Mechanisms Underlying the Metabolic Actions of Testosterone in the Human: a Narrative Review

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ABSTRACT

Background. The role of testosterone in improving sexual symptoms in men with hypogonadism is well known. However, recent studies indicate that testosterone plays an important role in several metabolic functions in males.

Methods. Multiple Pubmed searches were conducted with use of terms, testosterone, insulin sensitivity, obesity, type 2 diabetes, anemia, bone density, osteoporosis, fat mass, lean mass, body composition. This narrative review is focused on detailing the mechanisms that underlie the metabolic aspects of testosterone therapy in humans.

Results. Testosterone enhances insulin sensitivity in obese men with hypogonadism by decreasing fat mass, increasing lean mass, decreasing free fatty acids and suppressing inflammation. At a cellular level, testosterone increases the expression of insulin receptor β subunit, insulin receptor substrate (IRS)-1, AKT-2 and Glucose transporter type 4 (GLUT-4) in adipose tissue and adenosine 5'-monophosphate-activated protein kinase (AMPK) expression and activity in skeletal muscle. Observational studies show that long term therapy with testosterone prevents progression from prediabetes to diabetes and improves hemoglobin A1c. Testosterone increases skeletal muscle satellite cell activator, fibroblast growth factor-2 and decreases expression of muscle growth suppressors, myostatin and Mrf4. Testosterone increases hematocrit by suppressing hepcidin and increasing expression of ferroportin along with that of transferrin receptor and plasma transferrin concentrations. Testosterone also increases serum osteocalcin concentrations, which may account for its anabolic actions on bone.

Conclusions. Testosterone exerts a series of potent metabolic effects which include insulin sensitization, maintenance and growth of the skeletal muscle, suppression of the adipose tissue growth and maintenance of erythropoiesis and hematocrit.

Testosterone, the major male hormone, has well established functions as a hormone regulating sexual function, including sexual performance, erectile function and libido (1). However, it also regulates other functions including muscle mass and muscle strength. This property has been abused by body builders, weightlifters and athletes for a long time. In addition, it is known that testosterone deficiency leads to anemia and testosterone therapy increases hemoglobin concentrations (2). It is known that males with hypogonadism suffer from osteoporosis which also improves with testosterone treatment(3). In addition, it has been shown that the hypogonadal state in males is associated with insulin resistance and that testosterone replacement restores insulin sensitivity (4). Clearly, therefore, testosterone has multiple metabolic functions beyond sexual function. This review covers these areas and the recently discovered molecular mechanisms involved underlying these functions.

To perform the review, we searched Pubmed using terms, testosterone, insulin sensitivity, obesity, type 2 diabetes, anemia, bone density, osteoporosis, fat mass, lean mass, body composition. Only studies conducted in human males were considered. This review is not meant to be description of clinical data obtained on above parameters in trials of testosterone replacement. Excellent reviews on that topic are available (1, 5). Instead, we have focused on detailing the mechanisms that underlie the metabolic aspects of testosterone therapy in humans.

Insulin sensitivity

Marin *et al* were the first to demonstrate that testosterone increased insulin sensitivity, using euglycemic hyperinsulinemic clamps in the obese, and that there was an inverse relationship between BMI and plasma testosterone concentrations (6, 7). The relationship of insulin resistance, obesity and low testosterone concentrations was confirmed by Pitteloud *et al* (8). After the discovery of a high prevalence of hypogonadotropic hypogonadism (HH) in type 2 diabetes (9), Kapoor *et al* (10) confirmed that insulin resistance tends to fall after testosterone replacement in this group of patients. However, the assessment of insulin resistance was carried out by using Homeostatic Model Assessment-Insulin Resistance (HOMA-IR), which may not accurately measure insulin resistance in patients with diabetes. Most recently, Dhindsa *et al* (4) demonstrated that patients with hypogonadotropic hypogonadism and type 2 diabetes have an increase of 35% in insulin resistance when compared with eugonadal patients with type 2 diabetes, as observed after euglycemic hyperinsulinemic clamps. This increase in insulin resistance was reversed following testosterone replacement.

The mechanisms that mediate the effect of testosterone on insulin sensitivity are multi-fold and likely intertwined. Testosterone treatment in men with HH and diabetes caused a loss of 3.3 kg of total fat mass and an increase in lean body mass of 3.4 kg over 24 weeks in men with hypogonadism and type 2 diabetes (4). There was no change in insulin sensitivity at 3 weeks, but a 32% increase was observed at 24 weeks (figure 1). There was a decline in circulating free fatty acids, starting at 15 weeks of treatment(4). This would tend to enhance insulin signaling since free fatty acids are known to induce oxidative and inflammatory stress and to interfere with insulin signal transduction(11). Adipose tissue biopsies from patients with hypogonadism showed an impairment in insulin signal transduction as reflected in the reduction of the expression of insulin receptor β subunit, insulin receptor substrate (IRS)-1, AKT-2 (protein kinase B) and Glucose transporter type 4 (GLUT-4) (4). While there was a reduction in mRNA of all four, a diminution in protein expression was demonstrated only in insulin receptor β subunit and AKT-2 since western blots could not be obtained for IRS-1 and GLUT-4.

Testosterone administration for 24 weeks reversed all four molecular defects in parallel with the restoration of insulin sensitivity (figure 2). Most recently, it has also been shown that testosterone replacement leads to an increase in adenosine 5'-monophosphate-activated protein kinase (AMPK) expression and phosphorylation (12). AMPK is known to stimulate AKT-2 and to increase the expression of GLUT-4 in turn(13). This would potentially increase glucose transport. AMPK is known to mediate the beneficial glucose transport effects of metformin and exercise(14). This action would be additive to that of the improvement in insulin signal transduction following testosterone replacement described above.

The insulin sensitizing effect of testosterone is comparable to the other well-known interventions that increase insulin sensitivity (as measured by hyperinsulinemic euglycemic clamps), such as weight loss, exercise, or treatment with thiazolidinediones. Pioglitazone administration in patients with type 2 diabetes increases insulin sensitivity by ~30% (15-17). Similar to testosterone, Pioglitazone reduces circulating free fatty acids and increases AMPK phosphorylation in skeletal muscle (17). The effect of weight loss on increasing insulin sensitivity is proportional to the degree of weight loss, and can range from 20-200% (18, 19). A few weeks of exercise can enhance insulin sensitivity by ~20% in previously sedentary obese adults, independently of weight loss (20, 21). However, the main effect of exercise on glucose uptake in the working skeletal muscle is insulin-independent and is partly mediated via AMPK (22). An interesting aspect to consider is that testosterone treatment may improve fatigue and physical activity which would also increase lean mass and AMPK activity. Testosterone therapy, however, does not improve fatigue or enhance routine physical activity in elderly men(23). The patients in above mentioned studies were not part of an exercise plan. Thus, it is not likely that undocumented increase in exercise contributed markedly to the changes in body composition, insulin sensitivity and AMPK changes described above. Nevertheless, this issue should be considered in planning future trials in this area.

In the context of insulin signal transduction, it is important that testosterone also exerts an anti-inflammatory effect with the suppression of C-reactive protein (CRP), Interleukin (IL)-1β and tumor necrosis factor (TNF)- α in the serum, IKKβ (inhibitor of nuclear factor kappa-B kinase subunit beta), SOCS-3 (suppressor of cytokine signaling) and PTEN (Phosphatase and tensin homolog) in mononuclear cells and TLR (Toll-like receptor)-4 and PTP-1B (protein-tyrosine phosphatase 1B) in adipose tissue (4). These inflammatory mediators interfere with insulin signaling (figure 2). SOCS-3 interferes with insulin signal transduction by causing the ubiquitination and proteasomal degradation of IRS-1, while IKKβ induces serine phosphorylation of IRS-1 and thus prevents insulin signal transduction though IRS-1. PTP-1B dephosphorylates the insulin receptor after the auto-phosphorylation by tyrosine kinase and thus limits insulin signaling. TNFα is known to interfere with insulin signaling at the level of IRS-1. IL-1β, on the other hand, is toxic to the β-cell in the pancreatic islet. Thus, the anti-inflammatory action of testosterone may contribute not only to the reversal of insulin resistance but also to the preservation of the β-cell and insulinogenesis. This combination of actions potentially has a comprehensive anti-diabetic effect. The classic inflammatory drug, salsalate, lowers HbA1c by 0.4% and modestly increases insulin sensitivity by 15% or less (24-26).

The above discussion lists various mechanisms that contribute to the insulin sensitivity after testosterone therapy: loss of subcutaneous fat, gain in muscle mass, decrease in circulating free fatty acids and suppression of inflammation (table 1). Since these changes occur simultaneously, it is not possible to tease out the relative contributions of either mechanism to overall insulin sensitivity. As shown in figure 1, there was no change in insulin sensitivity at 3 weeks after starting testosterone. Thus,

it is likely that the insulin sensitization of testosterone is not an immediate effect and may be mediated by changes in body composition. The reduction in AMPK provides an additional pathway for glucose uptake in muscle. A reduction in free fatty acids may reflect reduction of lipolysis by testosterone. This is an area that needs further molecular investigation. Testosterone therapy consistently diminishes subcutaneous fat mass. However, longer duration of treatment may be needed to demonstrate a decrease in hepatic or visceral fat in obese men.

It is important to mention that while the insulin sensitizing effect of testosterone has been consistently observed in obese insulin resistant men, studies in non-obese men or in those with low normal testosterone (instead of subnormal) concentrations sometimes fail to show an impact on insulin sensitivity (27-29). A trial of testosterone replacement in 790 elderly hypogonadal men showed a small reduction in HOMA-IR after one year of transdermal testosterone therapy (30). In contrast, two studies in elderly men with low normal testosterone showed no change in insulin sensitivity after testosterone administration for 2-3 years (28, 31). These studies were composed largely of men with low normal testosterone (instead of subnormal) concentrations. It thus appears that the "inconsistency" in the effect of testosterone replacement on insulin sensitivity arises from studying testosterone replacement in men who are neither hypogonadal nor insulin resistant (32).

Approximately 50% of total testosterone in the circulation is tightly bound to sex hormone binding globulin (SHBG), a glycoprotein that is produced in the liver. Epidemiological studies consistently demonstrate an inverse association of SHBG with obesity, insulin resistance and type 2 diabetes (33, 34). In fact, SHBG polymorphisms that predict higher levels of SHBG are protective against type 2 diabetes in both males and females (35). Since obesity is associated with lower SHBG concentrations, there is a physiological lowering of total testosterone concentrations in obese men. Hence, free or bioavailable (non-SHBG bound) testosterone measurement is essential in obese men to assess the gonadal status. It is important to clarify that insulin resistant states such as obesity, type 2 diabetes and metabolic syndrome in males are associated with lower free and bioavailable testosterone concentrations (5). A quarter of obese men and a third of men with type 2 diabetes have subnormal free testosterone concentrations (9, 36). The insulin sensitizing effect of testosterone therapy is, therefore, especially pertinent to this population.

Diabetes and Prediabetes

The effects of testosterone therapy on glycemic control in men with type 2 diabetes has been evaluated in some studies. Kapoor *et al* showed a decrease in fasting glucose (28 mg/dl) and HbA1c (0.37%) as compared to placebo with 3 months of testosterone therapy in a small trial (37). A trial in men with new onset type 2 diabetes with transdermal testosterone also showed a decrease in HbA1c from 7.5% to 6.3% over a period of one year(38). This was in conjunction with diet and exercise, but no hypoglycemic medications. A randomized placebo controlled trial in men with type 2 diabetes showed a placebo subtracted decrease of 0.7% in HbA1c (from baseline HbA1c of 8%) after one year of intramuscular testosterone therapy (39). In contrast, HbA1c does not change significantly after short term testosterone therapy in men with well controlled diabetes (A1c <7%) (4, 40).

Indeed, a recent study has shown that long term testosterone therapy in patients with hypogonadism may reverse prediabetes altogether and normalize glucose homeostasis. Yassin *et al* reported on the effects of long-term (8 years) testosterone therapy in men with prediabetes and hypogonadism in an

observational registry study (41). The investigators compared 229 men with hypogonadism who were treated with testosterone undecanoate with 87 men who had opted against testosterone therapy (untreated controls). At baseline, 51% of these men were obese, 43% were overweight and only 6% had normal weight. Testosterone therapy resulted in weight loss and reduction in waist circumference. Testosterone Normal glucose regulation (HbA_{1c} <5.7%) was restored in 90% of the testosterone treated patients. In contrast, 40% of untreated patients progressed to frank diabetes with HbA_{1c} >6.5%. These data suggest that long-term testosterone therapy in men can prevent the progression of prediabetes to overt diabetes and reverse the prediabetes state into a normoglycemic state. .

The most recent study on the effect of testosterone replacement in HH patients with type 2 diabetes has demonstrated encouraging results (42). In this real world prospective registry study, 178 men who were receiving long acting testosterone undecanoate were compared with 178 men who had subnormal testosterone but were not treated with testosterone. The mean follow-up was 8 years and maximum follow-up was 11 years. The mean age at start of the study was 62 years in testosterone group and 64 years in untreated group. Testosterone replacement resulted in a sustained reduction of HbA1c and fasting plasma glucose concentrations and HOMA-IR. Ninety % of patients achieved an HbA1c of <7%, 83% achieved <6.5% and 46% achieved <5.7% while 34% had total remission of diabetes with freedom from all anti-diabetes drugs. During the further follow up of 2.5 years, there was no recurrence of diabetes in the group with remission. In contrast, those men with diabetes and HH who were not treated had a steady increase in glycemia and HbA1c with an increase in HOMA-IR .

Loss of adiposity

Testosterone has been shown to exert an inhibitory effect on the incorporation of dietary fat into adipose tissue, especially the intra-abdominal fat, both omental and retroperitoneal. Male subjects were given 250 mg of testosterone intramuscularly 5 days prior to abdominal surgery. Marin *et al* demonstrated that when milk fat combined with radiolabeled oleic acid was administered 24 hours prior to surgery, the amount of label found in omental and retroperitoneal fat was significantly diminished while that in the subcutaneous fat was increased following testosterone (43). Clearly, thus, testosterone has an inhibitory effect on fat deposition in visceral adipose tissue. Another study examining the effect of transdermally administered testosterone over the period of one year demonstrated that it reduced intra-abdominal fat but not subcutaneous or total body fat. In addition, there was a significant increase in the skeletal muscle mass (44). In a recent study, patients with HH and diabetes had significantly higher total body fat when compared with eugonadal patients with diabetes (4). When these patients were treated with testosterone for 6 months, total body and truncal subcutaneous fat was reduced while the lean body mass was increased by a similar amount. Therefore, the body weight did not change. A longer duration of testosterone therapy is needed for a reduction in body weight. In an observation study of obese men with hypogonadism, 8 years of testosterone therapy reduced body weight by ~20% and waist circumference by 10% (45).

At a cellular level, *in vitro*, testosterone has been shown to suppress adipocytic differentiation of 3T3 preadipocytes through the activation of Wnt pathway with an increase in the expression of β-catenin (46). Testosterone seems to promote the conversion of mesenchymal pluripotent stem cells into myogenic lineage and inhibit their conversion to adipocytes (47). These mechanisms have yet to be

confirmed in humans. Testosterone may also contribute to loss of adiposity through increased oxidation of fatty acids by the skeletal muscle whose growth is stimulated and sustained by testosterone.

A recent study has shown an impressive effect on body weight following testosterone therapy in patients with HH(48). Treatment with testosterone in HH patients led to weight loss which was proportional to the weight at baseline, being the greatest in the obese (20%), intermediate in the overweight (10%) and the least in those with normal weight (5%) when compared to those not treated with testosterone over a follow up period of 11 years. In addition, there was a proportionate reduction in waist circumference, systolic, diastolic, pulse blood pressures and plasma lipids. Associated with these changes was a reduction in the occurrence of acute myocardial infarction, stroke and death. These reductions were also the greatest in the obese, intermediate in the overweight and the least in the normal weight group.

Role of estrogens in regulating body fat in males

Estrogens have a role in mediating the anti-obesity effects of testosterone. Estrogen receptor deletion in mice leads to weight gain and obesity (49). Men rendered hypogonadal with injections of depot gonadotropin-releasing hormone agonist lose fat when given testosterone, but they do not lose body fat if they are treated with an aromatase inhibitor which is responsible for converting testosterone to estradiol (50). These findings are consistent with the observation that hormone replacement therapy in women leads to less weight gain after menopause. In this context, it is relevant that male patients with diabetes have a diminished expression of estrogen receptor and aromatase, both of which are restored following testosterone replacement (51). Consistent with these observations, estradiol concentrations are low in men with HH and type 2 diabetes, and increase after testosterone administration (52).

Muscle growth

The effect of testosterone on skeletal muscle has been known for a long time and has been abused by body builders and athletes to develop extra muscle bulk, strength and speed (53). Testosterone therapy increases the number of satellite stem cells and net protein balance, supporting muscle hyperplasia and hypertrophy (54, 55). A study in healthy men who had been rendered transiently hypogonadal by GnRH agonist and then replaced with intramuscular testosterone for 6 months showed an increase in satellite cell number (54). There was an increase in the cross-sectional area of myofibers and the number of myonuclei per myofiber, suggesting that testosterone-induced muscle hypertrophy is accompanied by addition of new nuclei from satellite cells. Recent work has shown that the expression of FGF2 (fibroblast growth factor 2) and FGFR2 (fibroblast growth factor receptor 2), one of its major receptors, is diminished in patients with HH and type 2 diabetes (56). FGF2 is known to mediate the growth and differentiation of skeletal muscle through the stimulation of satellite cells integral to the muscle (57). These cells are responsible for the growth of the skeletal muscle during development and for repair following injury later in life. The replacement of testosterone led to the restoration of normal levels of expression of FGF2 but not FGFR2 (56). In addition, there was an increase in plasma concentrations of FGF2. In contrast to FGFR2, the expression of the other receptor, FGFR1 was not altered. Clearly, thus, testosterone deficiency leads to a reduction in FGF2 and FGFR2 expression and the replacement of testosterone restores FGF2 expression while also increasing the plasma concentrations of FGF2.

In addition to this, there was also an increase in plasma concentrations of IGF1, a general growth factor which also promotes muscle growth (56). The increase in IGF1 was significantly related to that of FGF2. On the other hand, following testosterone replacement, there was a suppression of two key inhibitors of muscle growth, myostatin and Mrf4 (myogenic regulatory factor 4) (56). There was no change in the expression of two major promoters of muscle growth, myogenin or myoD, in the hypogonadal state. Nor was there a change after testosterone replacement. Clearly, the action of testosterone on the development of skeletal muscle is a complex one and will require further elucidation, especially in the context of the interaction of the various factors described above.

Testosterone may also potentially have an effect on the myocardium. One study investigating the effect of testosterone over 12 weeks has shown that the administration of testosterone to patients with congestive cardiac failure improves their mobility and muscle strength (58). However, these benefits were independent of any improvements in left ventricular ejection fraction and myocardial function and could be attributed to better skeletal muscle function allowing for more mobility. Long term studies are required to assess an independent effect on myocardial function.

Testosterone modulates androgen and estrogen receptor and aromatase expression

The deficiency of a hormone leads to the expectation that there will be a compensatory increase in its receptor expression so as to maximize the effect of the limited hormone available. However, in patients with HH and type 2 diabetes, the expression of androgen receptor was found to be diminished both in mononuclear cells and in adipose tissue (51). This was also associated with a decrease in the expression of estrogen receptor in the adipose tissue which was again contrary to expectations since estradiol concentrations were also diminished in these patients. In addition, there was also a reduction in the expression of aromatase, the enzyme which converts testosterone to estradiol. Testosterone replacement led to the increase/restoration of androgen receptor, estrogen receptor and aromatase. The men with hypogonadism also had lower protein content of androgen receptor in the total cell lysates of skeletal muscle and in the nuclei of mononuclear cells. There was an increase in the androgen receptor following testosterone therapy. Thus, the state of HH leads not only to a lack of testosterone and estradiol but also to a deficiency in their respective receptors and thus possibly the ability of the patient to respond to these hormones. Testosterone replacement reverses these defects to potentially restore these actions. It appears that the tissue androgen and estrogen receptors follow the availability of their ligands: decreasing in the hormone deficient state and increasing in the hormone replete state. It is not known if these changes have a role in mediating the signs and symptoms of hypogonadism and the response to hormone replacement therapy.

Hematocrit

The stimulatory effect of testosterone on hematocrit has been known for a long time (59). Hypogonadal states are characterized by a mild normocytic normochromic anemia which reverses following testosterone treatment (2). The mechanism underlying this effect has been thought to be due to an increase in erythropoietin synthesis in the kidney. However, more recently, it has been shown that hepcidin concentration is suppressed by testosterone (60, 61). Hepcidin suppresses the expression of ferroportin, the membrane protein responsible for the absorption of iron by the enterocyte and the release of iron stored in the monocytes and macrophages of the reticuloendothelial system (62). Thus,

ferroportin has a cardinal role in increasing the bio-availability of iron. Recent investigations have also revealed that with the suppression of hepcidin, testosterone therapy increases the expression of ferroportin along with that of transferrin receptor and plasma transferrin concentrations (63). Plasma iron and ferritin concentrations fall. These findings are consistent with the release of iron from the stores with increase in ferroportin and the transport of iron to erythropoietic cells through transferrin and the uptake of iron by erythropoietic tissues through the transferrin receptor. These effects, in addition to the stimulatory effect of testosterone on erythropoietin production, enhance hemoglobin production following testosterone therapy.

Bone Growth

The state of hypogonadism in the male is associated with osteoporosis (64). The response to testosterone replacement is dramatic in adolescents and young men, as reflected in BMD. In a registry study in 45 men with a mean age of 53±7 years and hypogonadism and osteoporosis receiving testosterone therapy with testosterone undecanoate for up to 6 years, almost all men experienced a change in diagnosis from osteoporosis to osteopenia. This improvement clearly depended on duration of treatment with progressive increase of T-scores over the full observation time (65). Since the demonstration that the congenital deficiency of estrogen receptor (66) or aromatase (67) leads to profound osteoporosis in the male, it has been assumed that the metabolic actions of testosterone on the bone are mediated by estradiol even in the male. The major known actions of estradiol on the bone are largely thought to be due to the suppression of osteolysis and osteoclastic activity (68).. One study carried out on obese patients with HH showed that testosterone suppressed the increase in bone breakdown that occurs after caloric restriction induced weight loss (69).

Men with type 2 diabetes have a higher risk of hip and non-vertebral fractures than non-diabetic men (70, 71). Paradoxically, the BMD is higher by ~5% in men with type 2 diabetes as compared to men without diabetes, possibly because they have a higher body weight and lean mass than non-diabetic men (72). A low bone turnover state exists in type 2 diabetes, and this contributes to the high fracture risk (73). Estradiol concentrations in men with type 2 diabetes are positively related to bone mineral density at hip and spine (74). In contrast, testosterone concentrations in these men were positively associated with bone strength index. This value is calculated using femur geometry and bone mineral density, and it provides a measure of resistance to bending. Testosterone therapy induces a dramatic increase in plasma osteocalcin concentrations consistent with an increase in osteoblastic activity in patients with HH and type 2 diabetes(74). The magnitude of this increase was similar to that following the administration of sodium fluoride or after teriparatide in patients with osteoporosis (75, 76). This was accompanied by a minor transient increase in CTx, consistent with an increase in bone turnover necessary for new bone formation. This study also reported that there was no change in plasma concentrations of sclerostin and receptor activator of nuclear factor kappa-B ligand (RANKL), or that of RANK expression. Thus, the beneficial action of testosterone on the bone may be due to a combination of osteoblastic and osteoclastic activity. These actions are potentially useful in reducing fracture risk in men with type 2 diabetes.

Androgen deprivation therapy

The metabolic actions of testosterone delineated above have been described in the setting of testosterone therapy. It is informative to review the metabolic changes in men after a drastic reduction of testosterone. Androgen deprivation therapy (ADT) with long acting GnRH agonists is routinely used as adjuvant therapy in intermediate and high risk localized or locally advanced prostate cancer. The benefits of ADT in prostate cancer have to be balanced against the adverse metabolic effects of its treatment. There is an increase in subcutaneous fat mass by 3.5 kg and a decrease in lean mass of 1.5 kg after 12 months of ADT (77, 78). Insulin sensitivity (measured by oral glucose tolerance test and HOMA-IR) declines by 13% following ADT (79, 80). It is assumed that the increase in fat mass or decrease in lean mass that happens after ADT account for the change in insulin sensitivity (78). These adverse effects of ADT are the likely reason for the increased incidence of diabetes in men receiving ADT. In a large retrospective cohort study, men receiving ADT had 60% higher relative risk of developing diabetes over 5 years (81). The absolute risks were 2.5 versus 1.6 events per 100 person-years.

These effects of ADT bring forth the consistency in metabolic actions of testosterone in various clinical scenarios: a) the gradual decline of testosterone in obesity and aging, b) the treatment of hypogonadism and c) the precipitous and dramatic loss of testosterone after ADT.

Concerns with testosterone treatment

Concerns regarding testosterone replacement therapy in elderly men generally relate to prostate hypertrophy, prostate cancer, cardiovascular events, erythropoiesis leading to polycythemia, lowering of HDL cholesterol and fluid retention.

Prostate: Men with subnormal testosterone concentrations have a smaller prostate and lower prostate-specific antigen (PSA) concentration in the blood than men with normal testosterone concentrations (82). Of note, men with obesity or type 2 diabetes are known to have ~ 20% lower concentrations than lean men without diabetes (83). The lower PSA concentrations may a combination of the lower testosterone concentrations in obesity and type 2 diabetes, as well as the larger plasma volumes and hence hemodilution (84). PSA concentrations are lower in hypogonadal than in eugonadal men with type 2 diabetes (0.89 vs. 1.1 ng/ml) (85). Testosterone is trophic to prostate and therefore, an increase in testosterone concentrations leads to a "normalization" in prostate size and PSA concentrations (82). Longer studies designed specifically to assess prostate risks after testosterone therapy in elderly men have not yet been conducted. While testosterone therapy has not been found to increase the incidence of benign prostatic hyperplasia (BPH) or a significant exacerbation of voiding symptoms attributable to BPH, it is prudent to avoid testosterone therapy in men with severe urinary symptoms till the BPH has been successfully treated (1).

Prostate cancer is well known to be, in the majority of cases, an androgen-sensitive disease. Testosterone therapy is an absolute contraindication in men with prostate cancer. However, there is no compelling evidence that testosterone has a causative role in prostate cancer. Epidemiologically, hypogonadal men do not have a lower incidence of prostate cancer than eugonadal men (86). The prevalence of prostate cancer in many studies on patients receiving testosterone therapy was similar to that in the general population (86, 87). However, these studies were not designed to evaluate the incidence of prostate cancer following testosterone therapy. Men on testosterone therapy should be screened for prostate cancer according to the local guidelines.

Erythrocytosis: Erythrocytosis is a known adverse effect of testosterone administration. A randomized placebo-controlled trial of transdermal testosterone therapy for one year in elderly men found a 2% incidence of polycythaemia(2). The effect is dose dependent and is seen more commonly in those with supra-normal levels of testosterone. Hematocrit above 55% increases blood viscosity and could exacerbate vascular disease in the coronary, cerebrovascular or peripheral vascular circulation. Periodic haematological assessment is therefore indicated (1). In those with other causes of secondary polycythaemia (such as smoking or sleep apnoea), dose adjustment and/or periodic phlebotomy may be necessary to keep the haematocrit below 55%.

Cardiovascular events and testosterone therapy: Epidemiological studies have shown that men with low testosterone are more likely to die from a major cardiovascular event (88). However, no randomized control trial (RCT) has been conducted to examine cardiovascular outcomes in following testosterone therapy. Cardiovascular outcomes have been sporadically reported in RCTs of testosterone therapy designed for other endpoints (such as muscle strength, glucose control) but these trials were underpowered to look at cardiac events (5). Meta-analyses of these trials do not find a consistent effect of testosterone therapy on cardiovascular events (89, 90). Increased "cardiovascular related events" were noticed in a trial of 209 elderly frail men, who had been randomly assigned a placebo gel or testosterone gel for 6 months (91). A total of 23 subjects in the testosterone group, as compared with 5 in the placebo group, had cardiovascular-related adverse events, including one death in the treatment group which led to the halting of the trial. It should be noted, however, that the study was not designed to investigate cardiovascular events and the majority of events would not be included as an "event" in any cardiovascular study due to questionable clinical significance, including peripheral edema, hypertension, and tachycardia, as well as non-specific EKG changes. Other studies in elderly population also have not shown an increase in cardiac events after testosterone replacement(23, 92-94). Major adverse cardiovascular events were similar in the testosterone and placebo groups in an RCT of transdermal testosterone replacement in 790 elderly men for one year (23), as well as in another RCT of 308 elderly men for 3 years. Similarly, RCTs performed in men with obesity or metabolic syndrome also do not show an increased cardiovascular event rate after testosterone therapy (4, 95, 96).

Most retrospective epidemiological studies have shown a benefit on cardiovascular events from long term testosterone use in elderly men (97-99). In one of the largest studies conducted on this issue, Sharma *et al* showed 56% reduction in total mortality and 24% reduction in myocardial infarction with use of testosterone therapy (98). An observational study with mean follow up of 8 years in men with type 2 diabetes showed that testosterone therapy was associated with a reduction in acute myocardial infarction (0% versus 31%), stroke (0% versus 25%) and mortality (7% versus 29%) when compared to untreated controls (42). Large-scale prospective randomized controlled trials of testosterone therapy, focusing on cardiovascular benefits and risks, are clearly needed.

Other side effects of testosterone therapy: There is a risk for gynecomastia in the first few months after initiation of testosterone therapy. A decrease in testicular size, spermatogenesis and compromised fertility can occur during testosterone therapy can occur because of the down regulation of gonadotropins (1). Specific to men using transdermal gels for testosterone therapy, there is a possibility of transferring the drug to others after skin-to-skin contact. Testosterone is anabolic and it can cause retention of sodium and water. Edema may be worsened in patients with pre-existing cardiac, renal or hepatic disease. Testosterone therapy should be avoided in men with decompensated heart failure (1).

Of note, testosterone therapy in men with compensated heart failure does not worsen ejection fraction and seems to improve physical performance (measured by distance walked before onset of shortness of breath)(58). In general, administration of testosterone in supraphysiological doses is more likely to lead to polycythemia, fluid retention and decrease in HDL cholesterol, and whereas physiological replacement is usually not accompanied with these effects (100).

In conclusion, testosterone exerts a series of potent metabolic effects which include insulin sensitization, the maintenance and growth of the skeletal muscle, the suppression of the adipose tissue growth, the maintenance and growth of the skeletal mass and the maintenance of erythropoiesis and the hematocrit. It is not surprising, therefore, that testosterone deficiency leads to a series of clinical effects including insulin resistance, anemia, adiposity, loss of muscle and bone loss. The replacement of testosterone leads to the reversal of these features.

Table 1: Metabolic effects of testosterone in males. "+" symbols in column 2 indicate the strength of evidence. "++" indicates that the effect is consistently observed in multiple randomized controlled trials (RCTs) while "+" indicates that effect is observed in many, but not all RCTs.
Mrf4 (myogenic regulatory factor 4)
FFA (Free fatty acids)

Parameter	Effect	Mechanism of action
Lean mass	Increase, ++	Muscle satellite cell activation, decrease in myostatin and Mrf4 (4, 54-56)
Subcutaneous fat mass	Decrease, ++	Decrease in adipocyte differentiation; ? fatty acid oxidation (4, 46, 47)
Insulin sensitivity	Increase, +	Increase in lean mass; decrease in fat mass, FFA and inflammation (4)
Glycemic control	Improves, +	Increased insulin sensitivity and glucose uptake; ? effect on beta cell function (37-40, 95)
Visceral and hepatic fat	?	Trend towards decrease in short term studies; trials of longer duration of treatment are needed. (4)
Bone density	Increases, ++	Increase in osteoblastic activity, effect on fracture is not known (3, 74)

Hemoglobin/hematocrit	Increase, ++	Increase in erythropoietin and transferrin, decrease in hepcidin (2, 63)

Legends

Figure 1: Insulin sensitivity, expressed as glucose infusion rate during hyperinsulinemic euglycemic clamps, after treatment with intramuscular testosterone or saline (placebo) for 24 weeks in men with hypogonadism and type 2 diabetes. Bars represent means ± S.D. The figure is based on data published in Diabetes Care (4). **P*=0.002 by *t*-test for change at 24 weeks as compared to placebo.

Figure 2: Cellular effects of testosterone that contribute to increase in insulin signaling and glucose uptake. Mechanisms noted in various tissues are depicted in a combined manner in the figure. Stimulatory effects of testosterone are shown as "+" in green square and inhibitory effects are shown as "-" in red oval shape. "+" or "-" in white ovals depict effects of insulin signaling mediators other than testosterone. It is not known how the stimulatory effect of testosterone therapy on androgen receptor expression and protein is linked to the mechanisms shown in the figure.
Abbreviations: inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β), Suppressor of cytokine signaling (SOCS)-3, Phosphatase and tensin homolog (PTEN), protein-tyrosine phosphatase 1B (PTP-IB), Toll-like receptor (TLR)-4, Insulin receptor (IR), insulin receptor substrate (IRS), Protein kinase B (AKT), Glucose transporter type 4 **(**GLUT-4), Free fatty acids (FFA), Phosphoinositide 3-kinases (PI3K), adenosine 5'-monophosphate-activated protein kinase (AMPK), phosphatidylinositol 3,4,5 trisphosphate (PIP3), 3-phosphoinositide-dependent protein kinase-1 (PDK1), Akt substrate (AS) 160, Rab (G protein member of Ras superfamily)

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