Author/s:
Grossmann, M;Ng Tang Fui, M;Cheung, AS

Title:
Late-onset hypogonadism: metabolic impact

Date:
2020-11-01

Citation:
Grossmann, M., Ng Tang Fui, M. & Cheung, A. S. (2020). Late-onset hypogonadism: metabolic impact. Andrology, 8 (6), pp.1519-1529. https://doi.org/10.1111/andr.12705.

DR MATHIS GROSSMANN (Orcid ID : 0000-0001-8261-3457)

Article type : Review Article

Late Onset Hypogonadism: Metabolic Impact

Short title: LOH: metabolic impact

Mathis Grossmann[1, 2], Mark Ng Tang Fui[1, 2], Ada S. Cheung[1, 2]

[1]Department of Medicine Austin Health, University of Melbourne, Melbourne, Victoria Australia

[2]Department of Endocrinology, Austin Health, Melbourne, Victoria Australia

Word Count: Abstract 247; Manuscript 6,369; References 76; Tables: 1, Figures: 1.

Correspondence: Dr Mathis Grossmann
Department of Medicine, Austin Health, University of Melbourne, 145 Studley Road, Heidelberg, VIC 3084, Australia
Email mathisg@unimelb.edu.au

Keywords: Obesity, diabetes, testosterone, Late onset hypogonadism

Abstract

Background: Obesity and dysglycemia (comprising insulin resistance, the metabolic syndrome and type 2 diabetes), i.e. diabesity, are associated with reduced circulating testosterone and, in some men, clinical features consistent with androgen deficiency.

Objective: To review the metabolic impact of late onset hypogonadism (LOH).

Methods: Comprehensive literature search with emphasis on recent publications.

Results: Obesity is one of the strongest modifiable risk factors for LOH, and coexisting diabetes leads to further hypothalamic-pituitary-testicular (HPT) axis suppression. The HPT axis suppression is functional and hence potentially reversible, and occurs predominantly at the level of the hypothalamus. While definitive mechanistic data are lacking, the evidence suggests that HPT axis suppression is mediated by dysregulation of pro-inflammatory cytokines leading to hypothalamic inflammation. Dysregulation of central leptin and insulin signalling may also contribute. In contrast, recent data challenge the paradigm that estradiol excess is a major contributor to HPT axis suppression. Instead relative estradiol signalling deficiency may contribute to metabolic dysregulation in men with diabesity. While weight loss and optimisation of comorbidities can reverse functional HPT axis suppression, testosterone treatment leads to metabolically favourable changes in body composition, and to improvements in insulin resistance.

Discussion: The relationship between diabesity and LOH is bi-directional. Preliminary evidence suggests that, in carefully selected men, lifestyle measures and testosterone treatment may have additive effects.

Conclusions: While recent research has provided new insights into mechanistic and clinical aspects of diabesity-associated LOH, more evidence from well-designed large trials is needed to guide the optimal clinical approach to such men.

Introduction

In this review, we will provide an update on the metabolic impact of late onset hypogonadism (LOH). Given several recent comprehensive reviews in this area (Dhindsa, et al., 2018, Gianatti and Grossmann, 2019, Grossmann, 2018, Rastrelli, et al., 2018), we will highlight recent progress focusing predominantly on relevant work published from 2016 onwards. In particular we will focus on the interactions between the hypothalamic-pituitary-testicular (HPT) axis and obesity and dysglycemia (comprising insulin resistance, the metabolic syndrome and type 2 diabetes (T2DM)). Of note, LOH can be subdivided, based on

biochemistry, into primary, secondary and compensated forms. While age-related HPT axis changes manifest predominantly as primary and compensated forms, LOH related to diabesity usually presents as the secondary (central) form (Kaufman, et al., 2019). However, metabolic disease may interact with, and modify the clinical presentation of all three forms of LOH. With respect to nomenclature and definitions, we will use 'diabesity' to denote the coexistence of obesity and dysglycemia, 'low testosterone' to denote endogenous testosterone concentrations below the reference range derived for healthy young men, 'biochemical hypogonadism' to denote low testosterone without reference to symptoms, and 'LOH' to denote low testosterone associated with androgen deficiency-like symptoms, noting that causality is not always certain and that sexual symptoms are the most specific (Wu, et al., 2010). However, chronic disease can have a significant impact on sexual symptoms independent of serum testosterone (Rastrelli, et al., 2019). We use 'functional hypogonadism' to denote a diagnosis of exclusion, defined as biochemical and/or clinical evidence of androgen deficiency in the absence of clear-cut organic HPT axis pathology (i.e. the absence of classical organic hypogonadism due to established medical disease of the HPT axis). We will consider epidemiologic, and mechanistic (both preclinical and in humans) studies, as well as clinical trials. The material covered in this review is based on multiple PubMed searches using the search terms "testosterone", "estradiol", "obesity", "diabetes", "insulin resistance", "metabolic syndrome", and "late onset hypogonadism" to June 2019.

Associations of testosterone with diabesity in observational studies

Obesity is arguably the single most important and strongest risk factor for biochemical hypogonadism (usually hypogonadotropic, see below) and LOH. In the European Male Ageing Study (EMAS), obese men (body mass index (BMI) >30 kg/m^2) had a 30% lower testosterone concentration (measured by liquid chromatography/mass spectrometry (LCMS)), a reduction equivalent to almost 3 decades of aging, and a 13-fold increase in LOH prevalence compared to men with a BMI of <25 kg/m^2 (Wu, et al., 2010). In the presence of obesity, type 2 diabetes (T2DM) is associated with an additional reduction in testosterone, although this reduction is comparatively modest; in one large study in obese men, those with diabetes had a 0.8 nmol/L lower total testosterone concentration (by LCMS) compared to obese men without diabetes (Dhindsa, et al., 2010). Indeed in the most recent meta-analysis, the relative reduction of total testosterone concentrations attributable to T2DM (irrespective of BMI) was relatively modest, 2.98 nmol/L, measured by immunoassays in most studies (Zhang, et al., 2019), quantitively similar to previous meta-analyses in men with T2DM

(Corona, et al., 2010) or the metabolic syndrome (Brand, et al., 2014). However, men in these meta-analyzed observational studies had generally fairly well controlled T2DM, and there is evidence that poorly controlled diabetes may have greater HPT axis-suppressive effects (Grossmann and Matsumoto, 2017). Of note, the association of diabetes with low testosterone is primarily driven by insulin resistance and adiposity rather than pancreatic beta cell failure, given that low testosterone concentrations are uncommon in men with type 1 diabetes, unless they have coexisting obesity and/or large insulin requirements (Holt, et al., 2014, Ng Tang Fui, et al., 2013). Overall these cross-sectional studies suggest that obesity is a more dominant risk factor for low testosterone than diabetes.

Role of SHBG

Whether the association of diabesity and low testosterone is solely mediated by lowered sex hormone binding globulin (SHBG) has been controversial, especially with respect to T2DM. Some studies have suggested that SHBG but not testosterone predicts T2DM (Bhasin, et al., 2011), and SBHG (Ding, et al., 2009) but not testosterone (Haring, et al., 2013) has been associated with T2DM risk in Mendelian randomization studies. In the aforementioned meta-analysis, men with diabetes also had lowered free testosterone concentrations (by 32 pmol/L) compared to men without T2DM (Zhang, et al., 2019), suggesting an SHBG-independent association. Free testosterone was derived differently in individual studies, calculated by various formulae and from different immunoassays, limiting the precision of these findings. In a prospective analysis of EMAS, a lower testosterone concentration was associated with an increased risk of developing the metabolic syndrome, even after adjustment for SHBG (Antonio, et al., 2015). In a meta-analysis of studies with a total of 16,709 men, higher free testosterone predicted a modestly reduced risk of T2DM (RR=0.94, 95%CI 0.90; 0.99, p=0.014)(Yao, et al., 2018). Likewise, free testosterone predicted incident metabolic syndrome in a patient level meta-analysis, although more weakly than total testosterone or SHBG (Brand, et al., 2014). In a prospective study of 1,597 men without T2DM, SHBG was no longer predictive of incident T2DM after adjustment for total testosterone (by LCMS) (Gyawali, et al., 2018). In contrast, in a prospective cohort study of 5,350 men, while low total testosterone and low SHBG were associated with an increased risk of future T2DM development, free testosterone and LH were not predictive (Holmboe, et al., 2016). However, in a small but well conducted study in 852 men free of diabetes using LCMS assay technology, after multivariable adjustment, the low concentrations of the pure androgen DHT

were most consistently associated with insulin resistance and T2DM development, compared to testosterone (whether total or free), and SHBG (Joyce, et al., 2017).

With respect to obesity, it is clear that while modest obesity is predominantly associated with a lowered total testosterone paralleling the reduced SHBG, more marked obesity (especially BMI>35-40 kg/m^2) is also associated with reductions in free testosterone. This suggests that more marked obesity is associated with genuine biochemical hypogonadism. In summary, while some of the low testosterone-diabesity associations reflect lowered SHBG, the observational data strongly suggest androgen-diabesity interactions that are independent of SHBG.

Testosterone threshold

Previous observational and experimental studies in men, reviewed elsewhere (Grossmann, 2018) have suggested that adverse metabolic impacts, such as insulin resistance occur largely at subnormal testosterone concentrations (<8-12 nmol/L). Interestingly, recent observational studies have reported that testosterone predicts T2DM even when concentrations are relatively high, with a cutoff point of <16 nmol/L in an Australian cohort on 1,665 men (Atlantis, et al., 2016). Likewise in a large UK database study of 70,541 men, compared to a reference cohort of men with total testosterone of ≥20 nmol/L, a significantly increased risk of T2DM was already evident in men with testosterone between 15-19.99 nmol/L, incident rate ratio=1.29, 95%CI 1.13; 1.47, p<0.001 after adjustment for age, BMI, and comorbidity index. The highest risk (2.71, 95%CI 2.34; 3.41, p<0.001) was observed in those with a testosterone <7 nmol/L (O'Reilly, et al., 2019).

In summary, observational studies suggest that low testosterone is associated with, and predicts future adverse metabolic outcomes, such as insulin resistance, the metabolic syndrome, T2DM and obesity. While this association is in part mediated via SHBG, evidence for independent associations with free testosterone and the pure androgen DHT, methodological caveats aside, suggest SHBG independent associations. Interestingly, diabetes risk may increase at higher testosterone concentrations than appreciated previously.

Previous observational studies, reviewed in detail recently (Gianatti and Grossmann, 2019, Grossmann, 2018), have reported that diabesity (including the metabolic syndrome) is associated with a more rapid decline in testosterone, suggesting a bi-directional association

(see below). Consistent with earlier work, in a recent prospective study of 141 younger (mean age 43 years) mostly insulin resistant men, insulin resistance (measured using an octreotide-based pancreatic suppression test) predicted hypogonadism (defined as a total testosterone <10.4 nmol/L) somewhat more strongly (risk ratio= 2.2) than hypogonadism predicted insulin resistance (risk ratio=1.3) (p=0.03) (Contreras, et al., 2018). However in a larger prospective study among 1,400 Swedish men (mean age 58 years), low baseline testosterone predicted insulin resistance (measured by HOMA-IR), but high insulin resistance at baseline did not predict low testosterone at follow-up (Ottarsdottir, et al., 2018). Although observational studies, even if prospective cannot establish causality, nor determine the direction of causality, the evidence overall is consistent with a bi-directional relationship. On the one hand, low endogenous androgens may promote diabesity risk, and on the other diabesity may accelerate the age-related decline in testosterone. Potentially low endogenous androgens and diabesity interact in a self-perpetuating cycle (**Figure**).

Intervention and experimental studies (see below) suggest that some of the effects of testosterone on fat mass and glucose metabolism may be dependent on aromatization to estradiol. Observational evidence associating circulating estradiol with diabesity is however scant and contradictory, in part because of the collinearity of circulating testosterone (the substrate) with estradiol (the product). Further, estradiol was measured by immunoassay in the majority of studies, which lacks accuracy in quantifying the relatively low circulating estradiol concentrations in men (Russell and Grossmann, 2019).

Of note, most of the studies discussed so far have reported on biochemical hypogonadism, rather than also considering clinical features of androgen deficiency, that is, focusing on the combined presence of androgen deficiency-like symptoms and biochemical hypogonadism, i.e. LOH. That said, generalized symptomatology in individuals with diabesity, especially if associated with comorbidities is almost impossible to distinguish from those of hypogonadism. In EMAS, using a stringent definition (syndromic association of low testosterone with the 3 most specific sexual symptoms), LOH was associated with multiple end organ deficits compatible with androgen deficiency. Associations were stronger in men with LOH compared to men that merely had reduced testosterone without symptoms (Tajar, et al., 2012). Interestingly, while numbers were small, among men with LOH only those with testosterone of < 8 nmol/L, but not those with higher testosterone, had an increased prevalence of central obesity, insulin resistance and the metabolic syndrome (Tajar, et al.,

2012). Moreover, in both the EMAS and the Boston Area Community Health (BACH) survey cohort, central obesity was the strongest predictor of symptomatic androgen deficiency, overriding the effects of age and comorbidities (Hall, et al., 2008, Wu, et al., 2010). One recent study of almost 5,000 Italian men sought to separate the effect of obesity and metabolic health on hypogonadal features by studying metabolically healthy and metabolically unhealthy (defined as presence of at least one of diabetes, dyslipidemia or hypertension) obese men, and healthy nonobese controls (Lotti, et al., 2019). Both metabolically healthy and metabolically unhealthy men had lower testosterone concentrations compared to nonobese men. Only metabolically unhealthy, but not metabolically healthy obese men had worse self-reported and objective (by penile doppler ultrasound) sexual function compared to nonobese men. This study suggests that even relatively specific sexual symptoms may, at least in a part, be aggravated by underlying vascular disease rather than solely caused by testosterone deficiency (Lotti, et al., 2019).

Directionality of the low testosterone-diabesity relationship

The relationship between diabesity and low testosterone is generally considered to be bi-directional. Overall, there is stronger clinical evidence that obesity (and to a lesser extent diabetes, especially if poorly controlled (Grossmann and Matsumoto, 2017)) has a greater effect on reducing testosterone, compared to the lesser effect that low testosterone has on promoting diabesity. In a recent bi-directional Mendelian study, a genetically instrumented increase in BMI was associated with a decrease in serum testosterone, whereas no association was seen for genetically instrumented testosterone with BMI (Eriksson, et al., 2017). The size effect of the genetic effect of increasing BMI on lowering testosterone was similar in magnitude to associations of actual BMI reductions with increases in circulating testosterone in observational studies (Camacho, et al., 2013), and effects of weight loss on testosterone in weight loss studies (Corona, et al., 2013, Grossmann, 2011). In adult men with biochemical hypogonadotropic hypogonadism, diet-associated weight loss leads to modest increases in testosterone, 2.87 nmol/L (95%CI 1.68; 4.07) with ~10% loss of body weight. Bariatric surgery (~30% loss of body weight) is associated with a more marked increase in total testosterone of 8.73 nmol/L (95%CI 6.51; 10.95) (Corona, et al., 2013). With sufficient weight loss (≥10% of body weight), increases in gonadotropins and free testosterone also occur, suggesting a genuine reactivation of the HPT axis (Camacho, et al., 2013, Corona, et al., 2013). Overall, the changes in endogenous testosterone achievable with non-surgical weight loss measures are relatively modest. In a recent RCT of testosterone treatment plus

diet in motivated obese men, a rigorous structured diet program leading to 11kg weight loss in the placebo group increased serum testosterone only modestly, by 2.9 nmol/L (Ng Tang Fui, et al., 2016). This effect was not sustained 18 months after trial end (Ng Tang Fui, et al., 2017). Consistent with HPT axis responsiveness to changes in body weight, biochemical hypogonadism is not a steady state; in EMAS over a mean follow-up of 4.3 years, 43% of men with biochemical hypogonadotropic hypogonadism recovered eugonadism. Weight loss and nonobesity at baseline were the strongest predictors (Rastrelli, et al., 2015).

Conversely, changes in endogenous testosterone concentrations are associated with relatively modest changes in body weight; even severe testosterone deficiency due to androgen deprivation therapy (ADT) which reduces testosterone to near zero has only minor effects on body weight. In a prospective study of older men with localised prostate cancer who were eugonadal at baseline (total testosterone 14 nmol/L), after 12 months of ADT (decreasing total testosterone to 0.4nmol/L), BMI increased by only 0.65 kg/m^2 (95%CI 0.14; 1.15) compared to matched prostate cancer controls not receiving ADT (Cheung, et al., 2016). However, ADT is clearly associated with metabolically adverse effects on body composition. Fat mass increased by 3.5 kg [95%CI 2.0; 5.0] and lean mass decreased by 1.5 kg (95%CI 0.2; 1.8), associated with a 30% increase in insulin resistance (measured by HOMA-IR) (Cheung, et al., 2016). The effects of testosterone treatment in clinical trials are discussed below, but are consistent with a bi-directional relationship between diabesity and low testosterone, with more dominant effects of the former.

Potential mechanisms of the low testosterone-diabesity relationship

Diabesity promoting low testosterone

While modest obesity/insulin resistance is primarily associated with a low total testosterone due to lowered SHBG, more marked obesity leads to genuine biochemical hypogonadism, evidenced by decreases also in free testosterone (Grossmann, 2018). Men with diabetes and/or obesity and low testosterone, as first reported by Dandona's group (Dhindsa, et al., 2018), rarely have elevated gonadotrophin concentrations. Therefore diabesity-associated reductions in endogenous testosterone are considered to be due to central gonadal axis suppression. This may occur largely at the level of the hypothalamus. As reviewed elsewhere, pro-inflammatory adipocytokines, impaired insulin signalling in the central nervous system (CNS) and dysregulated leptin signalling may contribute to hypothalamic suppression, likely, at least in part, via effects on KNDy (kisspeptin/neurokinin B/dynorphin) neurons in the

arcuate nucleus of the hypothalamus (Dhindsa, et al., 2018, Gianatti and Grossmann, 2019, Grossmann, 2018, Rastrelli, et al.,, 2018).

A role for hypothalamic inflammation was first suggested by a study in male rabbits by Maggi's group. This study demonstrated that high fat diet-induced hypogonadotropic hypogonadism was associated with increased hypothalamic inflammation, increased hypothalamic expression of pro-inflammatory cytokines and reduced expression of the kisspeptin-1 receptor (Morelli, et al., 2014). More recently in a cross-sectional study of 41 adult men, hypothalamic inflammation (estimated by validated magnetic resonance imaging) was inversely associated with serum testosterone concentrations, even after adjustment for visceral adiposity, BMI, age and insulin resistance (Berkseth, et al., 2018). Such studies, while hypothesis-generating cannot untangle potentially causal relationships, or directions thereof, between testosterone, (visceral) adiposity and hypothalamic inflammation. A recent experimental study in adult men reported that infusing the proinflammatory cytokine interleukin-2 (IL-2) at a relatively low dose reduced LH stimulated testosterone secretion and augmented testosterone-mediated negative LH feedback (Veldhuis and Bowers, 2009). Moreover, a small 4-week RCT in men (n=33) with the metabolic syndrome reported that anti-inflammatory treatment with an IL-1 antagonist modestly increased serum testosterone (0.96 nmol/L, 95%CI 0.3; 1.9, p=0.04). Greater treatment effects were seen in men with a baseline C-reactive protein >2mg/L (2.14 nmol/L, 95%CI 0.1; 4.9, p=0.04) or a BMI>40 kg/m^2 (2.64 nmol/L, 95%CI 0.2; 5.9, p=0.04) (Ebrahimi, et al., 2018). While these studies cannot identify the exact site(s) where interleukins act in the hypothalamic-pituitary unit, they are consistent with a role of inflammation, both acute (Veldhuis and Bowers, 2009) or chronic (Berkseth, et al., 2018), in the pathogenesis of hypogonadotropic hypogonadism. A study in healthy men has reported that a low-dose endotoxin challenge produced a transient inflammatory response associated with a decline in serum testosterone (Tremellen, et al., 2018), leading the authors to speculate that inflammation originating from gut microbiota might contribute to hypogonadism, although further work is needed to explore this hypothesis. While evidence for a role of hypothalamic inflammation is accumulating, more preliminary evidence points to a role for genetic susceptibly, and perhaps auto-immunity in some men, although confirmatory studies are required. Whether functionally milder sequence variants in genes associated with congenital hypogonadotropic hypogonadism (CHH) may play a role for differential susceptibility to diabesity-associated HPT axis suppression in older men, is an intriguing hypothesis that requires further study. Rare variants of such genes have

been described in some obese men, but larger studies are needed (Cangiano, et al., 2019). Interesting, such rare variants have also been described in small series of men with functional hypogonadism due to weight loss and/or excessive exercise (Dwyer, et al., 2019, Wong, et al., 2019). This suggests that CHH-associated gene variants may modulate susceptibility to hypogonadotropic hypogonadism even in men without frank CHH. This may occur at extremes of both energy excess as well as energy deficit. Finally, a study in older obese men with lowered testosterone reported that a small fraction (4/100) had pituitary gonadotroph antibodies with granular positivity on cytosolic staining, a pattern relatively specific for auto-immunity (Ricciuti, et al., 2016). Such findings have not yet been confirmed in other, independent cohorts. Overall, these recent studies are consistent with the notion that, while generally considered functional, at least in some men, LOH may be caused by novel organic pathology, blurring the distinction from classical hypogonadism caused by medical disease of the HPT axis (e.g. Klinefelter, pituitary tumor). Indeed, in observational studies, lifestyle interventions or improvement of comorbidities such as diabetes is associated with normalization of circulating testosterone in only about 50% of men with secondary hypogonadism (Rastrelli, et al., 2015).

Low testosterone promoting diabesity

Studies discussed so far provide potential mechanisms by which diabesity may promote hypogonadism, but there are likewise studies providing potential mechanistic evidence for the reverse, i.e. low testosterone leading to diabesity. For example, reviewed in detail elsewhere (Dhindsa, et al., 2018, Gianatti and Grossmann, 2019, Grossmann, 2018, Rastrelli, et al., 2018), testosterone has been reported to reduce pro-inflammatory cytokines *in vitro* and *in vivo*, to increase insulin sensitivity in both muscle and adipose tissue and to promote catecholamine-induced lipolysis. Targeted deletions of the androgen receptor also suggest that androgen signalling in the liver (Grossmann, et al., 2019), brain and adipose tissue (Wang and Xu, 2019), prevents obesity development in male mice. Moreover, a recent study examining male mice with a deletion of the androgen receptor targeted to pancreatic beta cells reported that androgenic signalling augments glucose-stimulated insulin secretion in beta cells via amplifying the incretin effect of glucagon-like peptide-1 (Navarro, et al., 2016). Thus, androgen signalling in multiple somatic tissues may favour both insulin sensitivity and secretion. The caveat of these studies is that they cannot distinguish developmental from acquired effects, nor exclude a phenotype related to the absence of the androgen receptor, rather than its ligand.

In men, the extent to which effects of testosterone treatment on glucose metabolism are dependent on promoting metabolically favourable changes in body composition (i.e. increase in lean mass and decrease in fat mass) is not clear. Of note, whether testosterone treatment has effects on metabolically active visceral adipose tissue is not certain (see below). Small studies reported that testosterone may regulate insulin sensitivity directly and acutely, before changes in body composition are expected to occur (Yialamas, et al., 2007). Moreover, short-term hyperinsulinemic-euglycemic clamp studies in healthy adult men with pharmacologically altered endogenous sex steroid concentrations reported changes in insulin sensitivity in the absence of changes in BMI (Gibb, et al., 2016, Lapauw, et al., 2010). These studies also examined differential effects of testosterone vs. estradiol on insulin sensitivity (further discussed below).Other potential mechanisms are changes in energy expenditure or increased motivation to engage in physical activity (Ng Tang Fui, et al., 2016), but no definite evidence exists.

Role of estradiol

Early studies, especially in men with BMI >40 kg/m^2 suggested that increased circulating estradiol, produced by adipose-tissue expressed aromatase, may, via negative hypothalamic-pituitary feedback, promote obesity-associated hypogonadotropic hypogonadism. However, (for review see, Dhindsa, et al., 2018, Russell and Grossmann, 2019), LCMS-based studies in men with lesser obesity have reported that, parallel with reduced testosterone, circulating estradiol levels are likewise reduced in obese men (Dhindsa, et al., 2011). Interestingly and unexpectedly, a study in men with T2DM reported that diabetic men with biochemical hypogonadotropic hypogonadism had lower adipose aromatase tissue expression than biochemically eugonadal diabetic men (Ghanim, et al., 2018). This study further supports the notion that estradiol-mediated negative feedback is not involved in the pathogenesis of hypogonadotropic hypogonadism. Of note, the reflex rise in circulating testosterone that occurs in obese men upon treatment with agents that inhibit estradiol-mediated negative feedback at the hypothalamic-pituitary unit (aromatase inhibitors, selective estrogen receptor modulators) cannot be invoked to infer a pathogenetic role of estradiol excess in obesity associated-hypogonadism because the increase in testosterone also occurs if lean men are treated with these agents (Grossmann and Matsumoto, 2017). In Ghanim's study (Ghanim, et al., 2018), the hypogonadal men also had reduced expression of the androgen receptor, and of the estrogen receptor alpha in fat and muscle, which was normalised with testosterone

treatment. This suggests that, if confirmed, reduced tissue receptor-mediated signalling may augment the effects of reduced circulating sex steroids in some hypogonadal men (Ghanim, et al., 2018).

While estradiol does not appear to play a major role in diabesity-associated hypothalamic pituitary suppression, at least in men with sub-massive obesity, there is increasing evidence that some of the effects of testosterone on fat mass and glucose metabolism may, at least in part, be indirect, via aromatization to estradiol (Russell and Grossmann, 2019). Consistent with estradiol-mediated metabolic protection, in a large study of experimentally induced hypogonadism in healthy young men (n=400) with graded testosterone add-back with or without concomitant aromatase inhibitor treatment concluded that low estradiol (but not low testosterone) was responsible for the hypogonadism-associated increase in fat mass, although there was no placebo control for the aromatase inhibitor (Finkelstein, et al., 2013). The finding that estradiol, rather than testosterone, primarily regulates fat mass was subsequently confirmed in a similarly designed study of 56 healthy men (Chao, et al., 2016). However, an RCT in healthy men older than 50 years reported that transdermal DHT treatment at a dose of 70 mg per day reduced fat mass despite suppressing estradiol (Idan, et al., 2010). This suggests that androgen receptor-mediated signalling may also be important for testosterone-mediated effects on adipose tissue. Juang et al (Juang, et al., 2013) conducted a RCT in obese men with experimentally induced hypogonadism given testosterone add-back with or without dutasteride (to block DHT production) or the aromatase inhibitor anastrazole (to suppress estradiol production). Using hyperglycemic-euglycemic clamps, they found that testosterone plus dutasteride reduced fat mass and improved insulin sensitivity whereas testosterone plus anastrozole did not. Testosterone alone reduced fat mass but had no effects on insulin sensitivity (Juang, Peng, Allehmazedeh, Shah, Coviello and Herbst, 2013). Based on these findings, the authors speculated that reduction of the ratio of testosterone to DHT attenuates the beneficial effects of estradiol on insulin sensitivity, but not on fat mass (Juang, et al., 2013). In a recent experimental RCT using hyperinsulinemic-euglycemic clamps, aromatase inhibitor treatment reduced insulin-stimulated glucose disposal, again inferring a beneficial metabolic role for estradiol (Gibb, et al., 2016). Insulin resistance has also been documented in aromatase-deficient men and in a variety of tissue-specific estrogen alpha receptor male knock out mice, collectively suggesting that estrogen alpha receptor signalling in liver, brain, skeletal muscle and adipose tissue may favour insulin sensitivity (Russell and Grossmann, 2019). The importance of estradiol in improving glucose metabolism is consistent with large

observational studies among community-dwelling men using LCMS demonstrating that estradiol concentrations parallel the reduced testosterone concentrations and are inversely correlated with measures of obesity and diabetes (Dhindsa, et al., 2011). However, not every study is fully consistent. In a small short-term (1 week) hyperinsulinemic-euglycemic study in men (n=10), aromatase inhibitor treatment (leading to increased serum testosterone and reduced estradiol) was associated with increased insulin sensitivity. In men (n=10) receiving both an aromatase inhibitor and an estradiol patch (leading to a reduction in serum testosterone and an increase in estradiol), insulin sensitivity remained unchanged (Lapauw, et al., 2010). Overall the bulk of the evidence suggests that in men, testosterone reduces fat mass by both androgen receptor and estrogen receptor signalling, and that aromatization of testosterone to estradiol is required to improve glucose metabolism. The bi-directional relationship between lowered sex steroids and diabesity is summarised in the **Figure**.

Metabolic impact of testosterone treatment in clinical trials

RCTs in men with T2DM and/or the metabolic syndrome published before 2016 have been extensively reviewed elsewhere (Dhindsa, et al., 2018, Gianatti and Grossmann, 2019, Grossmann, 2018, Rastrelli, et al., 2018). Overall, findings suggested that testosterone treatment consistently and modestly increases lean mass (by about 2kg), decreases fat mass (by about 2kg), and modestly improves insulin resistance in most but not all studies. In contrast, testosterone treatment has no consistent effects on visceral adipose tissue mass and on glycemic control, measured by HbA1c. A meta-analysis restricted to placebo-controlled double-blind RCTs (n=7) concluded that testosterone treatment modestly improved insulin resistance, compared to placebo, pooled mean difference (MD) -1.58 (95%CI -2.25; -0.91, $p<0.001$) but did not improve glycemic (HbA1c) control, MD -0.15 (95%CI -0.39; 0.10), p=0.25 (Grossmann, et al., 2015). However, studies were relatively small (total n= 833 men), and short-term (≤ 12 months). Inclusion criteria were heterogeneous, and participants generally had well controlled diabetes at baseline. Moreover, given erythropoietic actions of testosterone, HbA1c may not reflect ambient glycemia accurately. A subsequent larger meta-analysis by Corona et al. included 59 trials totaling almost 5,100 older men (mean age 62 years) that reported effects of testosterone on body composition and glucometabolic outcomes. Trials were included irrespective of baseline testosterone concentrations or presence of metabolic disease. Uncontrolled trials without a placebo group were also eligible (Corona, et al., 2016). While testosterone treatment did not reduce body weight, waist circumference or BMI, fat mass decreased (standardized means -0.34, 95%CI -0.48; -0.20,

p<0.001) and lean mass increased (0.55 (95%CI 0.3- -0.72; p<0.001). HOMA-IR improved, (-0.80 (95%CI 1.16; -0.45, p<0.001), as did fasting glycemia (-0.34 mM (95%CI -5.1; -0.17, p<0.001). Effects on HbA1c were not reported. Overall, effect sizes were modest. Limitations inherent in the meta-analyzed studies include heterogeneity in inclusion criteria and testosterone treatment formulations. Men participating in the trials were not uniformly hypogonadal (mean baseline total testosterone was 11.6 nmol/L) and mean trial duration was only 8.7 months (Corona, et al., 2016).

More recently, additional evidence has been forthcoming both from RCTs and nonrandomized registry studies. In uncontrolled observational studies, the effects of testosterone therapy (using long acting intramuscular testosterone undecanoate) have been impressive; in a cohort of 411 obese men selected from a urology registry with a baseline mean age of 59 years and hypogonadism (defined as testosterone <12.1 nmol/L and presence of symptoms measured with the Aging Males' symptom scale (AMS)), testosterone therapy given over a mean of 6 years was associated with weight loss ranging from ~15 to 30 kg and concomitant decreases in waist circumference of >10cm (Saad, et al., 2016). The fact that weight loss has not been documented in RCTs was attributed to their shorter duration, albeit in this observational study (Saad, et al., 2016), a significant decline in body weight was already observed at 12 months. However, weight loss was considerably more marked with longer duration of treatment. In a recent 8-year follow up of a subgroup (n=316) of this urology registry cohort with baseline prediabetes (defined as HbA1c 5.7-6.4%) 229 received testosterone while 87 men untreated were used as controls (Yassin, et al., 2019). HbA1c decreased by 0.39% in testosterone treated men and increased by 0.63% in untreated men. 90% of men in the testosterone group achieved a HbA1c <5.7%, whereas in 40% of controls HbA1c increased to more than 6.5%. Men receiving testosterone had improvements in lipid parameters, reduced incidence of acute myocardial infarction and reduced mortality relative to controls (Yassin, et al., 2019). Limitations of these studies include the observational nature with lack of randomization or blinding, baseline imbalances in clinical characteristics (Yassin, et al., 2019) and lack of information regarding concomitant cardiometabolic medications and their changes.

Several recent placebo-controlled double blind RCTs reporting glucometabolic outcomes have been reported. In one small 12-month RCT of 55 obese men with T2DM on oral antidiabetic mediations, long acting intramuscular undecanoate reduced HOMA-IR by 4.64,

and HbA1c by 0.94% relative to placebo, with no significant between group differences in BMI or waist circumference (Groti, et al., 2018). In a secondary analysis of a subgroup of 134 nondiabetic men enrolled in the Testosterone Effects on Atherosclerosis in Aging Men (TEAM) trial, with a mean baseline age of 66 years, BMI of 28 kg/m^2 and total testosterone of 11.4 nmol/L, 3 years of topical testosterone gel (achieving mean serum testosterone 16.6 nmol/L in the testosterone group) had no effect on body weight, or on insulin sensitivity, evaluated by octreotide insulin suppression testing (Huang, et al., 2018). In the large Testosterone trial which included 788 men older than 65 years (at baseline, 72% were obese, and 37% had diabetes) with a serum testosterone of <9.51 mmol/L averaged from 2 measurements, 12 months of testosterone treatment (adjusted to mid-normal concentrations for healthy men) modestly reduced insulin resistance, HOMA-IR -0.6, p=0.03, but did not have an effect on body weight or waist circumference (Mohler, et al., 2018).

Finally, two RCTs using hyperinsulinemic-euglycemic clamps have reported discrepant results. In one 24-week study of 39 men with a bioavailable testosterone of 7.3 nmol/L (mean age 60 years, BMI 31 kg/m^2, HOMA-IR of 3.5 and HbA1c of 6.5%), testosterone gel treatment, despite expected changes in body composition (increase in lean mass by 1.9kg and decrease in fat mass by 1.3kg), had no effect on insulin sensitivity assessed by clamp, nor on HOMA-IR or on HbA1c (Magnussen, et al., 2016). A subsequent magnetic resonance analysis of this cohort demonstrated no effect of testosterone treatment on metabolically adverse hepatic or visceral fat, but testosterone decreased the potentially insulin sensitizing adipokine adiponectin (Magnussen, et al., 2017). In contrast, a 24-week clamp study enrolling 44 hypogonadal men (defined as free testosterone < 225 pmol/L with a mean age 55 years, BMI 40 kg/m^2 , HOMA-IR of 3.9, and HbA1c of 7.0%) while similarly demonstrating no change in visceral hepatic fat, found a 32% increase in insulin sensitivity with intramuscular testosterone treatment (p=0.03 for comparison with placebo), in association with upregulation of insulin signaling genes in adipose tissue (Dhindsa, et al., 2016). The reasons for the differences between these two studies are not clear, given enrolled men had broadly similar metabolic characteristics (apart from BMI) and despite different testosterone preparations achieved similar on treatment testosterone concentrations (22.1 nmol/L (Magnussen, et al., 2016) vs 19.5 nmol/L (Dhindsa, et al., 2016)); given the modest study sizes type 1 or 2 errors may have occurred.

One recent trial tested the hypothesis that testosterone treatment may have metabolic benefits over and above that of diet alone. In this 56-week RCT obese men with a baseline total testosterone of ≤12 nmol/L were randomized to long acting intramuscular testosterone undecanoate or placebo. All participants were subjected to a structured weight loss program. While both groups lost similar amount of body weight (11.4 kg in testosterone and 10.9kg in placebo group, p for comparison=0.80), men in the testosterone group lost more fat mass (between group difference 2.9kg (–5.7 to –0.2; p=0.04), more visceral fat (–2678 mm^2; –5180 to –176; p=0.04) but had an attenuated reduction in lean mass compared to controls (3.4 kg (1.3 to 5.5), p=0.002). Thus, testosterone treatment prevented diet-associated loss of lean mass, and while placebo-treated men lost both muscle and fat, the weight loss in testosterone treated men was almost entirely loss of body fat (Ng Tang Fui, et al., 2016). While both groups had improvements in HOMA-IR, HbA1c and lipid parameters, testosterone treatment, despite resulting in metabolically favorable changes in body composition did not improve metabolic parameters relative to placebo. However, the study was not powered for this outcome. With respect to potential mechanisms, testosterone treatment had no effect on gut-derived hormones implicated in appetite regulation including ghrelin, glucagon like peptide-1, gastric inhibitory polypeptide, peptide YY, pancreatic polypeptide and amylin. Testosterone treatment reduced leptin concentrations beyond those achieved by diet-associated weight loss, suggesting that testosterone treatment may reduce leptin resistance in obese men (Ng Tang Fui, et al., 2017). Moreover, men randomized to testosterone had, compared to placebo, higher levels of physical activity (Ng Tang Fui, et al., 2016). Despite the fact that these men were not selected for the presence of hypogonadal symptoms, testosterone treatment, compared to placebo, improved androgen deficiency symptoms over and above the effects of weight loss alone (Ng Tang Fui, et al., 2017). The effects of testosterone treatment on body composition were not sustained, with no between group differences 18 months after trial cessation (Ng Tang Fui, et al., 2017).

With respect to lipid parameters, consistent with previous trials reporting small reductions in serum total cholesterol, HDL cholesterol and LDL cholesterol concentrations (Isidori, et al., 2005), a recent analysis of the T-trials reported that testosterone treatment was associated with slight, but significant decreases in total cholesterol (-0.16 mmol/L), in HDL cholesterol (-0.05 mmol/L), and in LDL cholesterol (-0.06 mmol/L). There was no significant effect on triglyceride concentrations. The authors concluded that the clinical significance of these changes is uncertain (Mohler, et al., 2018). A recent meta-analysis of clinical studies of

testosterone treatment in hypogonadal men (defined as total testosterone <12 mmol/L) with T2DM included 7 studies (total n=612 men), 5 of which were RCTs (Zhang, et al., 2018). Again, a slight reduction in total cholesterol (-0.17 mmol/L) was reported, but in contrast to the T-trials, testosterone treatment was associated with a significant reduction in triglyceride concentrations (-0.32 mmol/L). Effects on HDL and LDL cholesterol were not significant in this metaanalysis (Zhang, et al., 2018). In the aforementioned metaanalysis by Corona, if only men with a baseline testosterone <12 nmol/L were considered, similar reductions in total cholesterol (-0.35 mmol/L) and triglycerides (-0.22 mmol/L) were observed, and again, no effect on HDL or LDL cholesterol (Corona, et al., 2016). More impressive improvements in lipid profiles have been reported in long term registry studies discussed in more detail above (Saad, et al., 2019). Cardiovascular outcome studies are required to determine the clinical relevance of these lipid effects. The metabolic effects of testosterone treatment in clinical trials are summarized in the **Table**.

In summary, the overall evidence from clinical trials suggests that testosterone has consistent effects on body composition that may be expected to be metabolically favorable, in particular increases in lean mass and decreases in total fat mass. Effects on metabolically active ectopic fat (liver and visceral fat) are less consistent. Body composition effects may be additive to lifestyle measures, such as a structured weight loss program. In most but not all studies testosterone treatment improves insulin resistance to a modest degree, but effects on glycemic control remain inconsistent. The overall evidence is compatible with hypotheses (yet to be tested) that men with lower testosterone, who are more overweight/obese and more insulin resistant may have more marked responses especially if they are treated for longer durations with effective testosterone treatment achieving consistently therapeutic testosterone concentrations. Moreover, the effects of testosterone in men with more poorly controlled T2DM remains unknown. In the light of recent studies, current Endocrine Society guidelines no longer recommend routine testosterone testing in men with T2DM, and recommend against testosterone treatment in asymptomatic men for the purpose of improving glucometabolic outcomes (Bhasin, et al., 2018).

Conclusions

In summary, there is a strong inverse association of endogenous testosterone with diabesity, including simple obesity, and, to a lesser extent, with the full spectrum of dysglycemia from insulin resistance to the metabolic syndrome and established T2DM. While the association is

bi-directional, the bulk of the evidence suggests that the effect of diabesity on HPT axis suppression (occurring predominantly at the hypothalamic-pituitary unit) is stronger than the effect of low endogenous testosterone on promoting dysglycemia and obesity. Mechanistic studies have reported plausible mechanisms by which diabesity suppresses the HPT axis, including hypothalamic inflammation, dysregulation of leptin and CNS insulin signaling. However, there are also many experimental preclinical and clinical studies suggesting mechanisms by which testosterone, either directly or via aromatization to estradiol, improves body composition and glucose metabolism by signaling in multiple somatic tissues. From a clinical perspective, while substantial weight loss achievable by bariatric surgery in obese men can restore eugonadism in a substantial proportion of men, the lesser weight loss generally achievable with lifestyle measures has only modest effect on serum testosterone. In clinical trials among men with dysglycemia overall, testosterone treatment has very consistent effects on body composition (increased lean mass, reduced fat mass, inconsistent effects on ectopic fat), less consistent effects on insulin resistance and no consistent effect on glycemic control. This is reminiscent of trials of testosterone therapy focusing on other outcomes where, for example effects on lean mass and strength are mostly consistent, effects on functional performance or fall frequency are not established (Bhasin, et al., 2018). The lack of evidence for glycemic outcomes in RCTs may be because of their short duration and the enrolment of men with well controlled diabetes who are unlikely to derive a benefit. T4DM is a large (>1,000 men) 2-year RCT that tests the hypothesis that testosterone treatment prevents the development of T2DM in high risk men over and above the effects of a lifestyle program alone. T4DM, with results expected in 2020, should provide further insights. However, this trial is not designed to evaluate long term outcomes such as cardiovascular events or mortality (Wittert, et al., 2018).

Acknowledgements

MNTF was supported an ESA-RACP research establishment fellowship and MG by a career Development Fellow (1024139) from the NHMRC.

Disclosures

MG has received research funding from Bayer, Novartis, Weight Watchers, Lilly and speaker's honoraria from Besins Health Care. MNTF has received research funding from

Bayer Pharma. ASC has received speaker's honoraria from Astra Zeneca and Merck Sharp & Dohme (Australia).

Figure Legend

Figure: Bidirectional relationship between lowered sex steroids and diabesity

Depicted is the bidirectional relationship between sex steroids, obesity and dysglycemia, and potential mechanisms. For details, see text.

References

Antonio L, Wu FC, O'Neill TW, Pye SR, Carter EL, Finn JD, Rutter MK, Laurent MR, Huhtaniemi IT, Han TS, Lean ME, Keevil BG, Pendleton N, Rastrelli G, Forti G, Bartfai G, Casanueva FF, Kula K, Punab M, Giwercman A, Claessens F, Decallonne B, Vanderschueren D, Group ES. Associations between sex steroids and the development of metabolic syndrome: a longitudinal study in European men. J Clin Endocrinol Metab. 2015;100:1396-404.

Atlantis E, Fahey P, Martin S, O'Loughlin P, Taylor AW, Adams RJ, Shi Z, Wittert G. Predictive value of serum testosterone for type 2 diabetes risk assessment in men. BMC Endocr Disord. 2016;16:26.

Berkseth KE, Rubinow KB, Melhorn SJ, Webb MF, Rosalynn BDLM, Marck BT, Matsumoto AM, Amory JK, Page ST, Schur EA. Hypothalamic Gliosis by MRI and Visceral Fat Mass Negatively Correlate with Plasma Testosterone Concentrations in Healthy Men. Obesity (Silver Spring). 2018;26:1898-1904.

Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, Snyder PJ, Swerdloff RS, Wu FC, Yialamas MA. Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2018;103:1715-1744.

Bhasin S, Ellenberg SS, Storer TW, Basaria S, Pahor M, Stephens-Shields AJ, Cauley JA, Ensrud KE, Farrar JT, Cella D, Matsumoto AM, Cunningham GR, Swerdloff RS, Wang C, Lewis CE, Molitch ME, Barrett-Connor E, Crandall JP, Hou X, Preston P, Cifelli D, Snyder PJ, Gill TM. Effect of testosterone replacement on measures of mobility in older men with mobility limitation and low testosterone concentrations: secondary analyses of the Testosterone Trials. Lancet Diabetes Endocrinol. 2018;6:879-890.

Bhasin S, Jasjua GK, Pencina M, D'Agostino R, Sr., Coviello AD, Vasan RS, Travison TG. 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.
Brand JS, Rovers MM, Yeap BB, Schneider HJ, Tuomainen TP, Haring R, Corona G, Onat A, Maggio M, Bouchard C, Tong PC, Chen RY, Akishita M, Gietema JA, Gannage-Yared MH, Unden AL, Hautanen A, Goncharov NP, Kumanov P, Chubb SA, Almeida OP, Wittchen HU, Klotsche J, Wallaschofski H, Volzke H, Kauhanen J, Salonen JT, Ferrucci L, van der Schouw YT. Testosterone, sex hormone-binding globulin and the metabolic syndrome in men: an individual participant data meta-analysis of observational studies. PLoS One. 2014;9:e100409.
Camacho EM, Huhtaniemi IT, O'Neill TW, Finn JD, Pye SR, Lee DM, Tajar A, Bartfai G, Boonen S, Casanueva FF, Forti G, Giwercman A, Han TS, Kula K, Keevil B, Lean ME, Pendleton N, Punab M, Vanderschueren D, Wu FC. 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.
Cangiano B, Duminuco P, Vezzoli V, Guizzardi F, Chiodini I, Corona G, Maggi M, Persani L, Bonomi M. Evidence for a Common Genetic Origin of Classic and Milder Adult-Onset Forms of Isolated Hypogonadotropic Hypogonadism. J Clin Med. 2019;8.
Chao J, Rubinow KB, Kratz M, Amory JK, Matsumoto AM, Page ST. Short-Term Estrogen Withdrawal Increases Adiposity in Healthy Men. J Clin Endocrinol Metab. 2016;101:3724-3731.
Cheung AS, Hoermann R, Dupuis P, Joon DL, Zajac JD, Grossmann M. Relationships between insulin resistance and frailty with body composition and testosterone in men undergoing androgen deprivation therapy for prostate cancer. Eur J Endocrinol. 2016;175:229-37.
Contreras PH, Serrano FG, Salgado AM, Vigil P. Insulin Sensitivity and Testicular Function in a Cohort of Adult Males Suspected of Being Insulin-Resistant. Front Med (Lausanne). 2018;5:190.
Corona G, Giagulli VA, Maseroli E, Vignozzi L, Aversa A, Zitzmann M, Saad F, Mannucci E, Maggi M. THERAPY OF ENDOCRINE DISEASE: Testosterone supplementation and body composition: results from a meta-analysis study. Eur J Endocrinol. 2016;174:R99-R116.

672 Corona G, Monami M, Rastrelli G, Aversa A, Sforza A, Lenzi A, Forti G, Mannucci E,
673 Maggi M. Type 2 diabetes mellitus and testosterone: a meta-analysis study. Int J Androl.
674 2010.
675 Corona G, Rastrelli G, Monami M, Saad F, Luconi M, Lucchese M, Facchiano E, Sforza A,
676 Forti G, Mannucci E, Maggi M. Body weight loss reverts obesity-associated
677 hypogonadotropic hypogonadism: a systematic review and meta-analysis. Eur J Endocrinol.
678 2013;168:829-43.
679 Dhindsa S, Furlanetto R, Vora M, Ghanim H, Chaudhuri A, Dandona P. Low estradiol
680 concentrations in men with subnormal testosterone concentrations and type 2 diabetes.
681 Diabetes Care. 2011;34:1854-9.
682 Dhindsa S, Ghanim H, Batra M, Dandona P. Hypogonadotropic Hypogonadism in Men With
683 Diabesity. Diabetes Care. 2018;41:1516-1525.
684 Dhindsa S, Ghanim H, Batra M, Kuhadiya ND, Abuaysheh S, Sandhu S, Green K, Makdissi
685 A, Hejna J, Chaudhuri A, Punyanitya M, Dandona P. Insulin Resistance and Inflammation in
686 Hypogonadotropic Hypogonadism and Their Reduction After Testosterone Replacement in
687 Men With Type 2 Diabetes. Diabetes Care. 2016;39:82-91.
688 Dhindsa S, Miller MG, McWhirter CL, Mager DE, Ghanim H, Chaudhuri A, Dandona P.
689 Testosterone concentrations in diabetic and nondiabetic obese men. Diabetes Care.
690 2010;33:1186-92.
691 Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, Buring JE, Gaziano JM, Liu S.
692 Sex hormone-binding globulin and risk of type 2 diabetes in women and men. N Engl J Med.
693 2009;361:1152-63.
694 Dwyer AA, Chavan NR, Lewkowitz-Shpuntoff H, Plummer L, Hayes FJ, Seminara SB,
695 Crowley WF, Pitteloud N, Balasubramanian R. Functional hypogonadotropic hypogonadism
696 in men: Underlying neuroendocrine mechanisms and natural history. J Clin Endocrinol
697 Metab. 2019.
698 Ebrahimi F, Urwyler SA, Straumann S, Doerpfeld S, Bernasconi L, Neyer P, Schuetz P,
699 Mueller B, Donath MY, Christ-Crain M. IL-1 Antagonism in Men With Metabolic Syndrome
700 and Low Testosterone: A Randomized Clinical Trial. J Clin Endocrinol Metab.
701 2018;103:3466-3476.
702 Eriksson J, Haring R, Grarup N, Vandenput L, Wallaschofski H, Lorentzen E, Hansen T,
703 Mellstrom D, Pedersen O, Nauck M, Lorentzon M, Nystrup Husemoen LL, Volzke H,
704 Karlsson M, Baumeister SE, Linneberg A, Ohlsson C. Causal relationship between obesity

and serum testosterone status in men: A bi-directional mendelian randomization analysis. PLoS One. 2017;12:e0176277.
Finkelstein JS, Lee H, Burnett-Bowie SA, Pallais JC, Yu EW, Borges LF, Jones BF, Barry CV, Wulczyn KE, Thomas BJ, Leder BZ. Gonadal steroids and body composition, strength, and sexual function in men. N Engl J Med. 2013;369:1011-22.
Ghanim H, Dhindsa S, Abuaysheh S, Batra M, Kuhadiya ND, Makdissi A, Chaudhuri A, Dandona P. Diminished androgen and estrogen receptors and aromatase levels in hypogonadal diabetic men: reversal with testosterone. Eur J Endocrinol. 2018;178:277-283.
Gianatti EJ, Grossmann M. Testosterone deficiency in men with Type 2 diabetes: pathophysiology and treatment. Diabet Med. 2019.
Gibb FW, Homer NZ, Faqehi AM, Upreti R, Livingstone DE, McInnes KJ, Andrew R, Walker BR. Aromatase Inhibition Reduces Insulin Sensitivity in Healthy Men. J Clin Endocrinol Metab. 2016:jc20154146.
Grossmann M. Low testosterone in men with type 2 diabetes: significance and treatment. J Clin Endocrinol Metab. 2011;96:2341-53.
Grossmann M. Hypogonadism and male obesity: Focus on unresolved questions. Clin Endocrinol (Oxf). 2018;89:11-21.
Grossmann M, Hoermann R, Wittert G, Yeap BB. 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.
Grossmann M, Matsumoto AM. A Perspective on Middle-Aged and Older Men With Functional Hypogonadism: Focus on Holistic Management. J Clin Endocrinol Metab. 2017;102:1067-1075.
Grossmann M, Wierman ME, Angus P, Handelsman DJ. Reproductive Endocrinology of Nonalcoholic Fatty Liver Disease. Endocr Rev. 2019;40:417-446.
Groti K, Zuran I, Antonic B, Forsnaric L, Pfeifer M. 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;21:158-169.
Gyawali P, Martin SA, Heilbronn LK, Vincent AD, Taylor AW, Adams RJT, O'Loughlin PD, Wittert GA. The role of sex hormone-binding globulin (SHBG), testosterone, and other sex steroids, on the development of type 2 diabetes in a cohort of community-dwelling middle-aged to elderly men. Acta Diabetol. 2018;55:861-872.

738 Hall SA, Esche GR, Araujo AB, Travison TG, Clark RV, Williams RE, McKinlay JB.
739 Correlates of low testosterone and symptomatic androgen deficiency in a population-based
740 sample. J Clin Endocrinol Metab. 2008;93:3870-7.
741 Haring R, Teumer A, Volker U, Dorr M, Nauck M, Biffar R, Volzke H, Baumeister SE,
742 Wallaschofski H. Mendelian randomization suggests non-causal associations of testosterone
743 with cardiometabolic risk factors and mortality. Andrology. 2013;1:17-23.
744 Holmboe SA, Jensen TK, Linneberg A, Scheike T, Thuesen BH, Skakkebaek NE, Juul A,
745 Andersson AM. Low Testosterone: A Risk Marker Rather Than a Risk Factor for Type 2
746 Diabetes. J Clin Endocrinol Metab. 2016;101:3180-90.
747 Holt SK, Lopushnyan N, Hotaling J, Sarma AV, Dunn RL, Cleary PA, Braffett BH, Gatcomb
748 P, Martin C, Herman WH, Wessells H, Diabetes C, Complications Trial/Epidemiology of
749 Diabetes I, Complications Research G. Prevalence of low testosterone and predisposing risk
750 factors in men with type 1 diabetes mellitus: findings from the DCCT/EDIC. J Clin
751 Endocrinol Metab. 2014;99:E1655-60.
752 Huang G, Pencina KM, Li Z, Basaria S, Bhasin S, Travison TG, Storer TW, Harman SM,
753 Tsitouras P. Long-Term Testosterone Administration on Insulin Sensitivity in Older Men
754 with Low or Low-Normal Testosterone Levels. J Clin Endocrinol Metab. 2018.
755 Idan A, Griffiths KA, Harwood DT, Seibel MJ, Turner L, Conway AJ, Handelsman DJ.
756 Long-term effects of dihydrotestosterone treatment on prostate growth in healthy, middle-
757 aged men without prostate disease: a randomized, placebo-controlled trial. Ann Intern Med.
758 2010;153:621-32.
759 Isidori AM, Giannetta E, Greco EA, Gianfrilli D, Bonifacio V, Isidori A, Lenzi A, Fabbri A.
760 Effects of testosterone on body composition, bone metabolism and serum lipid profile in
761 middle-aged men: a meta-analysis. Clin Endocrinol (Oxf). 2005;63:280-93.
762 Joyce KE, Biggs ML, Djousse L, Ix JH, Kizer JR, Siscovick DS, Shores MM, Matsumoto
763 AM, Mukamal KJ. Testosterone, Dihydrotestosterone, Sex Hormone-Binding Globulin, and
764 Incident Diabetes Among Older Men: The Cardiovascular Health Study. J Clin Endocrinol
765 Metab. 2017;102:33-39.
766 Juang PS, Peng S, Allehmazedeh K, Shah A, Coviello AD, Herbst KL. Testosterone with
767 Dutasteride, but Not Anastrazole, Improves Insulin Sensitivity in Young Obese Men: A
768 Randomized Controlled Trial. J Sex Med. 2013.
769 Kaufman JM, Lapauw B, Mahmoud A, T'Sjoen G, Huhtaniemi IT. Aging and the Male
770 Reproductive System. Endocr Rev. 2019;40:906-972.

Lapauw B, Ouwens M, t Hart LM, Wuyts B, Holst JJ, T'Sjoen G, Kaufman JM, Ruige JB. Sex steroids affect triglyceride handling, glucose-dependent insulinotropic polypeptide, and insulin sensitivity: a 1-week randomized clinical trial in healthy young men. Diabetes Care. 2010;33:1831-3.

Lotti F, Rastrelli G, Maseroli E, Cipriani S, Guaraldi F, Krausz C, Reisman Y, Sforza A, Maggi M, Corona G. Impact of Metabolically Healthy Obesity in Patients with Andrological Problems. J Sex Med. 2019;16:821-832.

Magnussen LV, Andersen PE, Diaz A, Ostojic J, Hojlund K, Hougaard DM, Christensen AN, Nielsen TL, Andersen M. MR spectroscopy of hepatic fat and adiponectin and leptin levels during testosterone therapy in type 2 diabetes: a randomized, double-blinded, placebo-controlled trial. Eur J Endocrinol. 2017;177:157-168.

Magnussen LV, Glintborg D, Hermann P, Hougaard DM, Hojlund K, Andersen M. 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.

Mohler ER, 3rd, Ellenberg SS, Lewis CE, Wenger NK, Budoff MJ, Lewis MR, Barrett-Connor E, Swerdloff RS, Stephens-Shields A, Bhasin S, Cauley JA, Crandall JP, Cunningham GR, Ensrud KE, Gill TM, Matsumoto AM, Molitch ME, Pahor M, Preston PE, Hou X, Cifelli D, Snyder PJ. The Effect of Testosterone on Cardiovascular Biomarkers in the Testosterone Trials. J Clin Endocrinol Metab. 2018;103:681-688.

Morelli A, Sarchielli E, Comeglio P, Filippi S, Vignozzi L, Marini M, Rastrelli G, Maneschi E, Cellai I, Persani L, Adorini L, Vannelli GB, Maggi M. Metabolic syndrome induces inflammation and impairs gonadotropin-releasing hormone neurons in the preoptic area of the hypothalamus in rabbits. Mol Cell Endocrinol. 2014;382:107-119.

Navarro G, Xu W, Jacobson DA, Wicksteed B, Allard C, Zhang G, De Gendt K, Kim SH, Wu H, Zhang H, Verhoeven G, Katzenellenbogen JA, Mauvais-Jarvis F. Extranuclear Actions of the Androgen Receptor Enhance Glucose-Stimulated Insulin Secretion in the Male. Cell Metab. 2016;23:837-51.

Ng Tang Fui M, Dupuis P, Grossmann M. Lowered Testosterone in Male Obesity: Mechanisms, Morbidity and Management. Asian J Androl. 2013;in press.

Ng Tang Fui M, Hoermann R, Grossmann M. Effect of Testosterone Treatment on Adipokines and Gut Hormones in Obese Men on a Hypocaloric Diet. J Endocr Soc. 2017;1:302-312.

Ng Tang Fui M, Hoermann R, Prendergast LA, Zajac JD, Grossmann M. Symptomatic response to testosterone treatment in dieting obese men with low testosterone levels in a randomized, placebo-controlled clinical trial. Int J Obes (Lond). 2017;41:420-426.

Ng Tang Fui M, Hoermann R, Zajac JD, Grossmann M. The effects of testosterone on body composition in obese men are not sustained after cessation of testosterone treatment. Clin Endocrinol (Oxf). 2017;87:336-343.

Ng Tang Fui M, Prendergast LA, Dupuis P, Raval M, Strauss BJ, Zajac JD, Grossmann M. 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.

O'Reilly MW, Glisic M, Kumarendran B, Subramanian A, Manolopoulos KN, Tahrani AA, Keerthy D, Muka T, Toulis KA, Hanif W, Thomas GN, Franco OH, Arlt W, Nirantharakumar K. Serum testosterone, sex hormone-binding globulin and sex-specific risk of incident type 2 diabetes in a retrospective primary care cohort. Clin Endocrinol (Oxf). 2019;90:145-154.

Ottarsdottir K, Nilsson AG, Hellgren M, Lindblad U, Daka B. The association between serum testosterone and insulin resistance: a longitudinal study. Endocr Connect. 2018;7:1491-1500.

Rastrelli G, Carter EL, Ahern T, Finn JD, Antonio L, O'Neill TW, Bartfai G, Casanueva FF, Forti G, Keevil B, Maggi M, Giwercman A, Han TS, Huhtaniemi IT, Kula K, Lean ME, Pendleton N, Punab M, Vanderschueren D, Wu FC, Group ES. Development of and Recovery from Secondary Hypogonadism in Aging Men: Prospective Results from the EMAS. J Clin Endocrinol Metab. 2015;100:3172-82.

Rastrelli G, Corona G, Maggi M. Both comorbidity burden and low testosterone can explain symptoms and signs of testosterone deficiency in men consulting for sexual dysfunction. Asian J Androl. 2019.

Rastrelli G, Filippi S, Sforza A, Maggi M, Corona G. Metabolic Syndrome in Male Hypogonadism. Front Horm Res. 2018;49:131-155.

Ricciuti A, Travison TG, Di Dalmazi G, Talor MV, DeVincentiis L, Manley RW, Bhasin S, Caturegli P, Basaria S. A Subset of Men With Age-Related Decline in Testosterone Have Gonadotroph Autoantibodies. J Clin Endocrinol Metab. 2016;101:1535-41.

Russell N, Grossmann M. MECHANISMS IN ENDOCRINOLOGY: Estradiol as a male hormone. Eur J Endocrinol. 2019.

Saad F, Yassin A, Doros G, Haider A. Effects of long-term treatment with testosterone on weight and waist size in 411 hypogonadal men with obesity classes I-III: observational data from two registry studies. Int J Obes (Lond). 2016;40:162-70.
Tajar A, Huhtaniemi IT, O'Neill TW, Finn JD, Pye SR, Lee DM, Bartfai G, Boonen S, Casanueva FF, Forti G, Giwercman A, Han TS, Kula K, Labrie F, Lean ME, Pendleton N, Punab M, Vanderschueren D, Wu FC. Characteristics of androgen deficiency in late-onset hypogonadism: results from the European Male Aging Study (EMAS). J Clin Endocrinol Metab. 2012;97:1508-16.
Tremellen K, McPhee N, Pearce K, Benson S, Schedlowski M, Engler H. Endotoxin-initiated inflammation reduces testosterone production in men of reproductive age. Am J Physiol Endocrinol Metab. 2018;314:E206-E213.
Veldhuis JD, Bowers CY. Factors other than sex steroids modulate GHRH and GHRP-2 efficacies in men: evaluation using a GnRH agonist/testosterone clamp. J Clin Endocrinol Metab. 2009;94:2544-50.
Wang C, Xu Y. Mechanisms for Sex Differences in Energy Homeostasis. J Mol Endocrinol. 2019;62:R129-R143.
Wittert G, Atlantis E, Allan C, Bracken K, Conway A, Daniel M, Gebski V, Grossmann M, Hague W, Handelsman DJ, Inder W, Jenkins A, Keech A, McLachlan R, Robledo K, Stuckey B, Yeap BB. Testosterone therapy to prevent type 2 diabetes mellitus in at-risk men (T4DM): Design and implementation of a double-blind randomized controlled trial. Diabetes Obes Metab. 2018.
Wong HK, Hoermann R, Grossmann M. Reversible male hypogonadotropic hypogonadism due to energy deficit. Clin Endocrinol (Oxf). 2019;91:3-9.
Wu FC, Tajar A, Beynon JM, Pye SR, Silman AJ, Finn JD, O'Neill TW, Bartfai G, Casanueva FF, Forti G, Giwercman A, Han TS, Kula K, Lean ME, Pendleton N, Punab M, Boonen S, Vanderschueren D, Labrie F, Huhtaniemi IT. Identification of late-onset hypogonadism in middle-aged and elderly men. N Engl J Med. 2010;363:123-35.
Yao QM, Wang B, An XF, Zhang JA, Ding L. Testosterone level and risk of type 2 diabetes in men: a systematic review and meta-analysis. Endocr Connect. 2018;7:220-231.
Yassin A, Haider A, Haider KS, Caliber M, Doros G, Saad F, Garvey WT. Testosterone Therapy in Men With Hypogonadism Prevents Progression From Prediabetes to Type 2 Diabetes: Eight-Year Data From a Registry Study. Diabetes Care. 2019;42:1104-1111.

867 Yialamas MA, Dwyer AA, Hanley E, Lee H, Pitteloud N, Hayes FJ. Acute sex steroid
868 withdrawal reduces insulin sensitivity in healthy men with idiopathic hypogonadotropic
869 hypogonadism. J Clin Endocrinol Metab. 2007;92:4254-9.
870 Zhang J, Li X, Cai Z, Li H, Yang B. Association between testosterone with type 2 diabetes in
871 adult males, a meta-analysis and trial sequential analysis. Aging Male. 2019:1-12.
872 Zhang KS, Zhao MJ, An Q, Jia YF, Fu LL, Xu JF, Gu YQ. Effects of testosterone
873 supplementation therapy on lipid metabolism in hypogonadal men with T2DM: a meta-
874 analysis of randomized controlled trials. Andrology. 2018;6:37-46.

Table: Metabolic Effects of Testosterone Treatment in Clinical Trials

Parameter	Effect
Body weight	No change*
Fat mass	Decrease
Lean mass	Increase
Hepatic fat	No change
Visceral fat	Decrease or no change
Insulin resistance (HOMA-IR)	Decrease or no change
Insulin resistance (hyperglycemic-euglycemic clamps)	Decrease or no change
HbA1c	No change*
Total cholesterol	Decrease
LDL cholesterol	Decrease
HDL cholesterol	Decrease
Triglycerides	Decrease or no change

*A decrease has been reported in nonrandomised, nonblinded studies.