (95%CI 0.68 to 0.79)] and femoral or hip fractures [HR 0.68 (95%CI: 0.48 to 0.96)] during the 6-year follow-up period, compared with their counterparts without a TTh prescription (Figure 20.5).

*Adjusted for age, race/ethnicity, marital status, BMI, co-pay requirement, zip code poverty level, and baseline status of the following clinical conditions: indications for pain, chronic pain conditions, use of glucocorticoids, congestive heart failure, cancers, coronary artery disease, hypertension, diabetes, hyperlipidaemia, liver disease, chronic kidney disease, stroke or transient ischaemic attack, dementia, depression, bipolar disease, substance use disorder, alcohol dependence, psychosis, and use of antipsychotic medications. **MACE: incident cases (new occurrence) of myocardial infarction or thrombotic stroke or death. BMI, body mass index; HIV, human immunodeficiency virus; HR, hazard ratio; MACE, major adverse cardiovascular events; SD, standard deviation; TTh, testosterone therapy.[29]

A small randomised trial also showed a positive result of the effect of testosterone replacement in men with opioid-induced androgen deficiency.

Figure 20.5. Likelihood of adverse outcomes after 6 years of follow-up in men with opioid-induced hypogonadism on chronic opioid therapy (N.=21,272) (modified from: Jasuja GK, *et al.*).[29]



Men, aged 18-64, with chronic non-cancer pain, morning testosterone <350 ng/dL (12.1 nmol/L) were recruited. Eighty-four men randomised, 43 men to AndroGel 1% and 41 men to placebo. This was a single-site investigation with assessment at baseline and week 14 with hormone assays, self-reported pain and quantitative sensory testing, sexual function and quality of life. Changes in body composition were reported.[30]

Hormone assays (Figures 20.6, 20.7)

Figure 20.6. Postintervention changes in serum concentrations of total and free testosterone, luteinizing hormone, and sex hormone binding globulin levels (reproduced with permission from: Basaria S, *et al.*).[30]

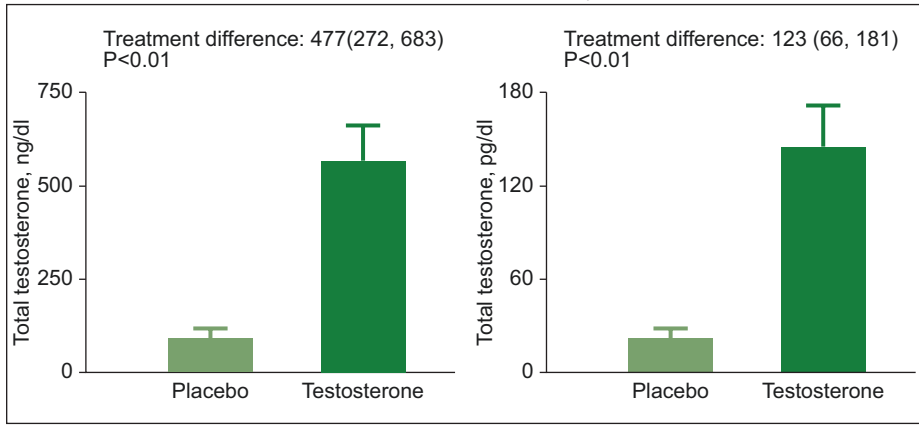
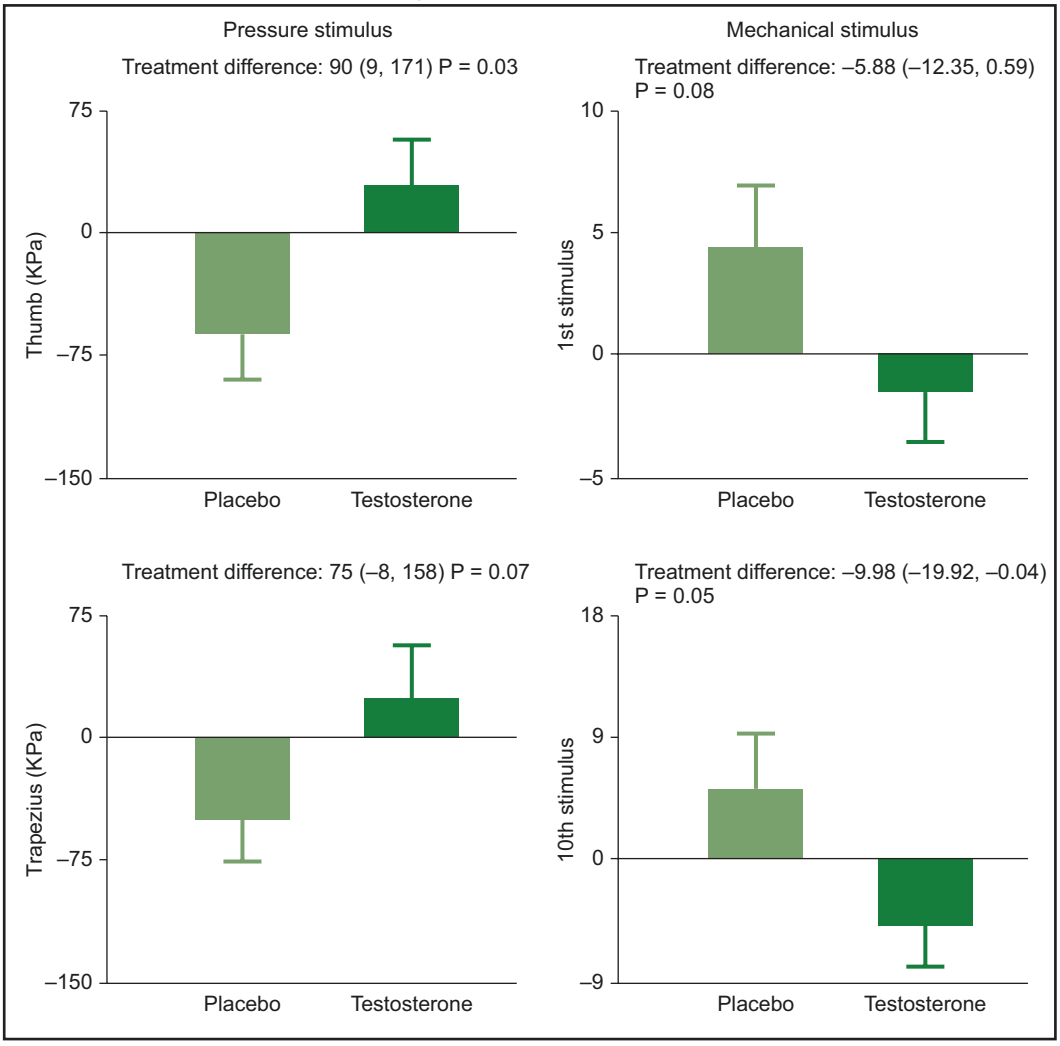
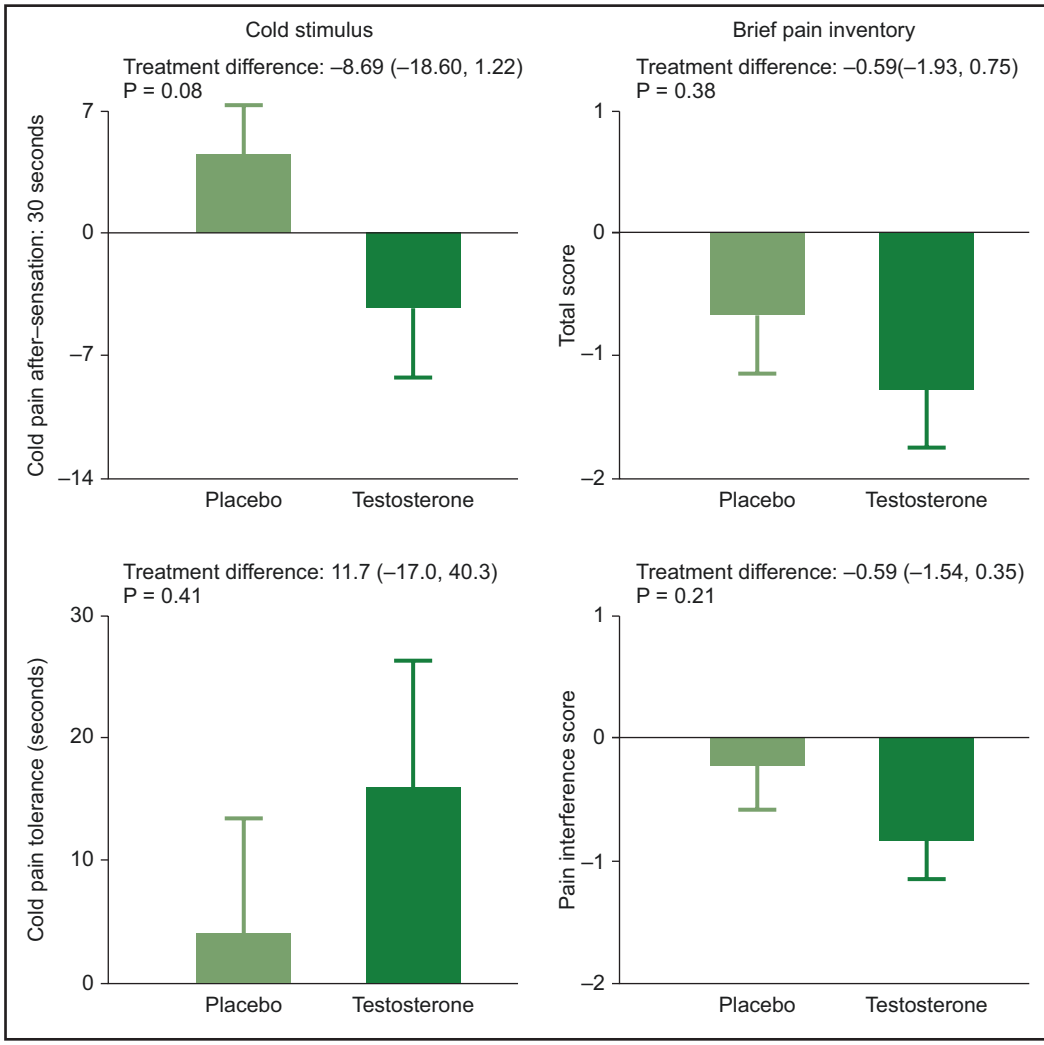


Figure 20.7. Self-reported pain and quantitative sensory testing - Thumb: postintervention changes in pain perception and tolerance with testosterone or placebo. Men in the testosterone arm exhibited greater tolerance to 1) algometer-induced pressure pain; 2) weighted pinprick stimulator-induced mechanical pain, and 3) ice water-induced cold pain and its after-sensations (reproduced with permission from: Basaria S, *et al.*).[30]





Conclusions from the Basaria study

- First randomized, double-blind placebo-controlled trial to determine the efficacy of testosterone replacement in men with opioid-induced androgen deficiency.
- Testosterone therapy improved pain sensitivity to a number of noxious painful stimuli, confirming its antinociceptive role.
- Testosterone therapy also improved sexual desire, body composition and aspects of QoL.

- There is a need for larger randomized trials of longer duration to further evaluate the efficacy of testosterone in chronic pain syndromes.

Opioid-induced hypogonadism is a lesser-known but highly prevalent adverse effect in patients on long-term opioid therapy. Narcotics have both central and peripheral effects causing reduced serum testosterone levels. The clinicians should look for these recognised adverse consequences and assess them clinically based on their signs and symptoms. Testosterone replacement therapy is a viable option for managing symptomatic males,[31] The Endocrine Society,[32] advise that, when assessing men for T deficiency it should include a general health evaluation to exclude systemic illness, eating disorders, excessive exercise, sleep disorders, and use of recreational drugs and certain medications (e.g., opioids or high-dose glucocorticoid therapy) that affect T production or metabolism. The British Society for Sexual Medicine[33] also advise screening for T deficiency in all men on long-term opiate, anticonvulsant or antipsychotic medication.

Conclusions

OPIAD is common and can impair satisfactory pain relief. OPIAD also impairs sexual activity, mood, especially depression, bone metabolism and is a risk factor for CVD and obesity.

Guidelines support screening for testosterone deficiency in this situation. Consideration should be given to screening for testosterone deficiency prior to an opioid prescription, to provide a baseline. From a clinical point of view, the effect is reversible and if the opioid is removed, the deficiency is reversed, usually within a month.

Wherever possible consider alternative pain management strategies, as per NICE guidance, but, if treatment is necessary consider using an opioid with a lower MOP affinity such as buprenorphine or tramadol, and enquire about relevant low testosterone symptoms, with testosterone measurements at subsequent follow-up.

Current evidence suggests testosterone replacement might be beneficial, and helpful with analgesics to improve the pain control in hypogonadal men.

OPIAD can have a profound effect on health and QoL, and it can hinder the clinician's ability to effectively treat chronic pain and manage complex co-morbidities, but it often goes unrecognised and untreated.

References

1. https://www.nytimes.com/2021/11/17/health/drug-overdoses-fentanyl-deaths.html https://www.bbc.co.uk/news/world-us-canada-59253091.
2. Vaduganathan M, Machado SR, DeFilippis EM, *et al.* Organ donation and drug intoxication-related deaths in the United States. N Engl J Med 2019;380:597-9.

3. Khera M. Patients with testosterone deficit syndrome and depression. Arch Esp Urol 2013;66:729-36.
4. Aydogan U, Aydogdu A, Akbulut H, *et al.* Increased frequency of anxiety, depression, quality of life and sexual life in young hypogonadotropic hypogonadal males and impacts of testosterone replacement therapy on these conditions. Endocr J 2012;59:1099-105.
5. https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/bulletins/deathsrelatedtodrugpoisoninginenglandandwales/2020.
6. Scott LJ, Kesten JM, Bache K, *et al.* Evaluation of a primary care-based opioid and pain review service: a mixed-methods evaluation in two GP practices in England. Br J Gen Pract 2020;70:e111-9.
7. Tsang A, Von Korff M, Lee S, *et al.* Common chronic pain conditions in developed and developing countries: gender and age differences and comorbidity with depression-anxiety disorders. J Pain 2008;9:883-91.
8. Breivik H, Eisenberg E, O'Brien T; OPENMinds. The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care. BMC Public Health 2013;13:1229.
9. https://www.nice.org.uk/news/article/nice-recommends-range-of-effective-treatments-for-people-with-chronic-primary-pain-and-calls-on-healthcare-professionals-to-recognise-and-treat-a-person-s-pain-as-valid-and-unique-to-them.
10. https://www.england.nhs.uk/south/info-professional/safe-use-of-controlled-drugs/opioids/.
11. Rosenblum A, Marsch LA, Joseph H, *et al.* Opioids and the treatment of chronic pain: controversies, current status, and future directions. Exp Clin Psychopharmacol 2008;16:405-16.
12. https://www.unodc.org/documents/wdr/WDR_2010/2.0_Drug_statistics_and_Trends.pdf.
13. https://www.wsj.com/articles/26-billion-opioid-settlement-among-states-and-drug-industry-expected-this-week-11626745448.
14. https://www.nbcnews.com/news/us-news/4-companies-near-26-billion-settlement-resolve-opioid-lawsuits-n1274520.
15. 2019 National Survey on Drug Use and Health, 2020. 2. NCHS Data Brief No. 394, December 2020.
16. NCHS, National Vital Statistics System. Provisional drug overdose death counts https://www.hhs.gov/opioids/sites/default/files/2021-02/opioids-infographic.pdf.
17. Coluzzi F, Billeci D, Maggi M, *et al.* Testosterone deficiency in non-cancer opioid-treated patients. J Endocrinol Invest 2018;41:1377-88.
18. Rubinstein AL, Carpenter DM, Minkoff JR. Hypogonadism in men with chronic pain linked to the use of long-acting rather than short-acting opioids. Clin J Pain 2013;29:840-5.
19. Rubinstein AL, Carpenter DM. Association Between Commonly Prescribed Opioids and Androgen Deficiency in Men: A Retrospective Cohort Analysis. Pain Med 2017;18:637-44.
20. Abs R, Verhelst J, Maeyaert J, *et al.* Endocrine consequences of long-term intrathecal administration of opioids. J Clin Endocrinol Metab 2000;85:2215-22.

21. Ajo R, Segura A, Inda MM, *et al.* Opioids Increase Sexual Dysfunction in Patients With Non-Cancer Pain. J Sex Med 2016;13:1377-86.

22. Coluzzi F, Billeci D, Maggi M, *et al.* Testosterone deficiency in non-cancer opioid-treated patients. J Endocrinol Invest 2018;41:1377-88.

23. Bawor M, Bami H, Dennis BB, *et al.* Testosterone suppression in opioid users: a systematic review and meta-analysis. Drug Alcohol Depend 2015;149:1-9.

24. Rubinstein A, Carpenter DM. Elucidating risk factors for androgen deficiency associated with daily opioid use. Am J Med 2014;127:1195-201.

25. Khera M, Adaikan G, Buvat J, *et al.* Diagnosis and Treatment of Testosterone Deficiency: Recommendations From the Fourth International Consultation for Sexual Medicine (ICSM 2015). J Sex Med 2016;13:1787-804.

26. Ramsey S. Opioids for back pain are linked to increased risk of erectile dysfunction. BMJ 2013;346:f3223.

27. Blick G, Khera M, Bhattacharya RK, *et al.* Testosterone replacement therapy outcomes among opioid users: the Testim Registry in the United States (TRiUS). Pain Med 2012;13:688-98.

28. Li L, Setoguchi S, Cabral H, *et al.* Opioid use for noncancer pain and risk of myocardial infarction amongst adults. J Intern Med 2013;273:511-26.

29. Jasuja GK, Ameli O, Reisman JI, *et al.* Health Outcomes Among Long-term Opioid Users With Testosterone Prescription in the Veterans Health Administration. JAMA Netw Open 2019;2:e1917141.

30. Basaria S, Travison TG, Alford D, *et al.* Effects of testosterone replacement in men with opioid-induced androgen deficiency: a randomized controlled trial. Pain 2015;156:280-8.

31. Marudhai S, Patel M, Valaiyaduppu Subas S, *et al.* Long-term Opioids Linked to Hypogonadism and the Role of Testosterone Supplementation Therapy. Cureus 2020;12:e10813.

32. Bhasin S, Brito JP, Cunningham GR, *et al.* Guidelines for Testosterone Therapy in Men J Clin Endocrinol Metab 2018, 103:1715-44.

33. Hackett G, Kirby M, Edwards D *et al.* British Society for Sexual Medicine Guidelines on Adult Testosterone Deficiency, With Statements for UK Practice. J Sex Med 2017;14:1504-23.

21

Testosterone case studies

Janine David

Reviewing real life case studies is very helpful to reinforce the benefits of correct diagnosis and treatment of testosterone deficiency. I have seen first-hand the dramatic impact testosterone deficiency has on the well-being of patients and their families, not to mention the improvements in their metabolic profiles. Some cases will describe where systems failed patients and how things could be improved.

I will describe a variety of real-life case studies which will help consolidate the knowledge gained in the previous chapters. Permission has been granted to share these stories, but all names have been changed for privacy reasons.

Case 1. The impact of a single question

John was 44 years old. He hadn't felt himself lately. He had been more tired, irritable, and grumpy, and lacking the usual energy for life he previously had. He was concerned there was something significantly wrong with him. His wife, Louise, was fed up with his complaining and so suggested he booked an appointment for a 40+ health check with his GP.
During the check, John highlighted the following complaints:

- Diminished energy.
- Reduced vitality/well-being.
- Increased fatigue.
- Depressed mood.
- Decreased concentration.
- Decreased exercise gains – less muscle mass and less strength.
- Falling asleep in the evenings.

His GP arranged some blood tests but omitted any discussion regarding sexual function. Consequently, John was not asked about:

- Libido.
- Erections.
- Loss of early morning erections.

His GP was therefore unaware that Louise and John had not had sex for over two years.

On examination:

- Weight: 106 kg.
- BP: 150/90 mmHg.
- Waist circumference: 108 cm.

Initial blood tests revealed:

- TC: 6.2 mmol/L (range: <5).
- LDL: 5.1 mmol/L (range: ≤3).
- HDL: 0.95 mmol/L (range:≥0.9).
- Triglycerides: 2.8 mmol/L (range:<2.3).
- HbA1c: 46 mmol/L (range:20-42).
- PSA: 0.525 ug/L (range: 0-2).
- Haematocrit: 0.39 (range: 0.37-0.5).

Putting these results into the JBS cardiovascular risk calculator[1] determined that John's heart age was 50 years, with a 4.5% annual risk of heart disease or a stroke.

John left with extensive lifestyle modification advice – most of which he already knew but could not be bothered to act on. On arrival home he delivered the news to his wife that he is 'obese and unhealthy'.

The impact of a single question

John then saw a different doctor who asked about his sexual function and from that one question the consultation took a very different direction. John acknowledged that he had erectile dysfunction, low libido and decreased early morning erections, which all contributed to a deterioration in his marriage and his well-being.

John was asked to complete 2 questionnaires. Firstly, the Ageing Males' Symptoms (AMS)[2] score and also the International Index of Erectile Function (IIEF).[3]

- AMS=62 (Severe symptoms of testosterone deficiency)
- IIEF Score=5 (Severe ED)

He then went onto have further blood tests:

- Total Testosterone (TT): 7.2 nmol/L (range: 12-35).
- SHBG: 19 nmol/L (range: 16-55).
- Calculated Free Testosterone (FT): 0.183 nmol/L (range: 0.225-0.62).
- LH: 2.0 IU/L (range: 1.7-8.6).

John was diagnosed with erectile dysfunction, testosterone deficiency and metabolic syndrome all of which significantly increased his cardiovascular risk. Following his consultation with the second doctor to discuss blood results, John returned

home with a prescription for a daily PDE5i and testosterone gel in addition to lifestyle advice.

He attended 6 weeks later for an initial follow-up consultation – on his way home from the gym!

Lessons learned from this case

- Importance of enquiring about sexual function in male health checks.
- Ask about erectile dysfunction and low libido in all men with metabolic syndrome.
- Check testosterone levels in all symptomatic men with obesity (WC>102 cm and or BMI>30 kg/m^2).[4]

Case 2. The man with long-term diabetes and ED, and an unsatisfied wife

Chris was a 54-year-old office worker with type 2 diabetes. He had had erectile dysfunction for several years which was putting a strain on his marriage. Whilst being managed by the secondary care diabetes team he was started on sildenafil 50mg for his erectile dysfunction which worked on the odd occasion, but hardly had any impact on his marriage or his wife's concerns over lack of desire. He did not think anything more could be done about it, so had given up on an active sex life.

His GP practice had recently initiated an audit of their male diabetes population which included a testosterone blood screen. Therefore, at Chris's next community diabetes review appointment he had a testosterone level assessed.

- Total testosterone: 5.6 nmol/L (range: 12-35).
- SHBG: 24 nmol/L (range: 16-55).
- Calculated Free testosterone: 0.125 nmol/L (range: 0.225 - 0.62).
- LH and FSH normal (range: 1.7-8.6) and (range: 1.5-12.4) respectively.
- Waist circumference: 42 inches.
- HbA1c: 68 mmol/mol (range: 20-42).

Chris was asked to attend his general practice to discuss these results. On further questioning it transpired that he was dissatisfied with his erections and had a low libido.

He had an AMS score of 49, representing moderate to severe symptoms.

Chris was counselled on the potential benefits and risks of testosterone deficiency and started testosterone therapy. In addition, his sildenafil was changed to a daily tadalafil regime. Chris was monitored for 12 months as per the British Society for Sexual Medicine (BSSM) guidelines on adult testosterone deficiency.[4] At his annual review, his total testosterone had increased to 15.9nmol/L and his AMS score had improved to 26. His wife also attended the 12-month review appointment with

him and said "thank you so much for helping Chris, he is like the man I fell in love with". Three years later Chris's HBA1c had decreased to 52 mmol/mol and his waist circumference reduced to 37 inches representing a total loss of 5 inches.

Lessons learned from this case

- Ask about ED and low libido in all men with type 2 diabetes.[4]
- Remember the impact of male sexual dysfunction on the partner.
- Prevalence of TD in T2DM men is around 40% so remember to check testosterone levels in this cohort as per national evidence-based guidelines.[4]
- Treatment with testosterone therapy can improve parameters of diabetes as well as sexual function.[5]

Case 3. The opioid user

Michael was a married 46- year- old plasterer with two young children.

He had been taking co-codamol 30/500mg regularly for the past 4 years for chronic back pain. He was unable to take any time out of his manual labour job and had little time to attend physiotherapy and was not motivated to exercise after working all day.

He presented to his GP with the following symptoms:

- Lethargy.
- Low mood.
- Lack of motivation.
- Struggling in work.
- Not engaging in family life.

His GP arranged for some blood tests including a FBC and thyroid function test. The GP also asked Michael to complete a PHQ-9 questionnaire[6] and based on Michael's responses he was diagnosed with depression and treated with an anti-depressant.

Michael was monitored on his anti-depressant over the course of 2 years (his anti-depressant medication was changed twice in this period), but he did not see any significant improvement in his symptoms. If anything, he felt even worse. There was increasing tension between Michael and his wife and he was concerned she may ask him for a divorce. His own internet research of his symptoms led Michael to believe that he needed to have his testosterone checked. He asked his GP to do so, and the GP agreed.

Michael's initial total testosterone was 7.1 nmol/L so his GP then repeated the test 2 weeks later to get a fuller picture.

The results were:

- Total testosterone: 6.8 nmol/L (range:12-35).
- LH, FSH and prolactin all normal.
- SHBG: 39 nmol/L (range:16-55).

- Calculated free testosterone: 0.117 nmol/L (range: 0.225-0.62).
- PSA 0.81 ug/L (range: 0-2).

Michael's family was noted to be completed so there were no concerns over fertility. He was started on testosterone therapy. Within 6 weeks Michael felt more motivated to exercise which improved his mobility and his back pain decreased substantially. At the three-month review, his back pain had improved to the extent that his GP was able to significantly reduce his opioid dose. At the 12-month review, Michael felt normal again and his marriage was back on track. He no longer had issues with his job and was no longer on regular pain medication.

Lessons learned from this case

- GP's need to be aware that regular opioid use in men with chronic pain can suppress testosterone levels leading to opioid-induced hypogonadism.[7, 8]
- As per British Society for Sexual Medicine Guidelines, all symptomatic patients on long-term opioids should be screened for testosterone deficiency.[4]
- Musculoskeletal pain can often be exacerbated by a low testosterone.

Case 4.1. Osteoporosis in men and the link with testosterone

Tony, a 64-year-old retired electrician, presented to his GP with recurrent falls and feeling off balance. On further questioning he also complained of the following symptoms:

- Irritability.
- Tiredness.
- Low mood.
- Low libido.
- Erectile dysfunction.

His AMS score was 44 which indicated moderate symptoms.

His past medical history included occasional angina on exertion following a myocardial infarct 15 years ago.

His drug history was recorded as:

- Diltiazem.
- Metoprolol.
- Simvastatin.
- Recently added in Calcium with vitamin D and risedronate.

In view of the falls, investigations started with bloods which showed the following:

- Testosterone: 3.6 nmol/L (initial) and 4.4 nmol/L (repeat) (range 12-35).
- SHBG: 42 nmol/L (range: 16-55).

- Free testosterone: 0.07 nmol/L (range 0.225-0.62).
- LH: 1.8 IU/L (range:1.7-8.6).
- FSH: 1.5 IU/L (range:1.5-12.4).
- Prolactin: 559 mIU/L (range:86-324)
- Short synacthen and TSH were normal.

Tony also had a DEXA bone scan revealing osteoporotic changes in his hip and spine.

Due to the low testosterone and low normal gonadotrophins a MRI of the pituitary was ordered. This revealed a 2.5 cm pituitary adenoma not reaching the optic chiasm. Tony was diagnosed with a non-functioning pituitary macroadenoma with hypogonadotropic (secondary) hypogonadism.

Treatment from the neurosurgeons involved a 'watch and wait' approach with a six-monthly review MRI due to Tony's co-morbidities. He was started on testosterone therapy for his hypogonadism, and he continued with the Calcium with Vitamin D and Risedronate for the osteoporosis.

At six-months review Tony felt much better, less fidgety, and less irritable. He was less fatigued, and his sleep quality had improved. His libido had returned, and his erections improved. He felt stronger physically and had even returned to occasional work. His AMS score had decreased to 25. The neurosurgeons were happy with his progress as the 6 monthly MRI showed no change in tumour size.

Tony continues to be under neurosurgical review, and annual review with regards to his testosterone deficiency.

Lessons learned from this case

- Hypogonadism is a common cause of osteoporosis in men.[9]
- There is no evidence at present that testosterone reduces fracture risk although a recent study, T4Bone,[10] showed that in men >50 years, testosterone treatment for 2 years increases volumetric bone density, predominantly via effects on cortical bone. This is in addition to the bone study in the testosterone trials (TTrials)[11] that demonstrated that in older men with low testosterone, testosterone therapy significantly increases volumetric BMD and estimated bone strength, especially in trabecular bone and the spine, but also in peripheral bone and the hip.
- At the time of writing, hypogonadism with osteoporosis should still be treated with testosterone therapy and standard therapy for osteoporosis.
- Gonadotrophins are more sensitive to pressure damage from tumours than other pituitary cells.
- In the absence of symptoms caused by the mass effect of the pituitary tumour, isolated hypogonadotropic hypogonadism may be the first manifestation of this condition.

Case 4.2. Osteoporosis in men and the link with testosterone

78- year-old Bernard had been married to his wife for 55 years. Sadly, she had been diagnosed as having breast cancer a year ago and Bernard sustained a hip fracture following a fall. He was referred for a DEXA and diagnosed with severe osteoporosis. He was subsequently referred to the 'falls' clinic, where all assessments were normal other than a low testosterone. He was then referred to endocrinology.

Symptoms

- No erections for 14 years (greatly reduced since staring atenolol).
- Decreased libido (but not absent).
- Muscle weakness in both legs.
- Walking distance reduced, and could not go dancing anymore (decreased quality of life).

Past medical history

- Angina following MI 6 years ago.
- Hypertension.
- Benign Prostatic Hyperplasia (BPH).

Drug history

- Atenolol, aspirin, atorvastatin, risedronate, finasteride, valsartan, terazosin and GTN spray as needed.

Physical examination

- BMI: 29 kg/m^2.
- WC: 116 cm.

Investigations

- Total testosterone: 6.7 nmol/L (initial) 8.3 nmol/L (repeat) (range 12-35).
- SHBG: 26.4 nmol/L (range: 16-55).
- Calculated free testosterone: 0.145 nmol/L (initial) 0.183 nmol/L (repeat) (range 0.225-0.62).
- LH: 6.8 IU/L (range: 1.7-8.6).
- FSH: 4.5 IU/L (range: 1.5-12.4).
- Vitamin D: normal.
- PSA: 0.49 ug/L (range: 0-2).

Treatment

Bernard was diagnosed as having osteoporosis secondary to late onset hypogonadism. He was initiated on testosterone therapy.

He was reviewed 4 months after starting TTh and felt generally better (TT 21.8 nmol/L). His wife unfortunately died 6 months later. The next time he attended clinic was 14 months later but had continued on TTh and had been monitored by primary care physician. He had been depressed as a result of his bereavement.

His strength in his legs had improved and he was not falling. He had found a new partner and returned to dancing 5 times every week. Notably he now rarely needed to use his GTN spray with only 2-3 episodes of angina (exercise induced) in the previous 12 months.

Lessons learned from this case

- Weakness in the legs, falls and hip fracture can be caused by hypogonadism.
- Testosterone therapy can make a significant improvement in quality of life in some men.[12]

Case 5. Testosterone deficiency following testicular cancer

Richard 46, an ex-army captain was diagnosed with testicular cancer aged 29. He was a very fit military man who was keen on boxing and downhill skiing. Following his diagnosis, he underwent an orchidectomy of his right testicle followed by a course of chemotherapy. Once he had recovered from his cancer, he never quite felt like the person he was previously.

His symptoms following treatment for his testicular cancer were:

- No energy.
- Low mood.
- Decreased libido.
- Weight gain.
- Lack of concentration and memory problems.
- Hot flushes.

He initially presented to his GP who conducted a check of Richard's testosterone level.

- Total testosterone: 8.7 nmol/L (range 12-35).

The GP referred Richard to an endocrinologist who told him that his testosterone levels were in the normal range. Two years later, Richard attended a Urology clinic appointment department with a renal stone, and he mentioned the above symptoms to the consultant.

The Urology consultant repeated Richard's bloods and the investigations revealed the following:

- Total testosterone: 7.1 nmol/L (range 12-35).
- FSH: 19.3 IU/L (range: 1.5-12.4).
- LH: 10.2 IU/L (range: 1.7-8.6).
- Prolactin: 211 mIU/L (range: 86-324).
- SHBG: 44 (range: 16-55).
- Calculated free testosterone: 0.141 nmol/L (range 0.225-0.62).
- HbA1c: 48 mmol/mol (range: 20-42).

Physical examination

- Weight: 104 kg.
- WC: 44 inches.

Richard was treated with testosterone therapy and a year later his total testosterone had increased to 18 nmol/L, his HbA1c had reduced to 41 mmol/mol, his weight was significantly lower as was his waist circumference. Most importantly, Richard felt like he was back to his pre cancer treatment level of wellbeing.

Lessons learned from this case

- Around 20% of testicular cancer survivors experience testosterone deficiency.[13]
- Almost 40% of testicular cancer survivors develop testosterone deficiency after receiving platinum-based chemotherapy.[14]
- Symptomatic men post orchidectomy need investigating for low testosterone.
- The effects of testosterone deficiency are not limited to erectile dysfunction and low libido; testicular cancer survivors who develop testosterone deficiency can suffer from metabolic syndrome and poor cardiac health.
- Early treatment with testosterone therapy may help prevent conversion of pre-diabetes to overt diabetes.[5]
- As long-term survival in testicular cancer remains high, effective follow-up monitoring should be a priority.
- The European Society for Medical Oncology (ESMO) recommends monitoring of total testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in all patients post orchidectomy.[15]

Case 6. Testosterone deficiency as a cause of unexplained anaemia

70-year-old Derek, who was happily married for 35 years presented to his GP with tiredness and general lethargy. On further questioning he had been feeling this way for a couple of years; however, his levels of fatigue had deteriorated over the past 6 months, and his wife had suggested he see his GP.

His main presenting symptoms were as follows:

- Tiredness.
- Falling asleep in the afternoon.
- Not enjoying life as he once did.
- Decreased libido.

His GP noted he had no 'red flag' symptoms:

- No weight loss.
- Normal bowels.
- No erectile dysfunction.
- Euthymic.
- No urinary symptoms.

Physical examination included a digital rectal examination which was unremarkable. A host of bloods were arranged to investigate his fatigue. These included a FBC, PSA, CRP, TFT, LFT, renal and bone profile.

All results were unremarkable other than a low haemoglobin of 116 g/L (range: 130-170). MCV normal. This was repeated with haematinics:

- Hb: 114 g/L (range: 130-170).
- All other tests normal.

At this stage with no obvious cause of his low-grade anaemia and due to his ongoing symptoms his testosterone level was checked.

- Total testosterone 6.7 nmol/L (range 12-35).
- Free testosterone 0.184 nmol/L (range 0.225-0.62).
- Gonadotrophins and prolactin all normal.

On the basis of the reduced total and free testosterone and with the presence of clinical signs of testosterone deficiency (low libido, fatigue), Derek was diagnosed with testosterone deficiency and subsequently treated with testosterone therapy.

At his 6-month review his bloods were as follows:

- HB: 149 g/L (range: 130-170).
- Total testosterone: 15.7 nmol/L (range 12-35).

With respect to his symptoms, his tiredness had reduced, he was now able to go out for his afternoon stroll and his libido had improved.

Lessons learned from this case

- Whilst sexual symptoms are the most frequent reason why men eventually present to the GP, not all men with testosterone deficiency will have sexual

problems. Many men will present complaining of feeling tired all the time, feeling a bit down, or feeling like they have lost their spark.

- In approx. 20-44% of elderly adults with anaemia, no recognised cause can be found.[16]
- Testosterone deficiency may be the cause of patients' unexplained anaemia.[17-19]
- Testosterone therapy can resolve unexplained anaemia in men with testosterone deficiency.[20]
- Screening for hypogonadism should form part of a standard anaemia work-up in men by all physicians, not just endocrinologists.[21]
- Assessment of testosterone levels should be considered in men complaining of tiredness.

Conclusions

Historically, healthcare professionals have been more inclined to check testosterone levels only in men complaining specifically of diminished libido or erectile dysfunction, but even then, that approach is far from universal. Sadly, many men presenting with these symptoms will have their testosterone deficiency left undiagnosed, especially following the advent of generic and over the counter PDE5 inhibitors.

What we have tried to demonstrate throughout this book and specifically in this chapter, is that the prevalence of testosterone deficiency is significant in cases that present with non-sexual symptoms and that if diagnosed and treated appropriately, testosterone therapy in men with testosterone deficiency can produce life-changing benefits in terms of men's sexual, psychological, somatic and metabolic profile.

Treating men for conditions such as hypertension or elevated cholesterol obviously improves their health, but men do not necessarily "feel" any different. The vast majority of hypogonadal men who are treated with testosterone therapy feel much better when their levels are restored to the normal physiological range and they are often extremely grateful to have had their symptoms addressed.

In addition, the positive impact that testosterone therapy, in appropriate patients, can have on relationships and family life is profound.

These are the reasons why doctors who regularly treat this condition have countless success stories which helps boost morale and job satisfaction.

As a take home message, we strongly encourage all healthcare professionals to screen for testosterone deficiency in the following groups of male patients:

- Erectile dysfunction.
- Low libido.
- Lack of spontaneous erections.
- Type 2 diabetes or pre-diabetes.
- Obesity (WC >102 cm or BMI>30 kg/m^2).

- Men taking long-term opioids, anticonvulsants or antipsychotics.
- Osteoporosis.
- Anaemia.
- Fatigue/tired all the time (TATT).
- Low mood.
- Testicular cancer survivors.
- Infertility.

Following screening, if a diagnosis of testosterone deficiency can be made based on the presence of symptoms and biochemically low levels of testosterone (total testosterone <12 nmol/L or free testosterone <0.225 nmol/L) on two separate early morning (7-11 am) samples, consider initiating treatment with testosterone therapy once contra-indications have been ruled out.

References

1. JBS 3. The Joint British Societies recommendations on the prevention of Cardiovascular Disease (JBS3). Available from: http://www.jbs3risk.com/
2. Heinemann LA, Saad F, Zimmermann T, *et al.* The Aging Males' Symptoms (AMS) scale: update and compilation of international versions. Health Qual Life Outcomes 2003;1:15.
3. Rosen RC, Riley A, Wagner G, *et al.* The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. Urology 1997;49:822-30.
4. Hackett G, Kirby M, Edwards D, *et al.* British Society for Sexual Medicine Guidelines on Adult Testosterone Deficiency, With Statements for UK Practice. J Sex Med 2017;14:1504-23.
5. Wittert G, Bracken K, Robledo KP, *et al.* Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021;9:32-45.
6. Patient Health Questionnaire (PHQ-9). Available from: https://patient.info/doctor/patient-health-questionnaire-phq-9
7. Bawor M, Bami H, Dennis BB, *et al.* Testosterone suppression in opioid users: a systematic review and meta-analysis. Drug Alcohol Depend 2015;149:1-9.
8. Coluzzi F, Billeci D, Maggi M, *et al.* Testosterone deficiency in non-cancer opioid-treated patients. J Endocrinol Invest 2018;41:1377-88.
9. Golds G, Houdek D, Arnason T. Male Hypogonadism and Osteoporosis: The Effects, Clinical Consequences, and Treatment of Testosterone Deficiency in Bone Health. Int J Endocrinol 2017;2017:4602129.
10. Ng Tang Fui M, Hoermann R, Bracken K, *et al.* Effect of Testosterone treatment on bone microarchitecture and bone mineral density in men: a two-year RCT. J Clin Endocrinol Metab 2021;106:e3143-e3158.
11. Snyder PJ, Kopperdahl DL, Stephens-Shields AJ, *et al.* Effect of Testosterone Treatment on Volumetric Bone Density and Strength in Older Men With Low Testosterone: A Controlled Clinical Trial. JAMA Intern Med 2017;177:471-9.

12. Behre HM, Tammela TLJ, Arver S, *et al.* A randomized, double-blind, placebo-controlled trial of testosterone gel on body composition and health-related quality-of-life in men with hypogonadal to low-normal levels of serum testosterone and symptoms of androgen deficiency over 6 months with 12 months open-label follow-up. The Aging Male 2012;15:198-207.

13. Abu Zaid M, Dinh PC, Monahan PO, *et al.* Platinum Study Group. Adverse Health Outcomes in Relationship to Hypogonadism After Chemotherapy: A Multicenter Study of Testicular Cancer Survivors. J Natl Compr Canc Netw 2019;17:459-68.

14. Haugnes HS, Wethal T, Aass N, *et al.* Cardiovascular risk factors and morbidity in long-term survivors of testicular cancer: a 20-year follow-up study. J Clin Oncol 2010;28:4649-57.

15. Oldenburg J, Fosså SD, Nuver J, *et al.* ESMO Guidelines Working Group. Testicular seminoma and non-seminoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24(Suppl 6):vi125-32.

16. Merchant AA, Roy CN. Not so benign haematology: anaemia of the elderly. Br J Haematol 2012;156:173-85.

17. Al-Sharefi A, Mohammed A, Abdalaziz A, *et al.* Androgens and Anemia: Current Trends and Future Prospects. Front Endocrinol (Lausanne) 2019;10:754.

18. Al-Sharefi A, Quinton R. Hiding in a plain sight: A high prevalence of androgen deficiency due to primary hypogonadism among acute medical inpatients with anaemia. Clin Endocrinol (Oxf) 2018;89:527-9.

19. Shahani S, Braga-Basaria M, Maggio M, *et al.* Androgens and erythropoiesis: past and present. J Endocrinol Invest 2009 Sep;32:704-16.

20. Roy CN, Snyder PJ, Stephens-Shields AJ, *et al.* Association of Testosterone Levels With Anemia in Older Men: A Controlled Clinical Trial. JAMA Intern Med 2017;177:480-90.

21. Al-Sharefi A, Wilkes S, Jayasena C, *et al.* How to manage low testosterone level in men: a guide for primary care. Br J Gen Pract 2020;70:364-5.

Appendix 1.1

AMS Questionnaire

Which of the following symptoms apply to you at this time? Please, mark the appropriate box for each symptom. For symptoms that do not apply, please mark "none".

	Symptoms:	none	mild	moderate	severe	extremely severe
	Score =	1	2	3	4	5
1.	**Decline in your feeling of general well-being** (general state of health, subjective feeling)	☐	☐	☐	☐	☐
2.	**Joint pain and muscular ache** (lower back pain, joint pain, pain in a limb, general back ache)	☐	☐	☐	☐	☐
3.	**Excessive sweating** (unexpected/sudden episodes of sweating, hot flushes independent of strain)	☐	☐	☐	☐	☐
4.	**Sleep problems** (difficulty in falling asleep, difficulty in sleeping through, waking up early and feeling tired, poor sleep, sleeplessness)	☐	☐	☐	☐	☐
5.	**Increased need for sleep, often feeling tired**	☐	☐	☐	☐	☐
6.	**Irritability** (feeling aggressive, easily upset about little things, moody)	☐	☐	☐	☐	☐
7.	**Nervousness** (inner tension, restlessness, feeling fidgety)	☐	☐	☐	☐	☐
8.	**Anxiety** (feeling panicky)	☐	☐	☐	☐	☐
9.	**Physical exhaustion / lacking vitality** (general decrease in performance, reduced activity, lacking interest in leisure activities, feeling of getting less done, of achieving less, of having to force oneself to undertake activities)	☐	☐	☐	☐	☐
10.	**Decrease in muscular strength** (feeling of weakness)	☐	☐	☐	☐	☐
11.	**Depressive mood** (feeling down, sad, on the verge of tears, lack of drive, mood swings, feeling nothing is of any use)	☐	☐	☐	☐	☐
12.	**Feeling that you have passed your peak**	☐	☐	☐	☐	☐
13.	**Feeling burnt out, having hit rock-bottom**	☐	☐	☐	☐	☐
14.	**Decrease in beard growth**	☐	☐	☐	☐	☐
15.	**Decrease in ability/frequency to perform sexually**	☐	☐	☐	☐	☐
16.	**Decrease in the number of morning erections**	☐	☐	☐	☐	☐
17.	**Decrease in sexual desire/libido** (lacking pleasure in sex, lacking desire for sexual intercourse)	☐	☐	☐	☐	☐

Have you got any other major symptoms? **Yes** ☐ **No** ☐

If Yes, please describe: _____

_____

THANK YOU VERY MUCH FOR YOUR COOPERATION

Appendix 2.1

INTERNATIONAL INDEX OF ERECTILE FUNCTION

Patient Questionnaire

HOSPITAL NUMBER (IF KNOWN) ☐☐☐☐☐☐☐

NAME .....

DATE OF BIRTH ☐☐ ☐☐ ☐☐☐☐ AGE ☐☐

ADDRESS .....

.....

.....

TELEPHONE .....

These questions ask about the effects that your erection problems have had on your sex life <u>over the last four weeks</u>. Please try to answer the questions as honestly and as clearly as you are able. Your answers will help your doctor to choose the most effective treatment suited to your condition. In answering the questions, the following definitions apply:

- **sexual activity** includes intercourse, caressing, foreplay & masturbation
- **sexual intercourse** is defined as sexual penetration of your partner
- **sexual stimulation** includes situation such as foreplay, erotic pictures etc.
- **ejaculation** is the ejection of semen from the penis (or the feeling of this)
- **orgasm** is the fulfilment or climax following sexual stimulation or intercourse

---

OVER THE PAST 4 WEEKS
CHECK ONE BOX ONLY

☐ **Q1**	**How often were you able to get an erection during sexual activity?**	0 No sexual activity 1 Almost never or never 2 A few times (less than half the time) 3 Sometimes (about half the time) 4 Most times (more than half the time) 5 Almost always or always
☐ **Q2**	**When you had erections with sexual stimulation, how often were your erections hard enough for penetration?**	0 No sexual activity 1 Almost never or never 2 A few times (less than half the time) 3 Sometimes (about half the time) 4 Most times (more than half the time) 5 Almost always or always
☐ **Q3**	**When you attempted intercourse, how often were you able to penetrate (enter) your partner?**	0 Did not attempt intercourse 1 Almost never or never 2 A few times (less than half the time) 3 Sometimes (about half the time) 4 Most times (more than half the time) 5 Almost always or always
☐ **Q4**	**During sexual intercourse, <u>how often</u> were you able to maintain your erection after you had penetrated (entered) your partner?**	0 Did not attempt intercourse 1 Almost never or never 2 A few times (less than half the time) 3 Sometimes (about half the time) 4 Most times (more than half the time) 5 Almost always or always
☐ **Q5**	**During sexual intercourse, <u>how difficult</u> was it to maintain your erection to completion of intercourse?**	0 Did not attempt intercourse 1 Extremely difficult 2 Very difficult 3 Difficult 4 Slightly difficult 5 Not difficult

Appendix 2.2

☐ Q6	**How many times have you attempted sexual intercourse?**	0 No attempts 1 One to two attempts 2 Three to four attempts 3 Five to six attempts 4 Seven to ten attempts 5 Eleven or more attempts
☐ Q7	**When you attempted sexual intercourse, how often was it satisfactory for you?**	0 Did not attempt intercourse 1 Almost never or never 2 A few times (less than half the time) 3 Sometimes (about half the time) 4 Most times (more than half the time) 5 Almost always or always
☐ Q8	**How much have you enjoyed sexual intercourse?**	0 No intercourse 1 No enjoyment at all 2 Not very enjoyable 3 Fairly enjoyable 4 Highly enjoyable 5 Very highly enjoyable
☐ Q9	**When you had sexual stimulation or intercourse, how often did you ejaculate?**	0 No sexual stimulation or intercourse 1 Almost never or never 2 A few times (less than half the time) 3 Sometimes (about half the time) 4 Most times (more than half the time) 5 Almost always or always
☐ Q10	**When you had sexual stimulation or intercourse, how often did you have the feeling of orgasm or climax?**	1 Almost never or never 2 A few times (less than half the time) 3 Sometimes (about half the time) 4 Most times (more than half the time) 5 Almost always or always
☐ Q11	**How often have you felt sexual desire?**	1 Almost never or never 2 A few times (less than half the time) 3 Sometimes (about half the time) 4 Most times (more than half the time) 5 Almost always or always
☐ Q12	**How would you rate your level of sexual desire?**	1 Very low or none at all 2 Low 3 Moderate 4 High 5 Very high
☐ Q13	**How satisfied have you been with your overall sex life?**	1 Very dissatisfied 2 Moderately dissatisfied 3 Equally satisfied & dissatisfied 4 Moderately satisfied 5 Very satisfied
☐ Q14	**How satisfied have you been with your sexual relationship with your partner?**	1 Very dissatisfied 2 Moderately dissatisfied 3 Equally satisfied & dissatisfied 4 Moderately satisfied 5 Very satisfied
☐ Q15	**How do you rate your confidence that you could get and keep an erection?**	1 Very low 2 Low 3 Moderate 4 High 5 Very high

Appendix 2.3

INTERNATIONAL INDEX OF ERECTILE FUNCTION (IIEF)

Guidelines on Clinical Application of IIEF Patient Questionnaire

Background

The 15-question International Index of Erectile Function (IIEF) Questionnaire is a validated, multi-dimensional, self-administered investigation that has been found useful in the clinical assessment of erectile dysfunction and treatment outcomes in clinical trials. A score of 0-5 is awarded to each of the 15 questions that examine the 4 main domains of male sexual function: erectile function, orgasmic function, sexual desire and intercourse satisfaction.

In a recent study[1],the IIEF Questionnaire was tested in a series of 111 men with sexual dysfunction and 109 age-matched, normal volunteers. The following mean scores were recorded:

FUNCTION DOMAIN	MAX SCORE	CONTROLS	PATIENTS
A. Erectile Function (Q1,2,3,4,5,15)	30	25.8	10.7
B. Orgasmic Function (Q9,10)	10	9.8	5.3
C. Sexual Desire (Q11,12)	10	7.0	6.3
D. Intercourse Satisfaction (Q6,7,8)	15	10.6	5.5
E. Overall Satisfaction (Q13,14)	10	8.6	4.4

Clinical Application

IIEF assessment is limited by the superficial assessment of psychosexual background and the very limited assessment of partner relationship, both important factors in the presentation of male sexual dysfunction. Analysis of the questionnaire should, therefore, be viewed as an adjunct to, rather than a substitute for, a detailed sexual history and examination. The following guide-lines may be applied:

1. Patients with low IEEF scores (<14 out of 30) in Domain A (Erectile Function) may be considered for a trial course of therapy with Sildenafil unless contraindicated. Specialist referral is indicated if this is unsuccessful.

2. Patients demonstrating primary orgasmic or ejaculatory dysfunction (Domain B) should be referred for specialist investigation.

3. Patients with reduced sexual desire (Domain C) require testing of blood levels of androgen and prolactin.

4. Psychosexual counselling should be considered if low scores are recorded in Domains D and E but there is only a moderately lowered score (14 to 25) in Domain A.

Reference

1. Rosen R, Riley A, Wagner G, et al. The International Index of Erectile Function (IIEF): A multidimensional scale for assessment of erectile dysfunction. *Urology*, 1997, **49**: 822-830.

Appendix 3.1

Sexual Health Inventory for Men (SHIM)

Instructions

Each question has five possible responses. Circle the number that best describes your own situation. Select only one answer for each question.

Over the last six months:

1. How do you rate your confidence that you could keep an erection?

1	2	3	4	5
Very low	Low	Moderate	High	Very high

2. When you had erections with sexual stimulation, how often were your erections hard enough for penetration (entering your partner)?

1	2	3	4	5
Almost never or never	A few times (much less than half the time)	Sometimes (about half the time)	Most times (more than half the time)	Almost always or always

3. During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?

1	2	3	4	5
Almost never or never	A few times (much less than half the time)	Sometimes (about half the time)	Most times (more than half the time)	Almost always or always

4. During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?

1	2	3	4	5
Extremely difficult	Very difficult	Difficult	Slightly difficult	Not difficult

5. When you attempted sexual intercourse, how often was it satisfactory for you?

1	2	3	4	5
Almost never or never	A few times (much less than half the time)	Sometimes (about half the time)	Most times (more than half the time)	Almost always or always

Appendix 3.2

Information for clinicians

Add together the numbers corresponding to the answers for all the questions. If the patient's score is 21 or less, erectile dysfunction (ED) should be addressed. The SHIM score measures the severity of the patient's ED in the following manner:

22 – 25:	No significant erectile dysfunction
17 – 21:	Mild erectile dysfunction
12 – 16:	Mild-to-moderate erectile dysfunction
8 – 11:	Moderate erectile dysfunction
5 – 7:	Severe erectile dysfunction

Score: ……

The purpose of SHIM

- With the advent of oral therapy for ED, the need for accurate diagnosis is greater than ever
- The SHIM questionnaire (also known as IIEF-5) is an abridged and slightly modified five-item version of the 15-item International Index of Erectile Function (IIEF), designed for easy use by clinicians to diagnose the presence & severity of ED in clinical settings
- This diagnostic tool may reduce the number of incorrectly diagnosed or underdiagnosed cases
- It is intended to complement the physical examination and patient history as a means of detecting ED

Adapted from:
Rosen RC, Cappelleri JC, Smith MD, Lipsky J, Peña BM
Development of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction
Int J Impot Res (1999); 11; 319 – 326

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Testosterone in cardiometabolic and other diseases

This book is very timely as several recent studies have demonstrated the important role of testosterone in the development of type 2 diabetes and metabolic syndrome. Testosterone is a highly controversial but vitally important hormone in men's health. The traditional approach has been to classify low testosterone, or hypogonadism as either primary, due to disorders of the testes and therefore "classical" and meriting testosterone therapy, or secondary, where treatment may be indicated when due to structural pituitary or hypothalamic disorders. These include so-called functional causes, such as obesity, diabetes and medications where management through lifestyle change might be considered appropriate. The problem with this approach is that lifestyle changes are often either ineffective or of short-term benefit. These functional cases suffer just as readily in terms of new onset diabetes, sexual dysfunction, depression, cognitive disorders and most importantly increased mortality. The authors discuss in detail the role of testosterone and other therapeutic approaches to these problems as demonstrated by recent studies, such as T4DM, the T trials and BLAST, along with the possible impact of the long-awaited TRAVERSE study. The recent Covid-19 pandemic has caused increased mortality in men associated with profound and acute falls in testosterone that are closely linked with adverse outcomes across multiple medical disciplines. The reality is that Covid-19 is now probably the major cause of hypogonadism, with acute and long-term implications. The authors have assembled a team of international experts in andrology to address highly controversial issues in the chapters that follow.

Testosterone in cardiometabolic and other diseases by Geoffrey I. Hackett and Michael Kirby is an outstanding addition to the medical literature. This book should be read and used as an active reference to any clinician who cares for men, especially those men with cardiometabolic disease and diabetes. Testosterone replacement therapy is no longer a controversial treatment for these men and can be expected to improve the quality of their lives, diabetes control and life expectancy.

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