Testosterone: a metabolic hormone in health and disease

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Abstract

Testosterone is a hormone that plays a key role in carbohydrate, fat and protein metabolism. It has been known for some time that testosterone has a major influence on body fat composition and muscle mass in the male. Testosterone deficiency is associated with an increased fat mass (in particular central adiposity), reduced insulin sensitivity, impaired glucose tolerance, elevated triglycerides and cholesterol and low HDL-cholesterol. All these factors are found in the metabolic syndrome (MetS) and type 2 diabetes, contributing to cardiovascular risk. Clinical trials demonstrate that testosterone replacement therapy improves the insulin resistance found in these conditions as well as glycaemic control and also reduces body fat mass, in particular truncal adiposity, cholesterol and triglycerides. The mechanisms by which testosterone acts on pathways to control metabolism are not fully clear. There is, however, an increasing body of evidence from animal, cell and clinical studies that testosterone at the molecular level controls the expression of important regulatory proteins involved in glycolysis, glycogen synthesis and lipid and cholesterol metabolism. The effects of testosterone differ in the major tissues involved in insulin action, which include liver, muscle and fat, suggesting a complex regulatory influence on metabolism. The cumulative effects of testosterone on these biochemical pathways would account for the overall benefit on insulin sensitivity observed in clinical trials. This review discusses the current knowledge of the metabolic actions of testosterone and how testosterone deficiency contributes to the clinical disease states of obesity, MetS and type 2 diabetes and the role of testosterone replacement.

Introduction

There has been an alarming increase, of epidemic proportions, in both obesity and diabetes in the general population with increased cardiovascular risk associated with type 2 diabetes mellitus (T2DM) and/or metabolic syndrome (MetS). MetS is defined as the presence of at least three of the following components: central obesity, hyperglycaemia (including T2DM), hypertension, hypertriglyceridaemia and low HDL-cholesterol (HDL-C). The MetS, a condition recognised by the World Health Organization, is associated with an increased risk of myocardial infarction, stroke and cardiovascular death. Reduced insulin sensitivity (known as insulin resistance) is the central biochemical defect associated with MetS and T2DM. Central obesity, hepatic steatosis and intracellular fat in muscle cells as well as lack of exercise and genetic factors precipitate the development of insulin resistance. Insulin resistance in turn promotes the development of glucose intolerance, hypertriglyceridaemia, low HDL-C, hypertension,

endothelial dysfunction and a proinflammatory milieu, which combine to promote atherogenesis.

Men develop coronary artery disease and experience premature coronary events earlier than women, increasing the risk of cardiovascular mortality by more than twofold. This led to the belief that testosterone *per se* exerts a detrimental influence upon the cardiovascular system; however, evidence has emerged over recent years to suggest that a number of the cellular mechanisms intimate to the atherosclerotic process are beneficially modulated by testosterone. Indeed, it is well established that total and biologically available testosterone in men decreases with age and the age-associated decline may be related to the increased prevalence of cardiovascular disease (CVD) and comorbidities. In fact, testosterone deficiency has been reported in population studies to be associated with an increase in all-cause mortality, and this has been shown to be accounted for mainly by CVD (Khaw *et al.* 2007, Vikan *et al.* 2009, Araujo *et al.* 2011). Moreover, accumulating evidence suggests that testosterone deficiency is an independent cardiovascular risk factor and many recent reviews have focussed on the link between hypogonadism, MetS, T2DM and CVD (Makhsida *et al.* 2005, Shabsigh *et al.* 2008, Yassin *et al.* 2008, Corona *et al.* 2009, Diaz-Arjonilla *et al.* 2009, Jones & Saad 2009, Stanworth & Jones 2009, Traish *et al.* 2009, Zitzmann 2009*a*, Grossmann *et al.* 2010, Jones 2010*a,b*, Muraleedharan & Jones 2010, Moulana *et al.* 2011, Wang *et al.* 2011, Saad *et al.* 2012, Salam *et al.* 2012).

Testosterone levels themselves are considered to be lowered by chronic disease, making the designation of a causal relationship difficult to delineate (Morris & Channer 2012). Androgen deprivation therapy (ADT) as treatment for prostate cancer is a unique situation where the direct effects of lowering testosterone can be observed. While ADT reduces tumour growth and survival, it also increases the risk of coronary heart disease, diabetes and cardiovascular death (Levine *et al.* 2010, Jones 2011). This supports a key role of testosterone in atheroprotection, noted by a science advisory from the American Heart Association (Levine *et al.* 2010). Testosterone replacement therapy (TRT) in androgen-deficient men is now slowly being recognised for its therapeutic potential in the management of MetS and T2DM as well as its known benefit on quality of life and sexual health. Remarkably, physiologically replacing testosterone in men with T2DM and low testosterone levels has been demonstrated to significantly improve survival (Muraleedharan *et al.* 2011, Shores *et al.* 2012). However, with some confounding results, a lack of long-term placebo-controlled trials and an uncertainty regarding the underlying mechanisms of action, TRT remains controversial and its use as a protective metabolic hormone in CVD is at the centre of great debate. This review focuses on some of the clinical, experimental and mechanistic evidence implicating a role for testosterone in cardio-metabolic disorders.

Testosterone deficiency and metabolic risk

Testosterone deficiency has a high prevalence in men with T2DM (Dhindsa *et al.* 2004, Corona *et al.* 2006, Ding *et al.* 2006, Kapoor *et al.* 2007*a*, Corona *et al.* 2009). Furthermore, low testosterone is associated with impaired insulin sensitivity, increased percentage of body fat, truncal obesity, dyslipidaemia, hypertension and CVD (see Wang *et al.* (2011)). Epidemiological studies have consistently reported that up to 40% of men with T2DM have testosterone deficiency (Dhindsa *et al.* 2004, Corona *et al.* 2006, 2009, Ding *et al.* 2006, Kapoor *et al.* 2007*a*). Two recent systematic reviews and meta-analyses support that endogenous total and free testosterone was lower in subjects with MetS compared with those without (Brand *et al.* 2010, Corona *et al.* 2011). Several longitudinal studies demonstrate a similar association with a low testosterone concentration independently predicting the future development of insulin resistance, MetS and T2DM (Haffner *et al.* 1996, Stellato *et al.* 2000, Oh *et al.* 2002, Laaksonen *et al.* 2004, Kupelian *et al.* 2006, Rodriguez *et al.* 2007, Selvin *et al.* 2007, Haring *et al.* 2009).

The causality of this relationship between low testosterone and metabolic disease is unclear with obesity-induced androgen deficiency and hypogonadism-induced obesity both likely contributing to a bidirectional effect on disease pathology. Indeed, increased body fat is a well-known clinical feature of hypogonadism, and men with MetS at baseline are at an increased risk of developing hypogonadism based on an 11-year follow-up (Laaksonen *et al.* 2005). The finding that obesity impairs testosterone levels while low testosterone levels promote increased fat deposition was initially proposed as the hypogonadal–obesity cycle hypothesis by Cohen (1999). Testosterone is converted to 17β oestradiol (E_2) by the enzymatic activity of aromatase in adipose tissue. Thus, with higher adipocyte expression of aromatase comes a subsequent reduction of circulating testosterone. Falling testosterone promotes increasing adipocyte number and fat deposition, which gradually leads to a further lowering effect on testosterone levels. In addition, the majority of the normal negative feedback of testosterone on the hypothalamo–pituitary axis occurs

via its aromatisation (either in peripheral adipose tissue or centrally) to E_2 (Hayes *et al.* 2000, 2001). Therefore, the excess aromatase activity from increased adipocyte numbers in obese men results in the suppression of gonadotrophin-mediated testosterone secretion leading to progressive hypogonadism.

The hypogonadal–obesity–adipocytokine hypothesis (Fig. 1) extends Cohen's theory and explains why the body cannot respond to low testosterone levels by the normal homoeostatic compensatory production of androgens via increased gonadotrophin secretion to stimulate the testis (Jones 2007). E_2 and the inflammatory adipocytokines tumour necrosis factor α (TNFα) and inter-leukin 6 (IL6) inhibit hypothalamic production of GNRH and subsequent release of LH and FSH from the pituitary. This, in turn, reduces gonadal stimulation and inhibits testosterone release, thus causing a state of hypogonadotrophic

hypogonadism. Leptin, an adipose-derived hormone with a well-known role in regulation of body weight and food intake, also induces LH release under normal conditions via stimulation of hypothalamic GNRH neurons. GNRH neurons, however, exhibit little or no mRNA for leptin receptors (Finn *et al.* 1998). Kisspeptins are peptides secreted by specific neurons in the hypo-thalamus and may provide the functional link between leptin and downstream gonadal regulation as they play a central role in the modulation of GNRH secretion and subsequent LH release. Indeed, GNRH neurons in the hypothalamus possess the kisspeptin receptor and kis-speptin neurons express the leptin receptor (Roseweir & Millar 2009). In human obesity, whereby adipocytes are producing elevated amounts of leptin, the hypothalamic–pituitary axis becomes leptin resistant (Isidori *et al.* 1999, Mantzoros 1999). In addition, oestrogen receptors (ERs)
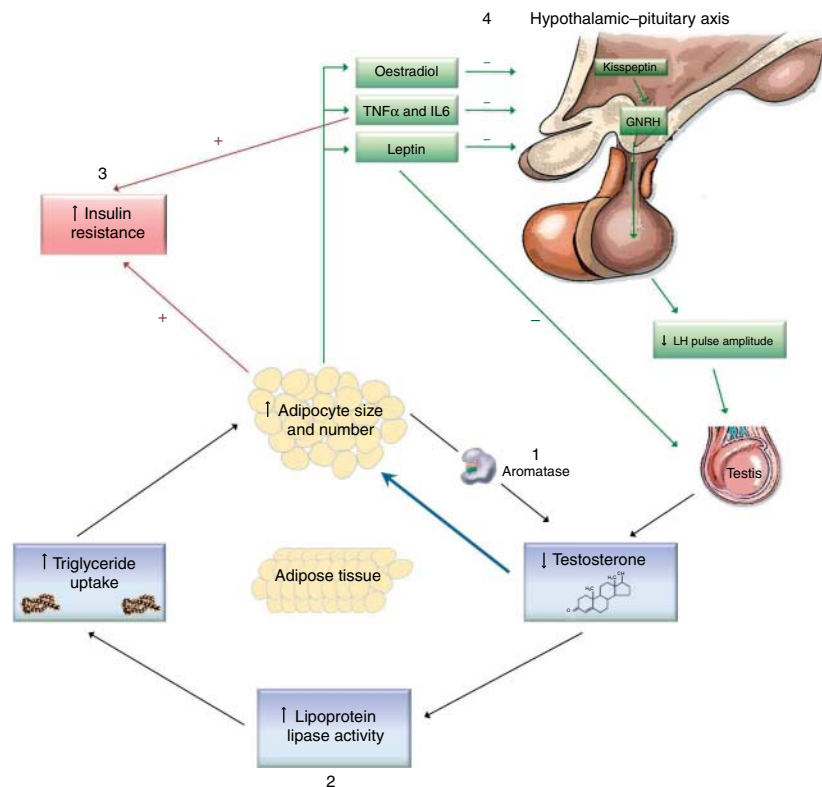

Figure 1

The hypogonadal–obesity–adipocytokine hypothesis. High aromatase activity in adipocytes converts testosterone to oestradiol (1). Reduced tissue testosterone facilitates triglyceride storage in adipocytes by allowing increased lipoprotein lipase activity (2) and stimulating pluripotent stem cells to mature into adipocytes (blue arrow). Increased adipocyte mass is associated with greater insulin resistance (3). Oestradiol and adipocyto-kines TNFα, IL6 and leptin (as a result of leptin resistance in human obesity) inhibit the hypothalamic–pituitary–testicular axis response to decreasing androgen levels (4). Kisspeptin neurons are inhibited by oestradiol, inflammation and leptin resistance and thus reduce GNRH stimulation of the pituitary and subsequent LH release. Reduced LH pulse decreases gonadal stimulation and testosterone release, thus causing a state of hypogonadotrophic hypogonadism. Furthermore, leptin also directly inhibits the stimulatory action of gonadotrophins on the Leydig cells of the testis to decrease testosterone production. $+$, positive effect; $-$, negative effect (Jones 2010*a*,*b*).

are demonstrated on kisspeptin neurons and there is evidence from animal studies that leptin resistance, inflammation and oestrogens inhibit neuronal release of kisspeptin (see George *et al.* (2010)). Beyond hypothalamic action, leptin also directly inhibits the stimulatory action of gonadotrophins on the Leydig cells of the testis to decrease testosterone production; therefore, elevated leptin levels in obesity may further diminish androgen status (Isidori *et al.* 1999). Moreover, increasing insulin resistance assessed by glucose tolerence test and hypoglycemic clamp was shown to be associated with a decrease in Leydig cell testosterone secretion in men (Pitteloud *et al.* 2005a).

ADT for the treatment of prostatic carcinoma in some large epidemiological studies has been shown to be associated with an increased risk of developing MetS and T2DM (Keating *et al.* 2006, Levine *et al.* 2010). Non-diabetic men undergoing androgen ablation show increased occurrence of new-onset diabetes and demonstrate elevated insulin levels and worsening glycaemic control (Keating *et al.* 2006, Derweesh *et al.* 2007). Prostate cancer patients with pre-existing T2DM show a further deterioration of insulin resistance and worsening of diabetic control following ADT (Haider *et al.* 2007). Indeed, a review of cross-sectional studies of men undergoing long-term (>12 months) ADT over the last 20 years reveals a higher prevalence of diabetes and MetS compared with controls (Shahani *et al.* 2008). The authors conclude that abdominal obesity and hyperglycaemia in relation to low testosterone were responsible for this higher prevalence, predisposing ADT patients to higher cardiovascular risk.

Testosterone therapy

Testosterone administration was first shown to improve glucose disposal and lower plasma insulin levels over a 3-month period in obese men (Mårin *et al.* 1992a). A follow-on study over 8 months by the same group confirmed that testosterone therapy in men with central obesity reduced insulin resistance as assessed using euglycaemic–hyperinsulinaemic clamp studies (Mårin *et al.* 1992b). TRT was first shown to improve insulin resistance in hypogonadal men with T2DM in 2006. Kapoor *et al.* (2006, 2007a) reported the first randomised double-blind placebo-controlled crossover study of testosterone therapy in hypogonadal men with T2DM. Once fortnightly i.m. injections of 200 mg testosterone esters over 3 months resulted in significant improvement in insulin sensitivity and glycaemic control (HbA1c −0.37%) as well as a reduction in waist circumference, serum leptin

and total cholesterol. The testosterone replacement in men with metabolic syndrome or type 2 diabetes (TIMES2) study, a large multi-centre European randomised double-blind placebo-controlled trial ($n=220$), demonstrated that testosterone replacement (2% testosterone gel) in hypogonadal men improved insulin resistance after 6 and 12 months of therapy by 15 and 16% compared with placebo (Jones *et al.* 2011). It is important to recognise that this benefit is similar to that of metformin, a first-line drug in the treatment of T2DM. Both the TIMES2 and the Moscow studies (randomised double-blind placebo-controlled trial; $n=184$; 30-week duration; intramuscular testosterone undecanoate) showed significant benefits on insulin resistance in men with the MetS (Kalinchenko *et al.* 2010, Jones *et al.* 2011).

Testosterone replacement has also been demonstrated to improve glycaemic control in some other studies in men with uncontrolled diabetes but these were not placebo controlled (Boyanov *et al.* 2003, Heufelder *et al.* 2009). One of these trials did compare the effects of a diet and exercise programme with the same programme in combination with testosterone replacement in hypogonadal newly diagnosed men with T2DM (Heufelder *et al.* 2009). This study found a greater benefit on glycaemic control with testosterone replacement than those with diet and exercise alone. The inclusion criterion for the TIMES2 study was the presence of hypogonadism in men with T2DM and/or the MetS, so the cohort included both men with good as well as poor diabetic control. A benefit on HbA1c of testosterone therapy compared with placebo was observed after 9 months but not earlier (Jones *et al.* 2011).

Chronic cardiac failure is associated with a state of insulin resistance and also of testosterone deficiency. The cause of the reduced insulin sensitivity is related to the chronic inflammation with elevated levels, in particular, of TNFα and not obesity as this disease is associated with cachexia. TRT improves the insulin resistance but has no effect on body weight (Malkin *et al.* 2007a, Caminiti *et al.* 2009). The response to testosterone replacement of insulin sensitivity is in part dependent on the androgen receptor (AR). In the TIMES2 study, those subjects with more sensitive AR, as demonstrated by shorter CAG repeats in exon 1 of the gene encoding the AR, had a greater improvement of Homeostasis Model of Assessment - Insulin Resistance (HOMA-IR) in response to testosterone (Stanworth RD, Akhtar S, Channer & Jones 2012, unpublished observations). There was no effect of transdermal TRT on hepatic insulin clearance in testosterone-deficient men (Basu *et al.* 2007).

Several studies have shown that TRT in obese men reduces BMI and visceral fat mass (Rebuffé-Scrive *et al.* 1991, Mårin *et al.* 1992*a*,*b*, 1995, Saad *et al.* 2007, 2008, Agledahl *et al.* 2008). In the double-blinded placebo-controlled Moscow study, 184 men suffering from both MetS and hypogonadism showed significant decreases in weight, BMI and waist circumference following 30 weeks of testosterone replacement to the normal range (Kalinchenko *et al.* 2010). Other TRT studies have also reported improved body weight, BMI and waist circumference in men with MetS, T2DM and/or hypogonadism (Wang *et al.* 2000, Boyanov *et al.* 2003, Woodhouse *et al.* 2004, Kapoor *et al.* 2006, Allan *et al.* 2008, Svartberg *et al.* 2008, Jones *et al.* 2011).

Testosterone effects on lipids

Circulating lipid profiles are linked with obesity as central body fat is associated with altered lipoprotein ratios and composition, contributing to the risk of atherosclerosis (James *et al.* 1997). Such a disruption of this balance between so-called 'good' and 'bad' lipids is associated with increased cardiovascular risk. Indeed, elevated triglycerides and a reduced HDL-C level are central to the MetS and elevated total and LDL-cholesterol (LDL-C) levels are common in T2DM, although they are not individual components of the MetS. This highlights the need for careful management of hyperlipidaemia in the prevention of cardiovascular events (Jones 2010*b*).

Low levels of testosterone have been associated with an atherogenic lipoprotein profile, characterised by high LDL and triglyceride levels (Wu & von Eckardstein 2003), and a negative correlation between serum levels of testosterone and total and LDL-C has been observed in cross-sectional studies (Barrett-Connor & Khaw 1988, Barrett-Connor 1992, Haffner *et al.* 1993, Simon *et al.* 1997, Barud *et al.* 2002). Additionally, a positive correlation between serum testosterone and HDL has been reported in both healthy and diabetic men (Simon *et al.* 1997, Van Pottelbergh *et al.* 2003, Stanworth *et al.* 2011). Moreover, patients undergoing ADT demonstrate increased total cholesterol and LDL-C (Dockery *et al.* 2003, Nishiyama *et al.* 2005, Braga-Basaria *et al.* 2006, Yannucci *et al.* 2006), elevated triglycerides and reduced HDL-C (Haffner *et al.* 1993), or all these indicators of atherogenic lipid profiles (Haider *et al.* 2007) when compared with baseline or controls. In contrast to these studies, however, Khaw *et al.* (2007) reported that LDL levels were increased in patients in the upper quartile of endogenous testosterone levels compared with the lower quartile, yet a positive association between HDL and testosterone level was apparent. A few cross-sectional studies, however, have found no association between serum lipid measurements and endogenous testosterone (Kiel *et al.* 1989, Denti *et al.* 2000).

The majority of data from studies investigating TRT in hypogonadal and eugonadal men show some improvements in lipid and lipoprotein profiles. Indeed, meta-analysis of clinical trials in hypogonadal men report that significant reductions in total cholesterol and LDL-C are associated with intramuscular TRT (Whitsel *et al.* 2001, Isidori *et al.* 2005, Jones & Saad 2009). Of particular interest, it has been reported that testosterone replacement reduces cholesterol and LDL-C in elderly men already treated with statins, indicating that TRT may be therapeutically beneficial beyond statin treatment alone (Jones & Saad 2009). Heufelder *et al.* (2009) were one of the few authors to demonstrate a significant reduction in serum triglycerides and a significant increase in serum HDL-C levels following 12 months of TRT in hypogonadal men with T2DM. Similarly, over a 1-year period of testosterone replacement, HDL levels were reported to increase in men with MetS (Saad *et al.* 2007), and in hypogonadal men over a shorter time period (Zitzmann & Nieschlag 2007). The majority of studies on the effect of testosterone on HDL-C, however, have generated contradictory results with either a decreases (Thompson *et al.* 1989, Bagatell *et al.* 1994) or no change (Zgliczynski *et al.* 1996, Uyanik *et al.* 1997, Boyanov *et al.* 2003, Malkin *et al.* 2004*a*, Kapoor *et al.* 2006). The reasons for the differences between the studies are unclear; however, it has been proposed that testosterone stimulation of reverse cholesterol transport may lead to increased consumption of HDL-C (Wu & von Eckardstein 2003). A long-term TRT investigation may support this hypothesis by demonstrating that after an initial fall in their serum concentrations, HDL-C levels return to baseline levels after 12 months (Jones *et al.* 2011).

Potential metabolic mechanisms of testosterone action

The mechanisms linking testosterone with insulin resistance and T2DM are still not fully understood and there are few published papers that have investigated potential mechanisms by which testosterone increases insulin sensitivity and regulates glucose and lipid metabolism. The major insulin-responsive target tissues, such as skeletal muscle, liver and adipose tissue, inadequately respond to the physiologic effects of circulating insulin in

T2DM. Impaired insulin sensitivity in these three tissues is characterised by defects in insulin-stimulated glucose transport activity, in particular into skeletal muscle, impaired insulin-mediated inhibition of hepatic glucose production and stimulation of glycogen synthesis in liver, and a reduced ability of insulin to inhibit lipolysis in adipose tissue. In addition, as a consequence of dissociated fatty acid (FA) release from adipose tissue surplus to energy requirements in other organs, fat deposition in non-adipose tissue including skeletal muscle and liver occurs as a 'spillover' effect (Yu & Ginsberg 2005). In turn, this lipid accumulation contributes to impaired insulin responsiveness and abnormalities in glucose control (Fig. 2). Indeed, fat in liver and muscle correlates more strongly with insulin sensitivity than abdominal fat in rats (Lim *et al.* 2008). A growing body of studies has pointed to the
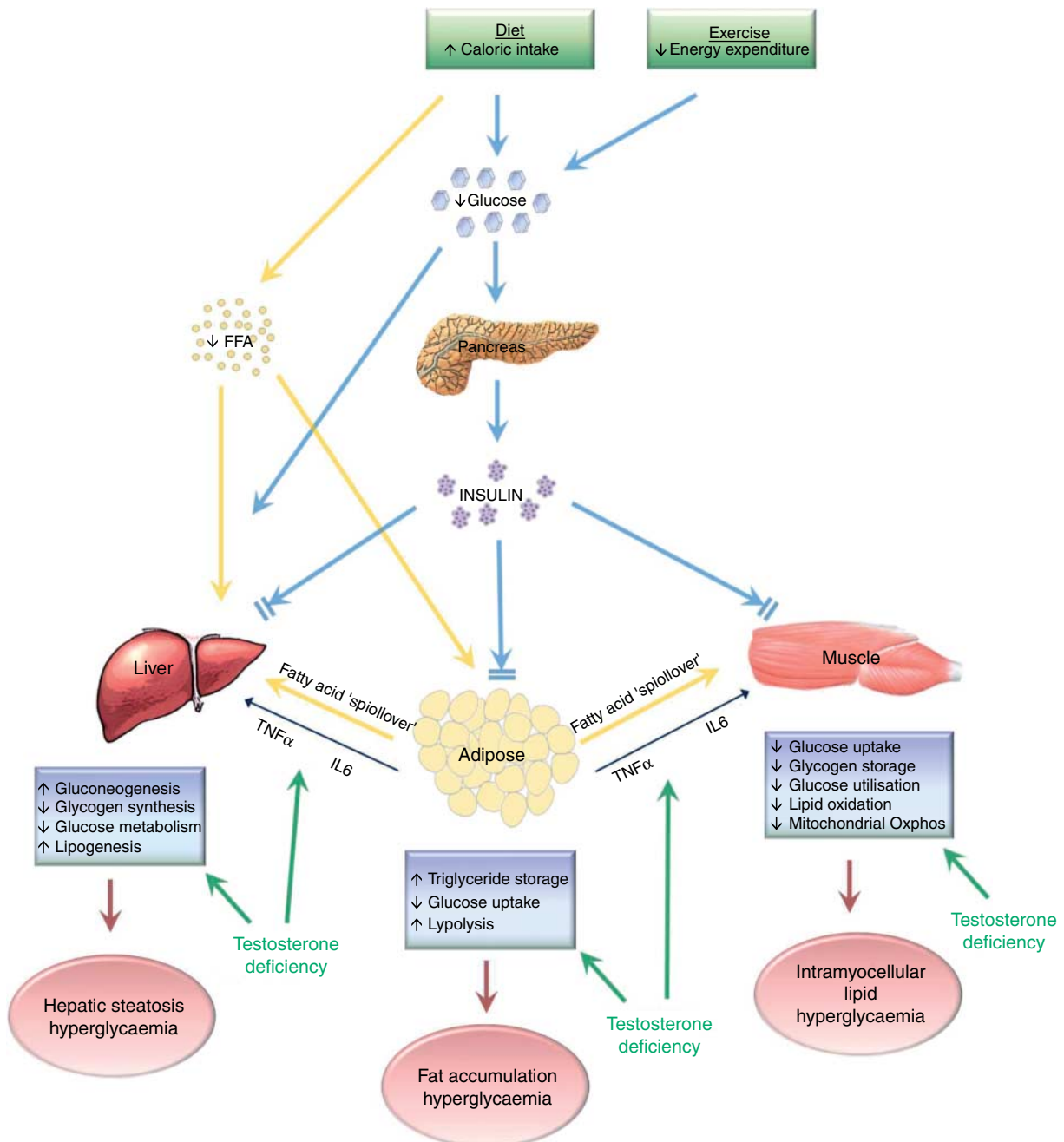
presence of heterogeneity regarding insulin resistance and insulin sensitivity among different tissues (Stumvoll *et al.* 2000); therefore, the mechanisms by which testosterone may impart beneficial actions on the development of T2DM and MetS are likely to be tissue dependent and involve multiple targets of lipid and carbohydrate metabolism (Table 1).

Muscle

Testosterone deficiency is associated with a decrease in lean body mass, and relative muscle mass is inversely associated with insulin resistance and pre-diabetes (Srikanthan & Karlamangla 2011). As up to 70% of the body's insulin sensitivity is accounted for by muscle, low testosterone may lead to insulin resistance via anabolic and/or metabolic effects on muscle (Pitteloud *et al.* 2005*b*). Currently, however, little is known regarding the influence of testosterone on insulin action in skeletal muscle and the subsequent consequences on glucose metabolism and T2DM.

Glucose metabolism It has been long known that castration is followed by decreased muscle glycogen levels in rat perineal and levator ani muscles and that the administration of testosterone induces a considerable increase in glycogen content (Leonard 1952, Apostolakis *et al.* 1963, Bergamini *et al.* 1968). An equal increase in skeletal muscle glycogen synthesis is apparent in castrated male rats supplemented with testosterone, diminishing the elevated blood glucose levels seen in untreated controls (Ramamani *et al.* 1999). A testosterone-induced increase in glycogen synthase activity was implicated for the alteration in the rate of glycogen synthesis from blood glucose. Concurrently, testosterone administration returned the enhanced glycogen phosphorylase activity in castrated rats to a normal level, thus reducing glycogen breakdown and the subsequent rise in free glucose. By contrast, the rate of glycogenesis was shown to be depressed by testosterone in rat skeletal muscle with increased glycogen content postulated rather as a result of decreased glycogenolysis (Bergamini 1975). In perineal muscle, however, glycogenesis was increased in response to testosterone, suggesting differential mechanisms in different tissue locations.

Insulin-stimulated glucose uptake into muscle and adipose tissue is largely mediated by the Glut4 glucose transporter isoform. Under normal resting conditions, most of the Glut4 molecules reside in membrane vesicles inside the cell. To increase glucose transport, *GLUT4* translocates to the cell membrane in response to insulin via signalling through the insulin receptor, subsequent binding of insulin receptor substrate 1 (IRS1) and activation of intracellular signalling pathways (Bryant *et al.* 2002). Thus, several studies have demonstrated that diminished *GLUT4* (*SLC2A4*) expression correlates with decreased insulin responsiveness, and defects at the level of the insulin receptor, *IRS1* and *GLUT4* expression have been observed in patients with T2DM (Pessin & Saltiel 2000). In agreement with androgen action on glucose control, GLUT4 and IRS1 were up-regulated in cultured adipocytes and skeletal muscle cells following testosterone treatment at low dose and short-time incubations (Chen *et al.* 2006). Similarly, testosterone treatment increased IRS2 mRNA in cells isolated from male human skeletal muscle biopsies (Salehzadeh *et al.* 2011). Sato *et al.* (2008)

Figure 2

Obesity-induced insulin resistance. Chronic excessive dietary fat and carbohydrate intake coupled with a decrease in energy expenditure leads to a sustained rise in circulating free fatty acids (FFA) and blood glucose concentration. Excess FFA (yellow arrows) are incorporated into adipocyte triglyceride storage increasing visceral and subcutaneous fat mass. Adipose accumulation promotes the release of FFA into the circulation via lipolysis and these are taken up by muscle and liver in a 'spillover' effect. With accumulation of intramyocellular lipid, insulin-mediated skeletal muscle glucose uptake and utilisation is impaired along with decreased glycogen synthesis and lipid oxidation. As a result, excess glucose is diverted to the liver. In the liver, increased liver lipid also impairs the ability of insulin to regulate gluconeogenesis and activate glycogen synthesis. Hepatic lipogenesis further increases lipid content and can lead to hepatic steatosis. Impaired insulin action in the adipose tissue allows for increased lipolysis, which additionally promotes re-esterification of lipids in other tissues (such as liver and muscle) and further exacerbates insulin resistance. At the same time, adipose-derived inflammatory mediators contribute to the development of tissue insulin resistance (dark blue arrows). In particular, IL6 and TNFα inhibit the normal tyrosine phosphorylation of IRS1 and downstream signalling in hepatic tissue reducing insulin sensitivity. Similarly, TNFα promotes insulin resistance in skeletal muscle via IRS1 degradation and inhibition of insulin signalling. Although IL6 has been shown to exert some insulin sensitising effects in muscle, evidence also indicates a negative impact on insulin action and glucose homoeostasis by decreasing gene transcription of *Irs1*, *Glut4* and *Pparγ* as well as IRS1 activity and thus reducing insulin-stimulated glucose uptake (see Wei *et al.* (2007)). Hyperglycaemia ensues. Testosterone deficiency contributes to tissue-specific mechanisms involved in the development of insulin resistance in liver, adipose and muscle tissue and promotes inflammation (green arrows). TRT may potentially improve the negative consequences of tissue-specific insulin insensitivity and improve metabolic function.

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Table 1 Influence of testosterone on targets of lipid and glucose homoeostasis

Testosterone action	Tissue	Target function
Glucose homoeostasis		
↑ Glut4	Muscle[a,b,c,d], liver[c] and adipose[a,b,c]	Glucose transporter protein involved in cellular glucose uptake
↑ IR	Liver[e,f] and larynx[g]	Insulin signalling
↑ IRS1	Muscle[a], adipose[a] and liver[f]	Insulin signalling
↑ IRS2	Muscle[h]	Insulin signalling
↑ Akt	Muscle[b]	Insulin receptor signalling pathway
↑ Protein kinase C	Muscle[b]	Insulin receptor signalling pathway
↑ Phosphofructokinase	Muscle[b]	Key regulatory enzyme in glycolysis
↑ Hexokinase	Muscle[b,d,i]	Key regulatory enzyme in glycolysis
↑ UQRCB	Muscle[j]	Oxidative phosphorylation in mitochondrial respiration
↑ Glycogen synthase	Muscle[k]	Glycogenesis
↓ Glycogen phosphorylase	Muscle[k]	Glycogen breakdown
↑ G6PD	Muscle[l,m]	Rate-limiting enzyme in the pentose phosphate pathway
Lipid homoeostasis		
↓ ACS	Adipose[n]	*De novo* lipogenesis
↓ ACC	Liver[o,p], sebaceous gland[q,r] and adipose[s]	FA synthesis
↑ ACC	Prostate cancer cells[t]	
↔ ACAT1	Sebaceous gland[r]	Conversion of cholesterol to cholesteryl esters
↔ LDLr	Sebaceous gland[r]	Receptor-mediated endocytosis of LDL
↑ ABC1	Sebaceous gland[r]	Cholesterol efflux
↓ FAS	Liver[p,u] and adipose[s,v]	FA synthesis
↔ FAS	Sebaceous gland[r]	
↑ FAS	Prostate cancer cells[t]	
↑ HMG-CoA	Sebaceous gland[q,r] and prostate cancer cells[t]	Cholesterol synthesis
↑ GPAT	Sebaceous gland[q,r]	Cholesterol synthesis
↓ HSL	Adipose[v,w]	Triglyceride breakdown
↑ β-Adrenergic receptor	Adipose[x]	Noradrenaline-stimulated lipolysis
↓ ATGL	Adipose[v]	Lipolysis
↓ LPL	Adipose[v,y,z,AA]	Triglyceride uptake
↑ ApoE	Liver[p]	Cholesterol efflux
↓ RBP4	Adipose[v]	Role in insulin resistance and lipid metabolism
↓ Scd1	Liver[o,u]	Key enzyme in FA metabolism
↑ SCD1	Liver[BB]	
↓ C7AH	Liver[BB]	Key enzyme in cholesterol conversion to bile acid
SR-1B	Liver[BB]	Selective uptake of cholesterol esters from HDL
MTTP	Liver[BB]	Central role in lipoprotein assembly; ApoB and VLDL secretion
Master regulators		
↑ LXR	Liver[p]	Regulator of glucose and cholesterol metabolism, FA synthesis and inflammation
↓ PPARγ	Liver[o] and stem cells[CC]	Whole-body energy homoeostasis adipogenesis and inflammation
↑ SREBP-1c	Sebaceous gland[r] and prostate cancer cells[DD,EE]	Regulator of *de novo* lipogenesis, cholesterol homoeostasis and glucose homoeostasis
↑ SREBP-1a	Sebaceous gland[r]	
↓ SREBP-1c	Liver[o]	

ABC1, ATP-binding cassette 1; ACAT1, acyl-CoA cholesterol acyl transferase-1; ACC, acetyl-CoA carboxylase; ACS, acyl-CoA synthetase; ApoE, apolipoprotein E; ATGL, adipose triglyceride lipase; C7AH, cholesterol 7α-hydroxylase; FAS, fatty acid synthase; GLUT4, glucose transporter-4; GPAT, glycerol 3 phosphate acyl transferase, HMG-CoA, hydroxymethylglutaryl coenzyme A; HSL, hormone-sensitive lipase; IR, insulin receptor; IRS, insulin receptor substrate; LDLr, LDL receptor; LPL, lipoprotein lipase; LXR, liver X receptor; MTTP, microsomal triglyceride transfer protein; PPARγ, peroxisome proliferator-activated receptor γ; RBP4, retinol-binding protein-4; Scd1, stearoyl-CoA desaturase 1; SR-1B, scavenger receptor class B member 1; SREBP-1c, sterol regulatory element binding protein 1c; UQRCB, ubiquinol cytochrome *c* reductase-binding protein.

[a]Chen *et al.* (2006); [b]Sato *et al.* (2008); [c]Muthusamy *et al.* (2009); [d]McLaren *et al.* (2012); [e]Parthasarathy *et al.* (2009); [f]Muthusamy *et al.* (2011); [g]Sesti *et al.* (1992); [h]Salehzadeh *et al.* (2011); [i]Bergamini *et al.* (1968); [j]Pitteloud *et al.* (2005b); [k]Ramamani *et al.* (1999); [l]Max & Knudsen (1980); [m]Max (1984); [n]Santosa & Jensen (2012); [o]Lin *et al.* (2008); [p]Kelly *et al.* (2012); [q]Hall *et al.* (1983); [r]Rosignoli *et al.* (2003); [s]Macotela *et al.* (2009); [t]Swinnen *et al.* (1997); [u]Chow *et al.* (2011); [v]McInnes *et al.* (2012); [w]Dicker *et al.* (2004); [x]De Pergola (2000); [y]Rebuffé-Scrive *et al.* (1991); [z]Mårin *et al.* (1995); [AA]Ramirez *et al.* (1997); [BB]Movérare-Skrtic *et al.* (2006); [CC]Singh *et al.* (2002); [DD]Huang *et al.* (2012); [EE]Heemers *et al.* (2001).

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demonstrated that GLUT4 expression and translocation was elevated following the addition of testosterone to cultured skeletal muscle cells of neonatal rats. Additionally, testosterone increased phosphorylation of Akt and protein kinase C (PKC), as key steps in the insulin receptor signalling pathways for regulation of GLUT4 translocation (Sato *et al.* 2008). These effects were blocked by a dihydrotestosterone (DHT) inhibitor, suggesting that local conversion of testosterone to DHT and activation of AR may be important for glucose uptake. Patients with Kennedy disease (a neuromuscular disorder) possess a genetic polymorphism with excess CAG repeats in exon 1 of the AR, leading to a condition associated with a relatively insensitive AR. In support of AR-dependent mechanisms, men with Kennedy disease have an increased risk of developing diabetes, thus providing further evidence of a link between impaired androgen status and diabetes (Stanworth *et al.* 2008, 2011, Zitzmann 2009*b*). *Glut4* expression in the AR-deficient testicular feminised (Tfm) mouse, however, was shown to be up-regulated following testosterone replacement, suggesting some AR-independent actions (McLaren *et al.* 2012).

In addition to insulin signalling and glucose uptake, testosterone has been shown experimentally to influence key enzymes involved in glycolysis. Sato *et al.* (2008) reported that testosterone increased activity of phosphofructokinase and hexokinase in cultured rat skeletal muscle cells. In an early study, Bergamini *et al.* (1968) demonstrated a maximal 40% increase in hexokinase activity in castrated male rats 12 h after testosterone administration, with levels constantly above those of castrated rats over time. Preliminary work in the Tfm mouse, whereby affected animals present low endogenous testosterone levels and a non-functional AR, has also demonstrated that hexokinase-2 expression was significantly reduced in skeletal muscle tissue of Tfm mice compared with wild-type (McLaren *et al.* 2012). Testosterone treatment did not significantly alter hexokinase-2 expression, suggesting an AR-dependent action.

In addition, glucose oxidation as an early step in glycolysis was increased in isolated sebaceous glands from testosterone-treated castrated male mice compared with castrated controls (Sansone *et al.* 1971). This increased oxidation, however, was considered to parallel increased glucose use in the pentose phosphate pathway for the generation of NADPH+ and its subsequent utilisation in FA synthesis. Indeed, triglyceride, FA and wax ester were increased in the glands in a time-responsive manner rising from 24 h and reaching highest levels at 6 days post-testosterone injection. It is not clear whether castrate control tissue was investigated at all time points to eliminate the possibility of sebaceous gland lipid accumulation over time rather than a treatment effect. Glucose-6-phosphate dehydrogenase activity, the rate-limiting enzyme in the pentose phosphate pathway, was shown to be increased in rat levator ani muscle following administration of testosterone propionate, suggesting a potential action on NADPH+ formation and possibly lipogenesis (Max & Knudsen 1980, Max 1984).

Another potential mechanism for the beneficial effect of testosterone in diabetic men may be to increase the metabolic rate in skeletal muscle, promoting the acquisition of energy from adipose tissue and thus reducing fat mass. Pitteloud *et al.* (2005*b*) describes an inverse correlation between testosterone levels and adverse mitochondrial function, assessed by measuring maximal aerobic capacity (VO₂max) in men with varying degrees of glucose control. In addition, the expression of genes involved in mitochondrial oxidative phosphorylation from skeletal muscle biopsies was related to testosterone levels, impaired glucose tolerance and the presence of T2DM. Of these genes, ubiquinol cytochrome *c* reductase-binding protein (UQRCB) had the largest expression difference between normal and diabetic muscle and was correlated with testosterone and insulin resistance (Pitteloud *et al.* 2005*b*). UQRCB is involved in the activation of ubiquinol cytochrome *c* reductase, which plays a critical role in oxidative phosphorylation.

Interestingly, orchidectomy of male Wistar rats and associated testosterone deficiency induced increased absorption of glucose from the intestine in a recent study (Olorunshola *et al.* 2012). A paradoxical significant decrease in cellular glucose uptake in the gut was observed when compared with sham-operated controls, suggesting a concurrent decrease in glucose utilisation by the gut. Subcutaneous testosterone propionate administration at a dose of 2.5 mg/kg body weight in castrated rats returned both gut absorption and utilisation of glucose to sham levels. This offers another alternate mechanism by which testosterone may contribute to the regulation of blood glucose levels.

Lipid metabolism Muscle tissue additionally has a role in lipid metabolism. Fat is oxidised in the liver and extra-hepatic tissue such as skeletal muscle and this component of lipid metabolism may be influenced by testosterone. Adverse effects on energy expenditure and fat oxidation were observed in elderly males suffering from prostate cancer and undergoing androgen deprivation (Reis *et al.* 2009). Decreased fat oxidation and increased

glucose oxidation was observed post-orchiectomy compared with pre-treatment. Likewise, testosterone deficiency induced by gonadal steroid suppression in healthy young men resulted in a significant decline in rates of lipid oxidation, with parallel changes in resting energy expenditure resulting in increased adiposity (Mauras *et al.* 1998). Furthermore, short-term (2 weeks) transdermal testosterone treatment stimulated whole-body fat oxidation and reduced the increased fat mass in testosterone-deficient men with hypopituitarism (Birzniece *et al.* 2009). Increased fat oxidation was not associated with an increase in resting energy expenditure or alterations in glucose oxidation leading the authors to postulate that a decrease in protein oxidation may be the source. Indeed, intramuscular TRT has been shown to reduce protein oxidation in hypogonadal men (Gibney *et al.* 2005). By contrast, orchidectomised mice treated with DHT resulted in obesity due to decreased fat oxidation and reduced energy expenditure while food consumption and locomotor activity remained unchanged (Movérare-Skrtic *et al.* 2006). The decrease in total body fat oxidation was not associated with a decrease in rate-limiting enzymes involved in β-oxidation in skeletal muscle or liver. The authors suggest that DHT may be acting indirectly on fat oxidation by reducing glucose oxidation. The disparity in these studies may be due to the differential influence of different hormones in different metabolic environments. The response to energy requirements by switching between lipid, glucose and protein oxidation in skeletal muscle is essential for metabolic homoeostasis, with impairment of this termed 'metabolic inflexibility' (Kelley & Mandarino 2000). Frederiksen *et al.* (2012*a*) recently demonstrated that testosterone may influence components of metabolic flexibility as 6 months of transdermal testosterone treatment in aging men with low–normal bioavailable testosterone levels increased lipid oxidation and decreased glucose oxidation during the fasting state.

Decreased lipid oxidation coupled with diet-induced chronic FA elevation is linked to increased accumulation of myocellular lipid, in particular diacylglycerol and/or ceramide in myocytes (Itani *et al.* 2002, Stratford *et al.* 2004). In turn, triglyceride and lipid build-up in muscle impairs insulin signalling leading to decreased insulin sensitivity, demonstrating the link between myocellular lipid and insulin resistance. By potentially enhancing lipid oxidation in muscle, improving circulating lipid profiles and sensitising myocyte insulin signalling and glucose metabolism, testosterone may protect against the detrimental myocellular consequence of dysregulated lipid metabolism in T2DM, obesity and MetS, although further investigation is required.

Liver

Glucose metabolism   The liver has a prominent role in the maintenance of glucose and lipid homoeostasis through the function of multiple metabolic pathways. In addition to insulin, experimental data indicate that many other hormones influence hepatic glucose control. In the Chang human adult liver cell line, insulin receptor mRNA expression was significantly increased following exposure to testosterone. This resulted in increased insulin binding, as measured by radio receptor assay and elevated glucose oxidation compared with basal response (Parthasarathy *et al.* 2009). Sesti *et al.* (1992) also reported that testosterone and DHT treatment of human larynx carcinoma cell line (HEp-2) significantly increased insulin receptor mRNA levels and insulin receptor number as well as enhancing insulin responsiveness through elevated glucose utilisation. Similarly, testosterone administration in orchidectomised adult male rats increased insulin receptor mRNA and protein expression in liver tissue and was associated with a normalisation of the castration-induced impairment of glucose oxidation (Muthusamy *et al.* 2011). Serine phosphorylation of IRS1, known to attenuate insulin signalling by inhibiting tyrosine phosphorylation (Aguirre *et al.* 2002), was elevated in castrated mice, with testosterone treatment significantly improving this effect even beyond control levels (Muthusamy *et al.* 2011).

Testosterone deprivation via castration of male rats led to decreased expression of *Glut4* in liver tissue, as well as adipose and muscle (Muthusamy *et al.* 2009). GLUT4 translocation, measured by the ratio of cytosolic to membrane protein fractions, was also inhibited. These cellular mechanisms were accompanied by an elevation of blood glucose, reduced insulin levels and decreased glucose uptake in adipose and skeletal muscle tissue. Physiologically, replacing testosterone in these animals restored *Glut4* expression and glucose uptake to wild-type levels. Oestrogen treatment had a limited effect on these parameters. The direct actions of testosterone were unclear and the authors indicate that the impairment of *Glut4* expression may result from castration-induced deficiency in insulin. By contrast, within the promoter region of the human insulin receptor gene, two functional oestrogen response elements have been identified, suggesting that testosterone actions may be

via aromatisation (García-Arencibia *et al.* 2005). Indeed, oestrogen was found to increase the expression of insulin receptors in insulin-resistant HepG2 human liver cell line (Xie *et al.* 2003).

Lipid metabolism   Liver fat is correlated with all components of MetS, and obesity directly impacts on liver function. Visceral fat blood supply is cleared into the hepatic portal vein, in contrast to the peripheral fat, which is drained by the systemic circulation (Mårin & Arver 1998). Consequently, the liver is exposed to products of adipocyte metabolism, such as free FA (FFA) and adipocytokines, in high concentrations and may induce metabolic disorders due to subsequent perturbation of liver metabolism. FFA decrease hepatic insulin binding and extraction, increase hepatic gluconeogenesis and increase hepatic insulin resistance. Ultimately, these effects lead to peripheral hyperinsulinaemia, systemic insulin resistance and hepatic steatosis. A reduction in adipocyte metabolism by testosterone would therefore reduce FFA production and, in turn, insulin resistance. The relationship between hepatic steatosis and serum testosterone concentrations has not yet received sufficient attention. Only one, albeit large-scale, population-based cross-sectional study reports an association between low serum testosterone concentrations and hepatic steatosis in men (Völzke *et al.* 2010). Two smaller studies report contrasting results in patients with hepatic steatosis as one reported low serum concentrations of testosterone and the other reported no significant differences in serum levels (Kley *et al.* 1975, Myking *et al.* 1987). Another study demonstrated through the generation of a hepatic AR knockout mouse model that a high-fat diet induced hepatic steatosis and insulin resistance in male but not in female mice (Lin *et al.* 2008). One of the contributing factors was hypothesised to be due to an increase in *de novo* lipid synthesis with loss of AR function, as hepatic expression of sterol regulatory element binding protein 1c (SREBP-1c), stearoyl-CoA desaturase 1 (SCD1), acetyl-CoA carboxylase (ACC (ACACA)) and proteasome proliferator-activated receptor γ (PPARγ (PPARG)), key enzymes in the regulation of FA synthesis, were up-regulated. Of interest, SCD1 is the rate-limiting enzyme of monounsaturated FA biosynthesis and increasing evidence implicates it as a novel control point in the pathogenesis of lipid-induced insulin resistance (Dobrzyn *et al.* 2010). Importantly, however, Lin *et al.* did not measure testosterone levels in high-fat diet-fed animals and thus could not determine whether increased hepatic steatosis was due to AR function or an obesity-related testosterone decline. By

contrast, hepatic *Scd1* mRNA expression was increased following DHT treatment in orchidectomised obese mice compared with placebo-treated controls (Movérare-Skrtic *et al.* 2006). Significantly elevated *Scd1* expression also remained when compared to sham-operated controls, suggesting possible pharmacological effects of DHT administration.

Movérare-Skrtic *et al.* (2006) showed a modest increase in scavenger receptor class B member 1 (SR-1B) expression in the liver of castrated obese mice treated with DHT. Hepatic SR-1B is involved in selective uptake of cholesterol esters from circulating HDL facilitating reverse cholesterol transport. At the same time, microsomal triglyceride transfer protein was decreased by DHT, thus reducing apolipoprotein B-mediated very LDL (VLDL) secretion. Cholesterol 7α-hydroxylase, a key enzyme in bile acid formation and therefore removal of cholesterol from the body, was decreased following DHT administration in this study, leading to hepatic lipid accumulation due to increased cholesterol uptake but decreased removal (Movérare-Skrtic *et al.* 2006). Contrariwise, AR-independent effects on hepatic steatosis and lipid metabolism have been demonstrated in the Tfm mouse (Kelly *et al.* 2012). A high-cholesterol diet induced significant hepatic lipid accumulation in Tfm mice compared to wild-type, and intramuscular testosterone replacement significantly abrogated this effect. An AR-independent action on *de novo* lipogenesis was considered as testosterone administration reduced the elevated expression of FA synthase (FAS) and ACC observed in the Tfm mouse in addition to increasing apolipoprotein E (apoE), an important protein in cholesterol transport/efflux. A concomitant increase in hepatic liver X receptor (LXR), as a master regulator of glucose and cholesterol metabolism and inflammation, was also described, suggesting that some of the beneficial actions of testosterone on hepatic lipid accumulation and metabolism may occur via a LXR effect that is independent of the AR (Kelly *et al.* 2012). In aromatase null (ArKO) male mice, hepatic transcript expression of *Fas* and *Scd1* was significantly elevated compared with wild-type males, but only *Fas* expression was lowered to wild-type level after ERα agonist treatment (Chow *et al.* 2011). This suggests that testosterone may confer some of its beneficial effects on hepatic lipid metabolism via conversion to E_2 and subsequent activation of ERα.

Adipose tissue

Adipose tissue plays a major role in glucose homoeostasis and insulin sensitivity through the regulation of lipid and

glucose metabolism. Rather than obesity *per se* being related to testosterone levels in men, adiposity and particularly the location of adipose distribution appear to influence both androgen concentrations and cardiovascular risk. Specifically, a strong inverse correlation between body fat and testosterone level is evident (Kapoor *et al.* 2005), with hypogonadal men exhibiting a reduced lean body mass and an increased fat mass, abdominal or central obesity (Seidell *et al.* 1990, Haffner *et al.* 1993, Phillips 1993, Couillard *et al.* 2000). Central adiposity may not fully correlate with the degree of visceral fat volume; therefore, more accurate methods for assessing visceral fat volume may provide more detailed investigation. Using computed tomography (CT) scanning, Tsai *et al.* (2000) showed an inverse correlation between the total testosterone levels of 110 men and accumulation of visceral fat, but not other fat depots, after a 7.5-year follow-up. Similarly, other studies using CT and MRI scanning confirmed this inverse relationship of testosterone with visceral fat (Seidell *et al.* 1990, Couillard *et al.* 2000, Garaulet *et al.* 2000). In a population of young men aged 20–29 years, Nielsen *et al.* (2007) showed by dual-energy X-ray absorptiometry and MRI that the level of visceral adipose tissue was inversely correlated with bioavailable testosterone. By contrast, there was no change in visceral fat mass in aged men with low testosterone levels following 6 months of transdermal TRT, yet subcutaneous fat mass was significantly reduced in both the thigh and the abdominal areas when analysed by MRI (Frederiksen *et al.* 2012*b*). However, ADT of prostate cancer patients increased both visceral and subcutaneous abdominal fat in a 12-month prospective observational study (Hamilton *et al.* 2011).

Lipolysis, or the breakdown of lipids for energy usage, in adipose tissue is influenced by androgens and may be a mechanism by which testosterone affects fat storage and obesity. Catecholamines are the major lipolysis regulating hormones in man and regulate adipocyte lipolysis through activation of adenylate cyclase to produce cAMP. The PKA complex is activated by cAMP which, in turn, stimulates hormone-sensitive lipase (HSL) to accelerate the breakdown of triglycerides in the process of lipolysis (Arner 2005). An early study undertaken in rodents demonstrated that testosterone enhanced noradrenaline-stimulated lipolysis in isolated fat cells from normal male rats (Hansen *et al.* 1980). Testosterone may also increase lipolysis by increasing the number of β-adrenergic receptors (De Pergola 2000).

Evidence from the AR knockout (ARKO) mouse model indicates that deficiency of androgen action decreases lipolysis and is primarily responsible for the induction of obesity (Yanase *et al.* 2008). Moreover, castration was found to reduce basal lipolysis in male hamsters (Pecquery *et al.* 1988). However, testosterone treatment also led to a 50% reduction in catecholamine-stimulated lipolysis in fully differentiated pre-adipocytes isolated from subcutaneous, but not visceral, human fat depots (Dicker *et al.* 2004). This reduction was matched by significantly decreased expression of HSL, as the final rate-limiting step for catecholamine-induced lipolysis. Taken together, these data suggest that there may be some regional differences in the action of testosterone on subcutaneous and visceral adipose function. Clinically, Abate *et al.* (2002) support this notion reporting that subcutaneous fat accumulation in the truncal area is highly predictive of low plasma concentrations of free testosterone rather than visceral adiposity. Dhindsa *et al.* (2007) also found a strong inverse relationship between subcutaneous fat mass and free and total testosterone in another study of men with T2DM. Furthermore, prostate cancer patients undergoing ADT present increased central adiposity and percentage body fat and decreased lean mass (Smith *et al.* 2001, 2002, 2006) with the change in body fat demonstrated to be mainly subcutaneous and not in visceral fat depots, which is atypical for the MetS (Faris & Smith 2010). To the best of our knowledge, there are no mechanistic studies that address the differential response to testosterone in different adipose tissues.

Androgens have been shown to affect the expression of several key enzymes involved in lipogenesis. Using the hamster ear sebaceous gland model, DHT strongly induced the synthesis of triglycerides, squalene and cholesterol esters with increased expression of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase and synthase, ACC and glycerol 3 phosphate acyl transferase (GPAT) (Hall *et al.* 1983, Rosignoli *et al.* 2003). No effect was seen for the expression of *FAS*, acyl-CoA cholesterol acyl transferase-1 (*ACAT1*) and LDL receptor mRNA (Rosignoli *et al.* 2003). The mechanism of lipogenic gene up-regulation was considered to be via the SREBP pathway as DHT increased *SREBF1* (both 1a and 1c isoforms) mRNA expression. SREBPs are considered master regulators of lipid homeostasis, controlling the expression of a range of enzymes required for endogenous cholesterol, FA, triacylglycerol and phospholipid synthesis (Brown & Goldstein 1999). Although DHT did not alter expression of SREBP cleavage-activating protein (SCAP), a chaperone protein responsible for SREBP activation, SCAP activity may still be a target for androgen action (Rosignoli *et al.* 2003). As this study only investigated the effects of DHT, any alternative AR-independent actions of testosterone may not have

been explicated. In addition, sebaceous glands are actively lipogenic, functioning to produce lipid containing sebum for the lubrication and waterproofing of the skin and hair. Indeed, expression of ATP-binding cassette 1 (ABC1), involved in cholesterol efflux, was also elevated by DHT, indicating that the increased lipids may be secreted from sebaceous glands (Rosignoli *et al*. 2003). Therefore, androgen stimulation of lipogenesis in this tissue may not be applicable to the major tissues involved in the pathogenesis of T2DM and MetS, although these studies do indicate the potential for androgen modulation of lipogenic gene expression.

In prostate cancer cell lines, a similar increase in SCAP expression and SREBP activity following androgen treatment has been described (Heemers *et al*. 2001). Indeed, androgen treatment of LNCaP cells resulted in a three- to fourfold stimulation of the steady-state mRNA levels of *FAS* and up to tenfold increase in FAS activity (Swinnen *et al*. 1997). HMG-CoA synthase and reductase as well as ACC expression was also elevated in the presence of synthetic androgen (Swinnen *et al*. 1997) reflective of a marked accumulation of cholesteryl esters (Swinnen *et al*. 1996). Up-regulation of these lipogenic genes was again mediated via SREBP and SCAP, and supporting this notion, Heemers *et al*. (2004) identified an androgen response element located in intron 8 of the *SCAP* gene. These findings suggest that rather than influencing the expression of lipogenic enzymes individually, androgens may modulate the expression and/or activity of common transcription factors regulating their coordinate control (see Swinnen & Verhoeven (1998)). Prostate cells display greatly enhanced AR expression and growth, and progression of prostate cancer cells is driven by the activation of AR and lipogenesis with SREBP-1 central to this interaction (Huang *et al*. 2012). Therefore, again this tissue is specific in its functioning and may not allow extrapolation of study findings to other tissues.

In addition, testosterone appears to inhibit lipid uptake potentially via affecting the expression of adipocyte lipoprotein lipase (LPL). LPL resides on the extracellular surface of adipocytes and hydrolyses circulating triglyceride-rich lipoproteins to FA, which are taken up into the adipocyte and then esterified back into triglycerides for storage (Eckel 1989). Owing to its important role in lipid uptake, the abnormal activity of this enzyme has been suggested as a potential contributing factor in the pathogenesis of obesity (Gruen *et al*. 1978). Ramirez *et al*. (1997) demonstrated that abdominal adipose tissue LPL activity in sedentary obese men at their usual weight was significantly inversely correlated with plasma levels of

bioavailable testosterone. In middle-aged men, an oral testosterone preparation caused a significant decrease in abdominal LPL activity when administered either in a single large dose or following a smaller four times daily dose for 6 weeks (Rebuffé-Scrive *et al*. 1991). Nine-month testosterone treatment of hypogonadal men has been reported to produce a marked decrease in both LPL activity and triglyceride uptake in subcutaneous abdominal adipose tissue (Mårin *et al*. 1995). Following further investigation, the inhibition of lipid uptake after testosterone administration was more apparent in visceral than in abdominal subcutaneous adipose tissue (Mårin *et al*. 1996). The authors suggest that this testosterone-mediated intra-abdominal inhibition of triglyceride assimilation directs lipid to subcutaneous fat in men (Mårin *et al*. 1996). Thus, when testosterone is reduced, this promotes enhanced triglyceride storage in visceral depots.

Santosa & Jensen (2012) have recently investigated the chronic effects of testosterone deficiency on adipose tissue FA storage. Men with chronic hypogonadism induced by at least 6 months of ADT for the prevention of prostate carcinoma reoccurrence were compared to eugonadal control men matched for age and body composition. Results of the study showed that hypogonadal men stored a greater proportion of both dietary FA and FFA in lower body subcutaneous fat than did eugonadal men and that this was associated with increased expression of acyl-CoA synthetase (ACS (ACSS2)) from femoral adipose biopsies. Indeed, increased ACS suggests a potential for an accumulation of intracellular acylated FA. Acylated FA are additionally considered to act as signalling molecules, modulating other enzymes involved in FA metabolism such as ACC, AMP-activated kinase-kinase, HMG-CoA reductase, carnitine palmitoyl transferase and HSL (Knudsen *et al*. 1999). Castration of male mice resulted in an increase in mRNA expression of *Fas* and *Acc* in subcutaneous adipocytes and a non-significant increase in these lipogenic enzymes in intra-abdominal/perigonadal adipocytes (Macotela *et al*. 2009). Increases in subcutaneous *Glut4* and intra-abdominal *Glut1* (*Slc2a1*) were also observed, however, suggesting that while testosterone deficiency increased lipogenic capacity, it also increased insulin sensitivity.

Beneficial influence of testosterone on adipogenesis is also demonstrated in studies investigating the direct effects of testosterone on cultured stem cells. Singh *et al*. (2002) reported that the treatment of isolated mouse pluripotent stem cells with testosterone stimulated the development of cells of myocyte lineage rather than adipocytes and that testosterone deficiency promoted the development of

adipocytes over myocytes. Down-regulated expression of PPARγ, which controls adipogenic differentiation and lipid metabolism, was also reported at the mRNA and protein level. These effects on adipogenesis were blocked by the AR antagonist bicalutamide, suggesting AR-dependent mechanisms. Indeed, adipocytes are recognised to express ARs, the density of which is positively regulated by testosterone. More recently, however, Du *et al*. (2009) reported that a pre-inhibition of the AR did not influence the down-regulation of PPARγ activity by testosterone.

Testosterone influence on adipose glucose and lipid homoeostasis are shown to be directed, at least in part, via the AR. Male mice with a selective adipocyte AR knockdown (fARKO) displayed metabolic dysregulation with early insulin resistance/hyperinsulinaemia on normal chow diet and accelerated insulin deficiency, hyperglycaemia and visceral obesity on high-fat diet (McInnes *et al*. 2012). These effects were associated with altered expression of metabolic targets, although a degree of adipose depot specificity was apparent. *Fas* and adipose triglyceride lipase (ATGL) mRNA levels were elevated in subcutaneous adipose from fARKO mice compared with controls, but no difference was observed in perigonadal fat depots. Expression of HSL and LPL, however, was elevated in both subcutaneous and perigonadal adipose tissue of fARKO mice. Although *Irs1* expression was not altered, IRS1 phosphorylation at the tyrosine residue as a measure of activation was significantly decreased in fARKO compared with wild-type mice, a mechanism potentially involving retinol-binding protein-4 (RBP4). Indeed, *Rbp4* expression was selectively elevated in adipose tissue of fARKO mice (McInnes *et al*. 2012), supporting the hypothesis that elevated expression of *Rbp4* may have an important role in lipid metabolism and insulin resistance (Graham *et al*. 2006, Ost *et al*. 2007). These AR-dependent mechanisms in adipose tissue may therefore contribute to glucose homoeostasis, and dysregulation of these pathways as a result of testosterone deficiency may contribute to the development of T2DM and MetS.

As mentioned previously, visceral fat is an active secretory tissue producing adipocytokines and other proinflammatory factors. In particular, proinflammatory adipocytokines IL1, IL6 and TNFα are increased in obesity with a downstream effect that stimulates liver production of CRP and other cytokine release (Schuster 2010). This chronic inflammation seen locally at the adipose tissue level also recruits monocytes that further contribute to the growing inflammatory activity. Overproduction of proinflammatory cytokines, other macrophage products and hormones by adipose tissue directly and indirectly induce insulin resistance (Kahn *et al*. 2006, Shoelson *et al*. 2006). In addition, adipokines are secreted into the circulation contributing to systemic and peripheral vascular inflammation and dysfunction associated with CVD (Guzik *et al*. 2006). As previously discussed, testosterone has several beneficial actions that decrease lipid deposition and therefore has the potential to reduce the release of adipocytokines by reducing fat tissue mass. Indeed, observational evidence suggests that IL1β, IL6, TNFα and CRP are inversely associated with serum testosterone levels in patients (Yang *et al*. 2005, Maggio *et al*. 2006, Kapoor *et al*. 2007a, Nettleship *et al*. 2007) and TRT has been reported to significantly reduce these proinflammatory mediators (Malkin *et al*. 2004b, Kalinchenko *et al*. 2010). RBP4, secreted by the adipose tissue, induces insulin resistance by reducing phosphatidylinositol-3-OH kinase signalling in the muscle, increasing the expression of phosphoenolpyruvate carboxykinase in the liver and local actions to inhibit tyrosine phosphorylation of IRS1 in adipose, along with diminished adiponectin and PPARα (PPARA) activity typical for obesity (Yang *et al*. 2005). In a mouse model of selective fat tissue AR knockout (fARKO), *Rbp4* mRNA expression was selectively increased in adipose tissue, along with an increased susceptibility to visceral obesity following high-fat diet feeding (McInnes *et al*. 2012). This suggests a role for AR in the metabolic actions of testosterone on fat accumulation and adipose tissue inflammatory response. McInnes *et al*. (2012) additionally demonstrated direct down-regulation of *Rbp4* expression in the adipose-differentiated 3T3-L1 cells following treatment with non-aromatisable androgen. Su *et al*. (2009), however, showed elevated secretion of IL6 and MCP-1 in 3T3-L1 cells treated with testosterone, a mechanism that was dependent on NF-κB signalling.

Adiponectin and leptin are also two important adipokines that have been linked to T2DM and obesity. Unlike other adipose tissue-derived peptides that are elevated in obesity, adiponectin levels are decreased in obese individuals with insulin resistance, and higher levels of adiponectin are associated with lower risk of T2DM in older men and women (Krakoff *et al*. 2003, Spranger *et al*. 2003). Leptin levels are high in obese individuals and it has been suggested that resistance to leptin is one of the contributing factors to obesity and testosterone deficiency, as noted earlier in the hypogonadal–obesity–adipocytokine hypothesis (Jones 2007). Kapoor *et al*. (2007b) demonstrated that both leptin and adiponectin are reduced following biweekly testosterone propionate treatment for 3 months. Similarly, TRT was shown to lower serum adiponectin (Lanfranco *et al*. 2004, Page *et al*.

2005). These interventional studies, however, were short-term and may reflect the reduction in total fat mass. Indeed, the reduction in these adipokines was correlated with a concomitant decrease in waist circumference (Kapoor *et al.* 2007b). The long-term effect of testosterone on adiponectin and leptin and the direct effects on adipocytokines remain unknown.

Consequently, testosterone treatment may have beneficial effects on preventing the pathogenesis of obesity by inhibiting adipogenesis, decreasing triglyceride uptake and storage, increasing lipolysis, influencing lipoprotein content and function and may directly reduce fat mass and increase muscle mass. This may, in turn, have a direct effect on circulating FA, the secretion of adipocytokines and subsequent insulin resistance. Some of these biochemical effects on lipid metabolism may be dependent on the location of the adipose depot and/or the tissue or cell type investigated. It is also worth noting that although the majority of studies report AR-dependent mechanisms, AR-independent effects in adipose tissue should not be discounted.

Conclusions

The high prevalence of clinical hypogonadism in men with T2DM and/or MetS has been shown in growing amounts of cross-sectional and epidemiological studies, and testosterone deficiency itself is a risk factor for the subsequent development of MetS and T2DM. Therefore, a bidirectional relationship between low testosterone with obesity and MetS is apparent. In parallel, the development of insulin resistance is multifaceted and involves multiple organs including liver, muscle and adipose tissue, which points to a heterogeneity among underlying mechanisms that could affect intervention strategies. This highlights the importance of taking multiple tissues into account and thus focussing on a systems biology approach. Early interventional studies suggest that TRT in hypogonadal men with T2DM and/or MetS has beneficial effects on lipids, adiposity and parameters of insulin sensitivity and glucose control. These effects of testosterone may be both acute and long-term in liver, adipose and muscle tissue with a rapid improvement in insulin sensitivity that occurs within a few days to few weeks of treatment and before loss of fat mass becomes evident and a prolonged effect associated with significant reduction of total and visceral body fat (Aversa *et al.* 2010b). The potential benefits of testosterone treatment must, of course, be balanced against its long-term risks. Large randomised, placebo-controlled clinical trials are therefore necessary to determine whether men with low levels of androgens can safely ingest long-term TRT and ultimately whether androgen replacement will reduce CVD risk in hypogonadal men over time.

Evidence that whole-body insulin sensitivity is reduced in testosterone deficiency and increases with testosterone replacement supports a key role of this hormone in glucose and lipid metabolism. These actions of testosterone are substantiated by demonstration of reported effects on fundamental and major biochemical regulators of glucose and lipid metabolism involved in insulin function. Furthermore, the different effects of testosterone in the major tissues involved in insulin sensitivity are consistent with the expected action of testosterone as an anabolic hormone. These underlying biochemical mechanisms of testosterone's potential therapeutic benefit remain unclear and require further and fuller investigation to understand the tissue-specific actions of testosterone and the influence of AR-dependent and -independent pathways in metabolic regulation.

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