# Androgen deficiency and mitochondrial dysfunction: implications for fatigue, muscle dysfunction, insulin resistance, diabetes, and cardiovascular disease

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## Abstract

Among the major physiological functions of steroid hormones is regulation of carbohydrate, fat, and protein metabolism. Mitochondria, through oxidative phosphorylation, play a critical role in modulating a host of complex cellular metabolic pathways to produce chemical energy to meet the metabolic demand for cellular function. Thus, androgens may regulate cellular metabolism and energy production by increased mitochondrial numbers, activation of respiratory chain components, and increased transcription of mitochondrial-encoded respiratory chain genes that code for enzymes responsible for oxidative phosphorylation. Androgen deficiency is associated with increased insulin resistance, type 2 diabetes (T2DM), metabolic syndrome, obesity, and increased overall mortality. One common link among all these pathologies is mitochondrial dysfunction. Contemporary evidence exists suggesting that testosterone deficiency (TD) contributes to mitochondrial dysfunction, including structural alterations and reduced expression and activities of metabolic enzymes. Here, we postulate that TD contributes to symptoms of fatigue, insulin resistance, T2DM, cardiovascular risk, and metabolic syndrome through a common mechanism involving impairment of mitochondrial function.

**Keywords:** cardiovascular risk; insulin resistance; mitochondria; testosterone deficiency; type 2 diabetes.

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## Introduction

### Testosterone deficiency and insulin resistance, type 2 diabetes, and vascular disease

Testosterone deficiency (TD) in men is associated with an increased risk of all-cause mortality independent of other risk factors [1–5]. Further, serum testosterone (T) levels are inversely related to mortality due to cardiovascular disease (CVD) and plasma T levels may represent a predictive biochemical marker. Interestingly, even after adjusting for several confounding clinical variables, significant evidence exists for an inverse relationship between serum T levels and mortality [5, 6]. Cardiomyocytes contain a large number of mitochondria providing the cell an aerobic respiration pathway through oxidative phosphorylation to generate approximately 60% of its energy from fatty acids and triglyceride metabolism and ~35% from carbohydrate metabolism and ~5% resulting from amino acid metabolism. T exerts beneficial effects on cardiac ischemia, angina, and chronic or congestive heart failure (CHF) [7–9]. Further, CHF is characterized by increased catabolic rate and reduced anabolic activity [10–12], and treatment with T improved oxygen consumption (VO$_2$) and physical activity in patients with CHF [9]. A number of studies have shown that T supplementation in men with TD reduced waist circumference, total cholesterol, and reduced circulatory pro-inflammatory cytokines [13, 14]. Men with low circulating T levels may exhibit impaired mitochondrial oxidative phosphorylation [7]. A recent review by Saad [15] summarized the data from several studies on T supplementation and improvement in body composition, and noted a marked increase in fat-free mass and a significant reduction in fat mass. Jockenhövel et al. [16, 17] reported that men with TD receiving T therapy consistently report reduced fatigue. This treatment is associated with concomitant stimulation of erythropoiesis and improvement in hematocrit levels [18].

A number of prospective studies have shown that low T is a precursor of the later development of type 2 diabetes mellitus (T2DM) [19–25]. Patients with prostate cancer (PCa) who were treated with androgen deprivation therapy (ADT) developed glucose intolerance within a 6-month period after ADT with concomitant elevated fasting insulin levels within 3 months after induction of TD [26, 27]. Furthermore, patients treated with ADT are at higher risk of myocardial infarction within the first 3–6 months of treatment [28, 29]. As discussed in Part 1 of this two-part series, ADT is associated with profound fatigue developing within 1 week of surgical or chemical castration; however, this side effect is not reported consistently within the scientific literature, and it is

viewed as a "quality of life issue." Thus, due to its subjective nature, no attempts have been made to objectively assess fatigue [30, 31] (see also Induced Testosterone Deficiency: From Clinical Presentation of Fatigue, Erectile Dysfunction and Muscle Atrophy to Insulin Resistance and Diabetes Part 1 of this two part series).

Bjorntorp [32] showed a significant relationship between low T and insulin resistance (IR). In recent studies, Pitteloud et al. [33] and Yialamas et al. [34] showed that acute sex steroid withdrawal reduces insulin sensitivity in young healthy men with idiopathic hypogonadotropic hypogonadism. They noted that the acuity of TD, in the absence of changes in body mass index or leptin levels, suggests that sex steroids modulate insulin sensitivity in the absence of changes in body composition. TD in the long term leads to IR, metabolic fat deposits, and T2DM [35]. As discussed in Part 1 of this two-part series, an extreme form of TD is seen in surgically or chemically castrated men with advanced PCa who experience an immediate onset of fatigue, muscle strength loss, and erectile dysfunction. These symptoms may be part of the spectrum of cellular changes due to a common denominator that is "mitochondrial dysfunction."

Here, we advance the hypothesis that in TD, "the common denominator between the development of IR in the long term and the immediate experience of fatigue may lie in the relationship between T and peak oxygen utilization ($VO_2$ max) and mitochondrial oxidative phosphorylation efficiency and gene expression of proteins and enzymes involved in this critical metabolic pathway."

Pitteloud et al. [33] showed that T levels correlate inversely with IR and positively with $VO_2$ max and *OXPHOS-CR* gene expression. Medical castration of healthy normal men decreases lipid oxidation and resting energy expenditures [36]. Androgens modulate mitochondrial functions, and reduced androgen levels contribute to inefficiency of energy utilization [37]. Thus, it is reasonable to propose that mitochondrial function controls our sense of energy and vitality, and the "pick-up-and-go" mentality, as well as possibly influences the pathogenesis of IR, T2DM, and CVD.

In subjects with IR, evidence exists for reduced expression of peroxisome proliferator-activated receptor γ (PPARγ) co-activator 1-α (PGC-1α) and down-regulation of the *OXPHOS* genes in skeletal muscle mitochondria [38, 39]. Morino et al. [40, 41] showed declining mitochondrial function in elderly men compared with young men. Whether this is related to the lower T levels in elderly men remains unknown. Elderly men exhibit a 40% decline in mitochondrial oxidative phosphorylation capacity, which may contribute to the development of IR.

The proposed link between mitochondrial dysfunction and increased IR, T2DM, increased body fat, decreased lean muscle mass, low energy levels, inefficient metabolism, increased low-grade inflammation, metabolic syndrome (MetS), and increased obesity may contribute to accelerated aging, CVD and even premature death. Although the proposed link between mitochondrial dysfunction and various pathological processes are evident as mitochondria are abundant in metabolically active tissues and cells, including the brain, skeletal muscle, heart, liver, and kidney, there are limited studies investigating androgen regulation of mitochondrial function.

Mitochondria are the sites of energy production from various fuel sources, such as carbohydrates, lipids, and proteins. A highly regulated set of complex biochemical pathways are involved in fuel oxidation to transform stored fuels into chemical energy in the form of ATP. During the process of energy production, reactive oxygen species (ROS) are also produced, and if not neutralized properly, these ROS will result in damage of mitochondrial DNA, proteins, and lipids. Mitochondrial dysfunction subsequent to mitochondrial DNA damage forms a vicious cycle whereby reductions in functional mitochondrial proteins leads to an increased accumulation of ROS and free radicals, which in turn causes further mitochondrial DNA damage.

Mitochondria are not only enclosed within their own membranes, but also possess their own DNA. The outermost compartment of mitochondria is its relatively permeable outer membrane. The outer membrane of the mitochondrion contains enzymes involved in the transport of lipids into the innermost compartment, the matrix, where they are used in the production of energy. The matrix is enclosed within the highly convoluted inner mitochondrial membrane. The series of folds and tubules that make up the inner mitochondrial membrane are known as cristae, and harbor the enzymes involved in ATP production. As such, it seems obvious that highly active cells possess a more complex inner mitochondrial membrane. ATP production occurs within the matrix where enzymes, such as ATP synthase are found. Some of these important enzymes are encoded by mitochondrial DNA, also found in the matrix.

Altered mitochondrial morphology has been associated with membrane potential heterogeneity [42, 43] and increased oxidative stress, whereby decreased membrane potential leads to increases in ROS production. Changes in mitochondrial morphology promote the opening of the mitochondrial permeability transition pores, a critical step that leads to reduced mitochondrial membrane potential accompanied by increased release of cytochrome *c* and ultimately committing cells to apoptosis. Studies have implicated mitochondrial fragmentation as the precursor to mitochondrial permeability transition, which is recognized as the "point of no return" for almost all signal transduction cascades leading to apoptosis. Therefore, we hypothesize that in cardiomyocytes, TD may increase oxidative stress and apoptosis leading to mitochondrial dysfunction [33, 44].

The question remains whether androgens regulate the number of mitochondria, as well as the expression of proteins and enzymes, which are involved in energy production. If this were the case, then this suggests that androgen deficiency contributes to mitochondrial dysfunction and disruption of normal cellular function, including production of ATP and promoting cell death. In the following sections, we discuss the relationship between TD and mitochondrial function and its implication in IR, T2DM, and CVD.

## Effects of androgens on mitochondrial biogenesis/morphology

Considerable information is available regarding the effect of T on mitochondrial biogenesis and morphology (see Table 1 and Figures 1 and 2). In a recent study, T up-regulated

serine-threonine kinase (Akt) phosphorylation and mitochondrial transcription factor A (Tfam) expression, exerting an anti-apoptotic effect against doxorubicin (Dox)-induced cardiotoxicity in cardiac myoblast cells [46]. Low levels of T are associated with reduced expression of PGC-1α in muscle [33]. Furthermore, androgen receptor (AR)-deficient mice express low levels of PGC-1α [47]. Electron microscopic examination revealed prominent vacuole formation of myocardial mitochondria in Dox-treated male AR knockout (ARKO) mice. In addition, cardiac oxidative stress and apoptosis of cardiomyocytes were more prominently increased by Dox treatment in male ARKO mice than in male wild-type (WT) mice [46].

The expression of a key mitochondrial transcriptional factor (Tfam) in cardiac tissues of male WT mice was not affected significantly by Dox treatment, while its expression was reduced significantly by almost half in male ARKO mice treated with Dox, suggesting an important role for the AR in modulating mitochondrial function [46]. Orchiectomy induced a severe decrease in levator ani muscle weight associated with increased apoptosis and accompanied by condensed mitochondria [66]. Reduced T induced anti-apoptotic protein expression, including Bcl-2 and survivin, in the androgen-sensitive human prostate adenocarcinoma cell line LNCaP in which depolarization of mitochondrial membrane potential was reported [67].

Evidence of high-grade swelling of mitochondria with loss of matrix density, disturbances of mitochondrial cristae, and disruption of mitochondrial membranes has been demonstrated in rat embryos exposed to maternal diabetes in vivo or to high concentrations of glucose, pyruvate, β-hydroxybutyrate, or α-ketoisocaproate in vitro [68]. Maternal diabetes caused swelling of the mitochondria in the embryonic neuroepithelium. The swollen mitochondria were characterized by markedly increased size, pale matrix, short distended cristae, and occasional disruptions of their membranes [68]. We have also demonstrated mitochondrial swelling in trabecular smooth muscle of penile tissue from castrated male animals [69] (Figure 3). It has been hypothesized that mitochondrial swelling is the result of peroxidation of the mitochondrial membrane lipids, which occurs through a free-radical chain reaction and can be inhibited by antioxidants that block this reaction [70].

### Effects of androgens on mitochondrial enzyme expression and activities

It has been shown that the anabolic response of the mouse gastrocnemius and soleus muscles to T is accompanied by a notable increase in the activity of mitochondrial cytochrome c oxidase as well as four lysosomal hydrolases [9]. T administration potentiates the exercise-induced increments in cytochrome c oxidase activity in the heart and soleus muscles, and has been shown to slightly increase cytochrome c oxidase activity in the fast-twitch extensor digitorum longus muscles of sedentary and exercised rats [48]. Previous findings reported higher (23%) cytochrome c oxidase activities in the glycolytic fragment of the gastrocnemius muscle and an ~17% increase in the soleus of sedentary male compared with sedentary female

mice. Orchiectomy abolished this sex difference, which was restored following T administration [71, 72].

T administration stimulates pyruvate dehydrogenase (PDH) activity and citrate production from pyruvate in the presence of oxaloacetate in castrated rats (Figure 1) [49]. The administration of androgens to castrated rats caused increased specific activity of a number of mitochondrial enzymes in the epididymis, including succinate dehydrogenase (SDH), glycerol phosphate dehydrogenase, and pyruvate carboxylase [42, 45]. T propionate induced a substantial increase in specific activity of the inner-mitochondrial-membrane enzyme cytochrome c oxidase in both red and white skeletal muscle as well as mouse kidney, heart, and aorta, without affecting the outer-mitochondrial-membrane enzyme monoamine oxidase [9]. T also increases both oxaloacetate and acetyl-CoA production, which results in increased citrate synthesis, in an as yet to be determined mechanism that enhances PDH activity [49]. In prostate-derived cell lines, transcriptional regulation of DNA-encoded mitochondrial enzyme aconitase, which interconverts citrate and isocitrate in the citric acid cycle, is mediated by the AR [50]. In skeletal muscle, it has been demonstrated that T levels correlated positively with both $VO_2$ max and *UQCRB* expression, a key enzyme in the oxidative phosphorylation pathway, suggesting that an association exists between serum T levels and mitochondrial function [33].

### Effects of androgens on oxidative metabolism

A recent study reported that androgens stimulate the utilization of glucose to undergo a metabolic conversion for production of ATP as well as lipogenesis in androgen-dependent PCa cells [51]. In a study investigating the effect of T treatment in patients with CHF, T supplementation improved glucose metabolism as well as functional capacity and large-muscle strength [9].

Androgen replacement therapy accelerates the conversion of fiber types from fast to slow oxidation [52], and increases the number and size of type I slow oxidative fibers [53]. These findings may explain the clinical observation of reduced muscle fatigability in response to T replacement, and the noted improved capacity of skeletal muscles may be attributed to an enhanced aerobic potential [9].

### Effects of androgens on fatty acid metabolism

T markedly stimulates hormone-sensitive lipolysis while inhibiting lipoprotein lipase (LPL) activity [75, 76]. This effect may enhance fatty acid release in tissues, causing an increase in plasma free fatty acid content and interfering with normal glucose utilization in muscles [77]. An increased flux of fatty acids may decrease muscle glycogen synthase activity [78] and increase muscle triglyceride stores [79]. T increases levels of fatty acid-binding protein (FABP) [48]. FABP plays an important role in the delivery of fatty acids to mitochondria for oxidation [80]. While the effect of T on FABP expression depends on the muscle type, it is clear that T treatment produces a positive influence on FABP availability [77]. T treatment in sedentary and exercised mice increased FABP content in cardiac muscle [48].

**Table 1**  Studies relating role of androgens in mitochondrial biogenesis, structural integrity, and biochemical function.

| Function | Study | Observations | Comments |
|---|---|---|---|
| Mitochondrial biogenesis/ morphology | Ikeda et al., 2010 [46] | T counteracts Dox-induced cardiotoxicity partly through activation of the Akt pathway and up-regulation of Tfam to protect cardiomyocytes from mitochondrial damage and apoptosis | T up-regulates Akt phosphorylation and Tfam expression |
| | Pitteloud et al., 2005 [33] | Low serum T levels appear to be associated with an adverse metabolic profile and impaired mitochondrial function promoting IR | Low levels of T are associated with decreased expression of PGC-1α |
| | Fan et al., 2005 [47] | AR plays an important role in metabolism affecting energy balance, and loss of AR has a negative impact on adiposity and insulin sensitivity | AR-deficient mice express low levels of PGC-1α |
| | Caminiti et al., 2009 [9] | T replacement therapy improves exercise capacity, muscle strength, and glucose metabolism in men with moderately severe CHF, and these effects are thought to be mediated by metabolic and peripheral effects | T causes an increase in muscle mitochondrial cytochrome *c* oxidase activity |
| Mitochondrial enzyme expression and activity | van Breda et al., 1992 [48] | FABP contents and mitochondrial activities of heart and skeletal muscle are affected by training and T, and these effects are different for heart and skeletal muscles | T potentiates training-induced increase in cytochrome *c* oxidase activity |
| | Koenig et al., 1980 [44] | Androgens regulate mitochondrial cytochrome *c* oxidase and lysosomal hydrolases in mouse skeletal muscle | Orchiectomy abolishes sex difference in cytochrome *c* oxidase activity |
| | Costello and Franklin, 1993 [49] | T stimulates PDH activity and citrate production from pyruvate in the presence of oxaloacetate (OAA). T increases both OAA and acetyl-CoA production, which results in increased citrate synthesis | T enhances PDH activity and citrate production |
| | Brooks, 1979 [42] | Castration resulted in a decrease in the concentration of nearly all enzymes associated with the tricarboxylic acid cycle (TCA), and administration of T restored concentrations to values similar to those in animals maintained by endogenous androgen. The most marked change was in that of pyruvate carboxylase (PC) | Androgen increased levels of SDH, glycerol 3-phosphate dehydrogenase (GPDH), and PC in castrated rats |
| | Juang et al., 2004 [50] | Dihydrotestosterone regulates mitochondrial aconitase gene expression | Transcriptional regulation of aconitase is mediated by AR |
| | Moon et al., 2011 [51] | Androgens increase glucose utilization for de novo lipogenesis in LNCaP cells through the activation of hexokinase 2 and activation of the cardiac isoform of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase | Androgens stimulate glucose utilization, ATP production, and lipogenesis |
| Oxidative metabolism | Czesla et al., 1997 [52] | Administration of androgens prevents severe muscle atrophy, and improves and accelerates fast to slow fiber type conversion necessary for successful cardiomyoplasty | Androgen replacement therapy (ART) accelerates conversion of fiber types from fast to slow oxidative |
| | Ustunel et al., 2003 [53] | T induces protein synthesis in gastrocnemius muscle fibers, and induces changes in shape and size, and also can change the appearance and the number of fibers | ART increases number and size of type I slow oxidative fibers |
| | Bjorntorp, 1991 [32] | Administration of T in moderate doses to middle-aged men leads to adaptations of the metabolism of adipose tissue expected to be followed by a diminution of this mass | T stimulates hormone-sensitive lipolysis |
| Fatty acid metabolism | Lanfranco et al., 2004 [54] | Hypogonadal men show higher adiponectin levels, which are reduced by T replacement therapy, suggesting that T exerts a regulatory role on adiponectin secretion in humans | T therapy can decrease adiponectin in hypogonadal men |
| | Basaria et al., 2006 [55] | Men with PCa receiving ADT are at risk for developing IR and hyperglycemia, thus leading to their increased risk of CVD | ADT contributes to development of MetS in men |
| MetS | Mauvais-Jarvis, 2011 [56] | An inverse relationship exists between total serum T and visceral adipose tissue in males with MetS | Estrogen and androgen receptors are potential targets in the prevention of age-related metabolic disorders |
| | Khaw and Barrett-Connor, 1992 [57] | T mobilizes the abdominal depot in males | Androgen plays a role in fat metabolism and adiposity |

(Table 1 continued)

| Function | Study | Observations | Comments |
|---|---|---|---|
| | Pitteloud et al., 2005 [33] | Low serum T levels are associated with an adverse metabolic profile and suggest a novel unifying mechanism for the previously independent observations that low T levels and impaired mitochondrial function promote IR in men | In males, high levels of T are associated with improved insulin sensitivity |
| | Lichtenstein et al., 1987 [58] | Insulin and T may have an interdependent regulatory effect on lipid metabolism and the effect of T on ischemic heart disease appears to be primarily mediated through its association with insulin | An inverse relationship exists between levels of T and fasting insulin levels in males independent of age, obesity, or body fat distribution |
| IR | Pasquali et al., 1991 [59] | Androgens play an important role in sugar and fat metabolism, and reduced T levels in diabetic men may contribute to the vicious cycle of altered glucose and lipid metabolism | Males with T2DM have lower levels of T than weight-matched non-diabetic controls |
| | Simon et al., 1992 [60] | Total plasma T decreased with each decade of age, and insulin increased with each decade of age. In these cross-sectional data, this significant graded inverse association between T and insulin was independent of age | A potential inverse relationship between T and IR may exist in diabetic men |
| | Andersson et al., 1994 [61] | T and sex hormone-binding globulin (SHBG) were lower in the diabetic men than in control groups | Men with T2DM have low levels of SHBG and low T values |
| | Barrett-Connor, 1992 [62] | Men with diabetes have significantly lower plasma levels of free and total T, and lower levels of endogenous T in diabetic men are associated with diabetic dyslipidemia | There is a positive relationship between total T levels and insulin sensitivity in normal males |
| | Wang et al., 2011 [63] | The presence of low T and/or SHBG predicts the development of MetS and T2DM | Men with obesity, MetS, and T2DM have low total and free T and low SHBG |
| | Haffner et al., 1994 [20] | Total whole-body glucose disposal is negatively associated with waist-to-hip ratio and positively associated with total T and SHBG | Higher waist-to-hip ratio and lower T are strongly associated with a decrease in total and non-oxidative whole-body glucose disposal in men |
| | Birkeland et al., 1993 [64] | There is a positive relationship between total T levels and insulin sensitivity in diabetic males | IR is associated with decreased levels of SHBG levels in men with T2DM |
| | Heufelder et al., 2009 [65] | T treatment of hypogonadal diabetic males markedly improves insulin sensitivity | T coupled with lifestyle modifications improves insulin sensitivity and reduces hemoglobin A1C (HbA1C) in diabetic men |

Akt, serine-threonine kinase; IR, insulin resistance; PGC-1α, coactivator 1-alpha; AR, androgen receptor; CHF, congestive heart failure; FABP, fatty acid-binding protein; PDH, pyruvate dehydrogenase; SDH, succinate dehydrogenase; PCa, prostate cancer; ADT, androgen deprivation therapy; CVD, cardiovascular disease; MetS, metabolic syndrome; T2DM, type 2 diabetes.

It has been reported that the high levels of adiponectin observed in men with TD can be reduced by T therapy [54]. T infusion decreases adiponectin levels in mice [81], most likely by an AR-mediated mechanism [47]. It remains unclear whether AR-mediated suppression of adiponectin reflects increased adiponectin sensitivity or a decreased number of adipocytes [56]. ADT contributes to the development of MetS in men [55, 56]. An inverse relationship exists between total serum T and the visceral adipose tissue in men with MetS [57]. This relationship was observed in age-related TD [14], inherited TD [82], and ADT during treatment [55]. It seems to follow that in men, high T levels are associated with improved insulin sensitivity [33].

## TD, mitochondrial dysfunction, and IR

Wang et al. [63, 74] recently reviewed the relationship between TD and T2DM. Cross-sectional studies have demonstrated an inverse relationship between T concentrations and fasting insulin levels in men independent of age, obesity, and body fat distribution [58–60, 83, 84]. The well-recognized observation that men with T2DM have lower T levels than weight-matched non-diabetic control subjects suggests a link between T and T2DM [61, 62, 74]. Moreover, several large prospective studies have demonstrated that low T levels are predictive of T2DM development in men [19–25]. In addition,
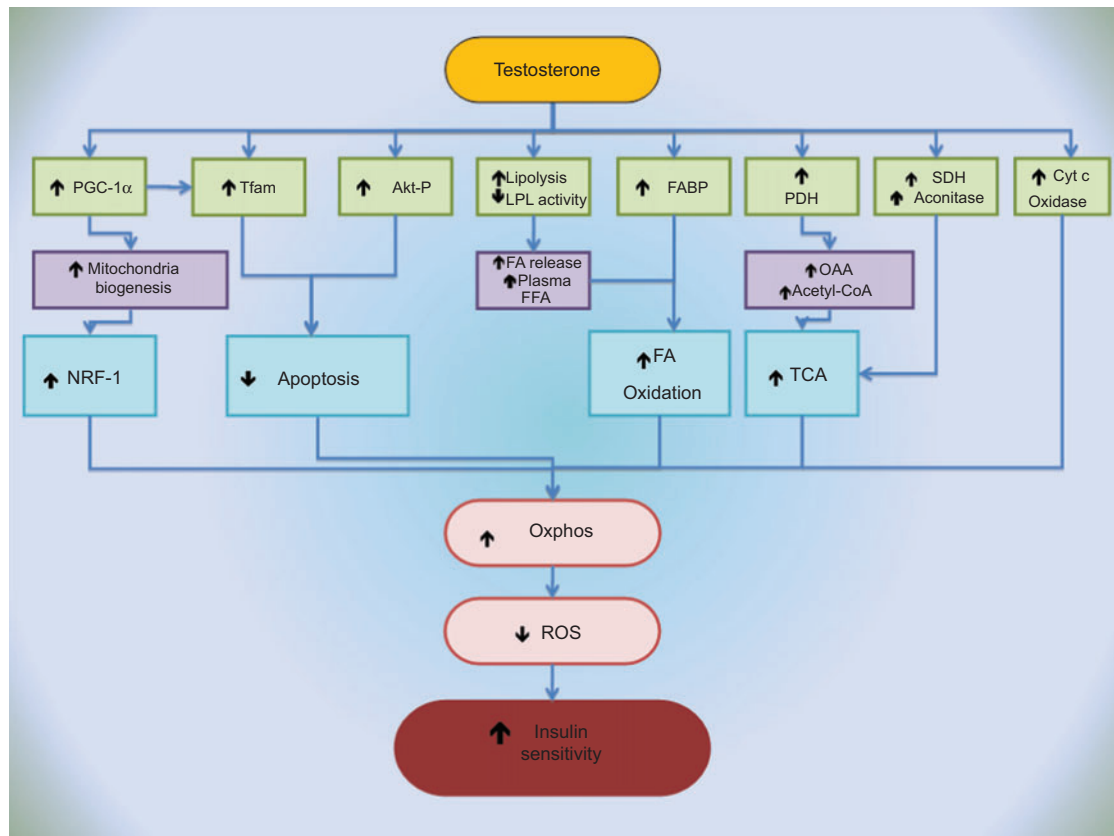
**Figure 1**   Effects of androgens on mitochondrial function.
T increases PGC-1α expression [33, 46, 47, 56], which in turn increases Tfam expression [46] as well as mitochondrial biogenesis [46]. The increase in mitochondrial biogenesis increases levels of NRF-1 [46], which in turn increases oxidative phosphorylation [7, 33]. T increases Tfam expression as well as Akt phosphorylation [46], both of which decrease apoptosis leading to an increase in oxidative phosphorylation. T stimulates lipolysis and down-regulates lipoprotein lipase (LPL) activity [36, 37] and increases expression of FABP [48] leading to an increase in fatty acid oxidation and in oxidative phosphorylation [33, 46, 48]. T increases expression of PDH [49], which increases production of OAA and acetyl-CoA [49] leading to a stimulation of the TCA. T also increases expression of SDH and aconitase [50], also up-regulating TCA and increasing oxidative phosphorylation [33, 46, 48, 50]. Finally, T increases the expression of cytochrome *c* oxidase [9, 48, 73], which leads to an increase in oxidative phosphorylation. The increase in oxidative phosphorylation leads to a decrease in ROS and an increase in insulin sensitivity [74].

two studies have shown a positive relationship between total levels of T and insulin sensitivity in normal [20, 21] as well as diabetic men [64]. Although the data with total T is well established, the relationship with free T is controversial. For instance, data on the relationship between free T levels and insulin sensitivity in some studies show a weakly positive relationship [20, 21] and other studies report no correlation [64, 85]. However, T treatment of men with T2DM and TD resulted in marked improvement of insulin sensitivity [65].

Morino et al. [40, 41] reported a 38% reduction in the number of mitochondrial density in patients with IR. This observation was consistent with previous studies reporting lower mitochondrial number in patients with T2DM [86]. Another study showed other morphological changes, such as impaired subsarcolemmal fraction in obese patients and patients with T2DM [87]. In agreement with these observations, it has been shown that the expression of cytochrome *c* oxidase I, SDH, and PDH is markedly reduced in subjects with IR [40, 41]. This reduction in skeletal muscle mitochondrial number may be responsible for the

diminished rates of mitochondrial oxidative phosphorylation, which predisposes to intramyocellular lipid accumulation [41].

Mitochondrial dysfunction has commonly been observed in muscles of patients with T2DM [86]. Several clinical studies in the past decade have reported that mitochondrial dysfunction, including the reduction in mitochondrial density and OXPHOS efficiency, is associated with T2DM [74]. IR in T2DM is associated with reduced oxidative capacity in skeletal muscle [88, 89] as well as diminished expression of a set of nuclear genes involved in oxidative metabolism [38, 39, 90, 91]. A significant reduction in the total activity of the mitochondrial electron transport chain has been observed in the skeletal muscle of T2DM patients compared with that of lean and healthy controls [86]. Furthermore, the specific activities of NADH-oxidase/cardiolipin, NADH-oxidase/citrate, and NADH-oxidase/β-hydroxyacetylcoAdehydrogenase (β-HAD) ratios are reduced by two- to three-fold in patients with T2DM [92]. The frequency of common and large-scale deletion in mitochondrial DNA was found to be higher in
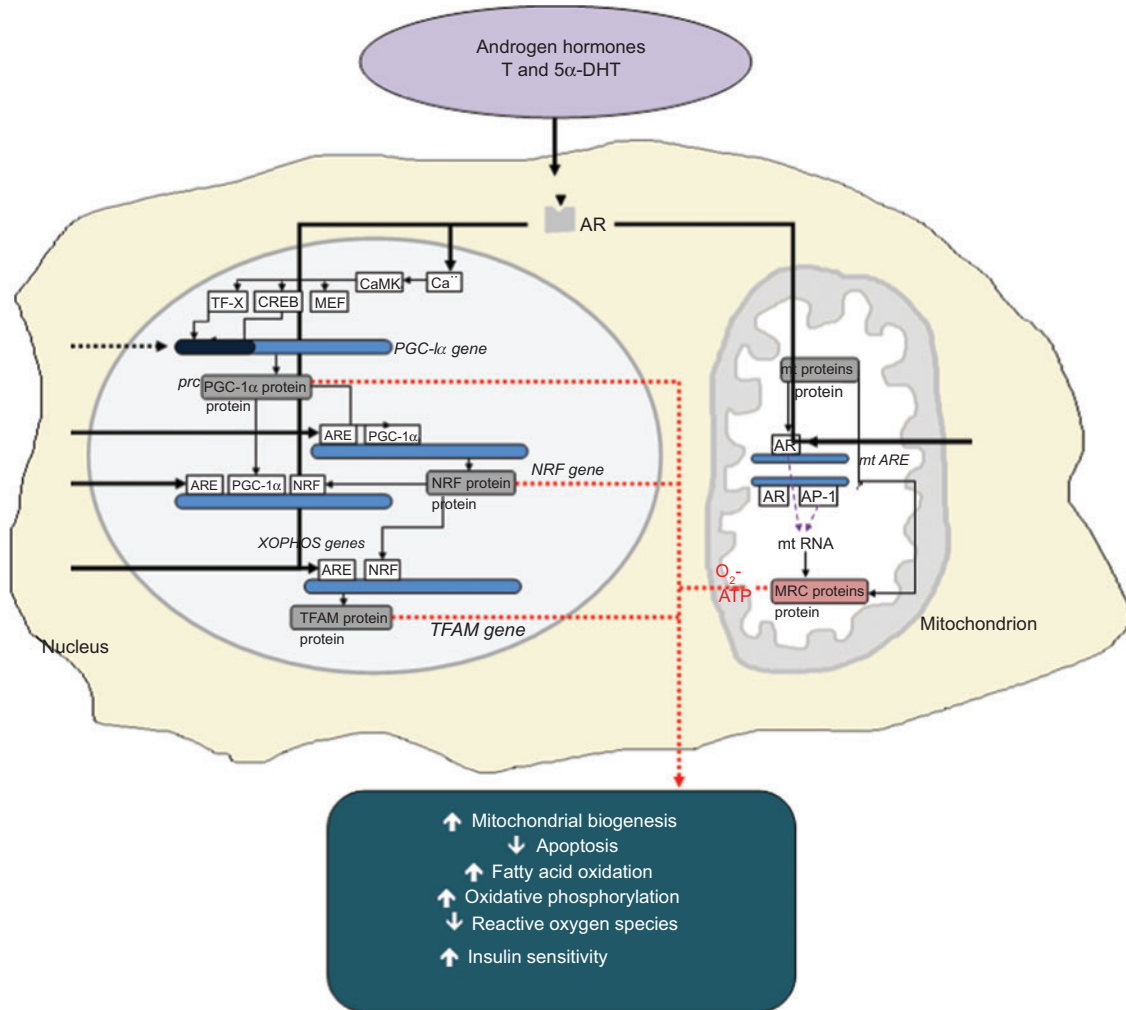
**Figure 2**  Modulation of mitochondrial function by androgens.
T and 5-α-dihydrotestosterone (5α-DHT) bind with high affinity to the AR. The ligand-AR complex modulates the expression of several nuclear and mitochondrial target genes that, together, promote fatty acid oxidation, oxidative phosphorylation, and mitochondrial biogenesis, attenuating the generation or build up of ROS resulting in an improvement in insulin sensitivity [33]. In the mitochondrion, the bound AR also up-regulates the expression of cytochrome *c* oxidase [9]. In the nucleus, the ligand-bound AR complex increases PGC-1α expression [46], which in turn up-regulates Tfam expression [46]. These pathways lead to mitochondrial biogenesis, which increases NRF-1 levels [46], the later in turn facilitates oxidative phosphorylation and increased expression of oxidative phosphorylation genes encoding PDH, SDH, GPDH, and PC [42, 49].

skeletal muscle of diabetic patients than in age- and sex-matched controls [93]. The impairment of oxidative phosphorylation as well as fatty acid metabolism has been observed in skeletal muscles of insulin-resistant offspring of patients with T2DM compared with age-matched insulin-sensitive controls [94]. Mogensen et al. [95] reported that ADP-stimulated respiration was diminished in obese subjects with T2DM with a compensatory increase in type 2 muscle fibers. Mouse models have been used to confirm that mitochondrial dysfunction can cause T2DM [74]. Other studies reported a decline in mitochondrial number and content of mitochondrial DNA and respiratory enzymes. Derangement of the mitochondrial network was also noted. These findings are consistent with the decline in oxidative phosphorylation and β-oxidation in adipose tissue of mice with T2DM [96].

### Oxidative stress and mitochondrial dysfunction

Mitochondrial dysfunction contributes to overproduction of ROS and dysregulation of the insulin signaling pathway leading to IR in muscle [74]. Overproduction of ROS caused by an imbalance of antioxidant enzymes and defective oxidative phosphorylation may damage intracellular components and impair normal cellular function [97]. Oxidative stress may activate multiple serine threonine kinase (Akt) cascades, including p38 mitogen activated protein kinase and Jun N-terminal kinase (JNK), and can act on a number of potential targets in the insulin signaling pathway, such as insulin receptor and the family of insulin receptor substrate (IRS) proteins [74]. Furthermore, inactivation of phosphoinositol-3-kinase has been shown to impair the translocation of the

insulin-dependent glucose transporter GLUT4 to the plasma membrane, leading to a decreased glucose uptake by muscle in response to insulin [97].

With advancing age, muscles exhibit lower mitochondrial number and lower efficacy of energy production [98]. It has been suggested that the lower efficiency in muscle mitochondria in the elderly is caused by ROS damage to the inner mitochondrial membrane, resulting in uncoupling of the electron transport chain [99]. The decreased efficiency and impaired energy production capacity of muscle mitochondria that develops with aging may be partially reversed [100, 101]. This suggests that mitochondrial dysfunction is not entirely due to irreversible mutations in mitochondrial DNA. Physical activity may stimulate mitochondrial biogenesis as well as improve the efficiency of existing mitochondria, possibly by reversing oxidative damage [98].

Impairment of mitochondrial function may be accompanied by a diminished activity of enzymes involved in β-oxidation of fatty acids, leading to an increase in intracellular lipid content [74]. Hotta et al. [102] reported that the plasma level of adiponectin, the adipokine released by adipose tissue, is diminished in obese patients or patients with T2DM. In mouse models, it has been established that adiponectin deficiency leads to IR [103]. This decrease in adiponectin expression in adipocytes with mitochondrial dysfunction occurs by activation of the JNK pathway [104]. Thus, mitochondrial dysfunction not only causes insulin insensitivity but also impairs the secretion of adipokines, by adipocytes, which in turn compromises other tissues with regard to glucose utilization [74].

Mootha et al. [38] reported decreased maximal aerobic capacity and reduced expression of mitochondrial genes involved in oxidative phosphorylation in men with impaired glucose tolerance and T2DM. Patti et al. [39] demonstrated a decreased expression of enzymes involved in oxidative phosphorylation in a group of T2DM patients as well as insulin-resistant first-degree relatives of T2DM subjects with normal glucose tolerance. Using magnetic resonance spectroscopy, it has been shown that decreased mitochondrial oxidative phosphorylation activity is the cause of age-related IR [105]. In addition, the lean offspring of patients with T2DM also exhibited mitochondrial dysfunction [94]. A recent study performed in an animal model bred to exhibit low aerobic capacity also implicated a causative role of impaired mitochondrial function in the development of the CVD risk profile associated with MetS [106].

## Molecular mechanism of androgen action in mitochondrial function

Androgens regulate gene transcription through a genomic pathway in which the AR, a ligand-activated transcription factor, binds the hormone and translocates into the nucleus where it interacts with specific DNA sequence known as androgen response element (ARE) in its target genes [107]. Evidence accumulating over the past two decades has also implicated rapid (non-genomic) responses to androgens, through a mechanism dependent or independent of AR action [108]. While limited information is available regarding the interaction of AR with mitochondria, it has been confirmed that the AR interacts directly with the Vb subunit of cytochrome $c$ oxidase [73]. In prostatic cells, the AR mediates the translocation of the pro-apoptotic factor Bax to the mitochondria in a transcriptionally dependent mechanism that is yet to be elucidated [109]. In contrast, direct receptor-independent activation of inner mitochondrial membrane ATP-sensitive $K^+$ channels has been observed following T administration in cardiomyocytes [110].

Several biochemical factors play an integral role in mitochondrial biogenesis. These include the PPARγ co-activator PGC-1α, a transcriptional factor co-activator [41], 5′ adenosine monophosphate-activated protein (AMP) kinase, which elicits its effects through myocyte enhancer factor-2 and cyclic AMP response element-binding protein-mediated increased PGC-1α expression [111–113]. Thus, increased expression of PGC-1α leads to an increase in target genes, such as the nuclear respiratory factor-1 (*NRF-1*), a transcription factor that stimulates many nuclear-encoded mitochondrial genes including *OXPHOS* genes and *Tfam*, which bind to the D-loop of the mitochondrial genome and increase transcription of mitochondrial genes and replication of mitochondrial DNA [114].

It has been suggested that the mechanism of IR induced by TD involves a down-regulation of the transcription factor PGC-1α in skeletal muscle [56]. As a stimulator of mitochondrial biogenesis as well as skeletal muscle oxidative fibers, PGC-1α is a molecular marker of insulin sensitivity, and a decrease in its expression has been observed in patients with T2DM [38]. Low T levels are associated with low PGC-1α expression in muscle [33]. This is supported by studies in mouse models in which similar association has been observed between TD and low levels of PGC-1α in tissues [47]. The reduced expression of PGC-1α compromises mitochondrial activity and energy production. Thus, it seems likely that TD promotes IR, at least partially, through an AR-dependent mechanism that involves a decrease in PGC-1α-mediated oxidative and insulin-sensitive muscle fibers [56].

T substitution in orchiectomized rats improved recovery of myocardial function after ischemia. While this effect might have been partly related to acute coronary vasodilation by T, the investigators of one study hypothesized that T may also exhibit direct cytoprotective actions on the myocardium. The results showed that T acutely and directly depolarized and oxidized cardiac mitochondria in a $K^+$-dependent, ATP-sensitive, and AR-independent manner (non-genomic pathway). By patch clamping the cardiac inner mitochondrial membrane, it was demonstrated that T induced activation of mitochondrial $K^+$ channels, which were inhibited by ATP, 5-hydroxydecainoic acid, and glibenclamide, but exhibited no effect on sarcoplasmic $K_{ATP}$ channels. T protected cardiomyocytes from ischemic cell death [110].

A recent study set out to clarify whether the AR system exerts a cardioprotective effect against Dox-induced cardiotoxicity. Electron microscopic examination of mitochondria in the hearts of animals treated with Dox revealed prominent mitochondrial damage, such as vacuolization in the ARKO

mice model compared with that of the WT mice. A basal mitochondrial dysfunction was noted in the myocardium of male ARKO mouse heart, in the absence of Dox treatment. This was attributed to loss of AR-mediated signaling, which may play a critical role in cardiac oxidative stress. Furthermore, superoxide production in response to Dox treatment of male ARKO mice was markedly enhanced compared with that of male WT mice, but the number of apoptotic cells in the ventricular tissues was significantly larger in male ARKO mice than in male WT mice. The expression of a key mitochondrial transcription factor (Tfam) in cardiac tissues of male WT mice was not affected significantly by Dox treatment while its expression was reduced by almost half in male ARKO mice treated with Dox. The results of this study suggest that the AR system may counteract Dox-induced cardiotoxicity partly through activation of the Akt pathway and up-regulation of Tfam to protect cardiomyocytes from mitochondrial damage and apoptosis [46].

A recent study comparing mitochondrial function of young obese and non-obese individuals showed more mitochondrial dysfunction in the cardiomyocytes of obese patients with excessive oxidative stress, mitochondrial damage, and apoptosis. This may be partially explained by the noted reduction in the expression of NRF-1 and its target Tfam [115] and may account for decreased expression of the complex I protein ND6 [116]. Since MetS and obesity are associated with reduced T levels, it is possible that reduced circulating androgens, which contribute to increased inflammatory cytokines, may play a role in mitochondrial dysfunction.

## Mitochondrial dysfunction and diseases

TD is increasingly recognized not only among older men but also in young men and in cancer survivors, who underwent ADT. However, the impact of TD on the quality of life remains poorly established. Among the common complaints of TD is fatigue, reduced energy, loss of self-esteem, and sexual dysfunction [30]. T replacement therapy in men with TD showed improvement in mood, sexual function, reduced depression and anxiety, increased concentration, self-confidence, improved mood, and decreased fatigue within a few weeks [17]. The mechanisms by which androgens affect many of these physiological processes are not clearly understood. One underlying hypothesis is that androgens modulate mitochondrial function and this may be a common link between TD and the various symptoms noted in men with TD. However, no specific mechanism has been provided to relate androgen deficiency to mitochondrial dysfunction.

Mitochondria play a critical role in cellular function by regulating biochemical pathways involved in lipid, protein, and carbohydrate metabolism as well as cell survival and apoptosis. Understanding androgen modulation of mitochondrial function is critical to understanding the role of TD in the pathophysiology of T2DM, IR, and CVD. As shown in Figure 1, androgen regulation of expression and activity of oxidative phosphorylation enzymes in the mitochondria may represent one critical mechanism, among others, in smooth and skeletal muscle in the control of

cellular processes. This regulation by androgens facilitates the increase in production of cellular energy depending on the physiological conditions, such as physical demand, stress, or acute illness.

As depicted in Figure 2, androgens regulate cellular metabolism and energy production through molecular and cellular mechanisms involving both genomic and non-genomic pathways and mitochondrial *OXPHOS* genes. Binding of activated AR complexes with nuclear and mitochondrial *OXPHOS* gene response elements has been demonstrated [117–119]. Another proposed pathway of androgen action is through direct interaction of AR complexes with AREs of *OXPHOS* genes in the mitochondria. Additional mechanisms may involve indirect interactions with ARE in the nucleus to activate transcription of genes encoding transcription factors, such as NRF and PGC-1α, which in turn activate *OXPHOS* genes in the mitochondria [120, 121].

## Testosterone effect on mitochondrial functions

As depicted in Figure 1, T up-regulates a host of enzymes and transcriptional factors. For example, T increases the expression of PGC-1α, in which the latter modulates Tfam transcriptional factor activity as well as stimulates mitochondrial biogenesis, leading to an increase in the expression of NRF-1. T also increases the expression and activity of Akt, hormone-activated lipases, FABP, cytochrome *c* oxidase, SDH, and PDH. These enzymes coordinate a host of integrated pathways leading to increased mitochondrial biogenesis and to increased energy production. Furthermore, the expression of nuclear transcription factors, which in turn control the expression of nuclear-encoded mitochondrial proteins, is also regulated by androgens.

Depletion of mitochondrial DNA by chronic treatment with ethidium bromide causes loss of response to insulin due to impaired insulin signaling pathways, and repletion of mitochondrial DNA restores insulin sensitivity of muscle cells [122]. In addition to this genetic mechanism of action, treatment with respiratory inhibitors has revealed a decrease in insulin-stimulated glucose uptake as well as inactivation of Akt and IRS-1 of the insulin signaling pathway [123]. One proposed mechanism by which mitochondrial dysfunction causes IR involves impairment in insulin secretion by β-islet cells in response to a decreased intracellular $Ca^{2+}$ concentration. It has been suggested that the ATP/ADP ratio is diminished in β-islet cells with mitochondrial defects, rendering the cell incapable of inducing closure of ATP-dependent $K^+$ channels or depolarization of the membrane [74].

Mitochondrial dysfunction is implicated in a number of pathophysiological processes in disease states, such as diabetes, IR, and CVD. Recent studies have demonstrated a strong association between TD, IR, diabetes, and CVD. However, the molecular and cellular mechanisms linking TD to these pathologies remain under investigation. Yialamas et al. [34] and Pittleoud et al. [33] have suggested that TD has a direct effect on glucose utilization in cases of increased IR. $VO_2$ max and *OXPHOS-CR* gene expression were shown to correlate positively with T levels [33]. In animal studies, castration
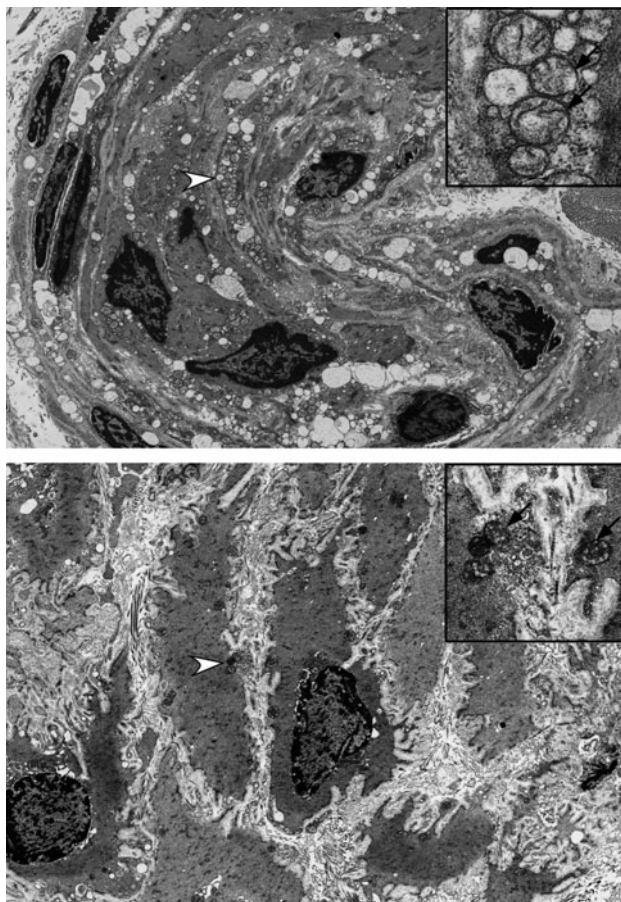
**Figure 3** Effects of androgen deprivation on mitochondrial morphology.

Penile corpus cavernosum tissue sections obtained from 2-week castrated mature male rabbits (top) or from sham-operated animals (bottom) were fixed, embedded in plastic, and sectioned for electron microscopic examinations as described previously [69]. Note that the trabecular smooth muscle cells from castrated animals have disorganized contours and have accumulated vacuoles and flocculating substances. Also note that the mitochondria (arrow) were increased in number and appear swollen (see magnified box at top right corner). In contrast, in the section from the sham-operated animals (bottom), the smooth muscle appears normal with no distortions or accumulation of vacuoles, and the mitochondria (arrow) appear normal with no increase in the number or swelling (see magnified box in the top right corner).

is also associated with IR and decreased glycogen synthase activity [37]. Investigations in obese rats, a genetic model of obesity and T2DM, strongly suggest a role for androgens in regulating mitochondrial function [37]. Interestingly, medical castration of young healthy men with gonadotropin-releasing hormone agonist resulted in reduced lipid oxidation and diminished resting energy expenditure [36]. These observations suggest that TD may contribute to IR by a mechanism that involves attenuated or altered fatty acid oxidation. T modulation of *OXPHOS* gene expression may represent an important therapeutic modality for preventing or treating mitochondrial dysfunction in men with TD.

## Summary

Androgens regulate fuel metabolism through mitochondrial function and modulate mitochondrial biogenesis, expression of mitochondrial enzymes, and oxidative phosphorylation. Androgen deficiency contributes to the pathophysiology of fatigue, IR, diabetes, and in turn CVD through the common link "mitochondrial dysfunction." Research on the molecular basis of androgen action in the mitochondria may provide novel strategies for the development of pharmacotherapeutic agents for the management of the aforementioned pathologies.

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