Testosterone therapy decreases subcutaneous fat and adiponectin in aging men

L Frederiksen1,2, K Højlund1,2, D M Hougaard1, T H Mosbech4, R Larsen4, A Flyvbjerg5,6, J Frystyk5,6, K Brixen1,2 and M Andersen1,2

1Department of Endocrinology, Odense University Hospital, Sdr. Boulevard 29, 5000 Odense C, Denmark, 2Institute of Clinical Research, University of Southern Denmark, Odense, Denmark, 3Department of Clinical Biochemistry and Immunology, Statens Serum Institut, Artillerivej, 2300 Copenhagen S, Denmark, 4DTU Informatics, Technical University of Denmark, Kgs. Lyngby, Denmark, 5The Medical Research Laboratories, Faculty of Health Sciences, Clinical Institute of Medicine, Aarhus University, Aarhus C, Denmark and 6Department of Endocrinology and Internal Medicine, Aarhus University Hospital, 8000 Aarhus C, Denmark

(Correspondence should be addressed to L Frederiksen at Department of Endocrinology, Odense University Hospital; Email: louise.frederiksen@ouh.regionsyddanmark.dk)

Abstract

Objective: Testosterone therapy increases lean body mass and decreases total fat mass in aging men with low normal testosterone levels. The major challenge is, however, to determine whether the metabolic consequences of testosterone therapy are overall positive. We have previously reported that 6-month testosterone therapy did not improve insulin sensitivity. We investigated the effect of testosterone therapy on regional body fat distribution and on the levels of the insulin-sensitizing adipokine, adiponectin, in aging men with low normal bioavailable testosterone levels.

Design: A randomized, double-blinded, placebo-controlled study on 6-month testosterone treatment (gel) in 38 men, aged 60–78 years, with bioavailable testosterone <7.3 nmol/l, and a waist circumference >94 cm.

Methods: Central fat mass (CFM) and lower extremity fat mass (LEFM) were measured by dual X-ray absorptiometry. Subcutaneous abdominal adipose tissue (SAT), visceral adipose tissue (VAT), and thigh subcutaneous fat area (TEA) were measured by magnetic resonance imaging. Adiponectin levels were measured using an in-house immunofluorometric assay. Coefficients (b) represent the placebo-controlled mean effect of intervention.

Results: LEFM was decreased (b = −0.47 kg, P = 0.07) while CFM did not change significantly (b = −0.66 kg, P = 0.10) during testosterone therapy. SAT (b = −3.0%, P = 0.018) and TEA (b = −3.0%, P < 0.001) decreased, while VAT (b = 1.0%, P = 0.54) remained unchanged. Adiponectin levels decreased during testosterone therapy (b = −1.3 mg/l, P = 0.001).

Conclusion: Testosterone therapy decreased subcutaneous fat on the abdomen and lower extremities, but visceral fat was unchanged. Moreover, adiponectin levels were significantly decreased during testosterone therapy.

Introduction

Testosterone therapy has been almost universally found to increase lean body mass (LBM) and decrease total fat mass (TFM) (1) in accordance with our data in aging men with low normal bioavailable testosterone levels (2). Theoretically, these beneficial changes would improve insulin sensitivity. However, we found no increase in insulin sensitivity using the hyperinsulinemic euglycemic clamp (2). The probable explanations for the lack of improvement in insulin sensitivity are complex and interrelated. Thus, the decrease in TFM during testosterone therapy may predominantly involve fat on the lower extremities, which has been reported to be positively associated with insulin sensitivity (3). Alternatively, testosterone therapy may reduce the insulin-sensitizing adipokine, adiponectin, and hence have counteracted the overall positive changes in body composition. Adiponectin levels could be decreased either indirectly through a decrease in fat on lower extremities, as fat on the lower extremities has been positively associated with adiponectin levels (4), or due to a direct effect of testosterone on adiponectin secretion (5). Regarding the effect of testosterone therapy on regional fat distribution, studies on abdominally obese, aging men have been conflicting, probably due to the use of oral preparations of testosterone (6, 7, 8). Studies that used sufficient testosterone doses reported a significant decrease in subcutaneous fat on the abdomen (SAT), but no change in visceral adipose tissue (VAT) during testosterone therapy (9, 10), whereas an old study on middle-aged obese men with
higher baseline testosterone levels reported no change in SAT and a decrease in VAT (11). Data on changes in lower extremity fat during testosterone therapy are limited. Regarding adiponectin, a direct suppressive effect of testosterone therapy on adiponectin levels has been suggested. Clinical studies on the effect of testosterone therapy have reported a decrease in adiponectin levels in men with type 2 diabetes mellitus (12) and in aging men using supraphysiological doses of testosterone (13), while no changes were observed in adiponectin levels in aging men treated with physiological doses of testosterone (14). However, changes in regional fat distribution or insulin sensitivity were not measured in these studies. Therefore, in this study, we assessed the effect of 6-month testosterone therapy on the regional fat distribution measured by dual X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) and adiponectin levels in abdominally obese, aging men with low normal bioavailable testosterone levels (15).

Methods
The study was a single-center, randomized, placebo-controlled, double-blinded study to assess the effect of testosterone gel and strength training on body composition, components of the metabolic syndrome, and quality of life in men aged 60–78 years with low normal bioavailable testosterone levels and increased body fat. In this study, we report only the results from the testosterone and placebo groups. The study was approved by the local ethics committee and declared in ClinicalTrials.gov (identifier: NCT00700024). All participants gave written informed consent. Recruitment of participants and study population have been described elsewhere (2).

Study design
Subjects were randomly assigned to receive testosterone (n = 23) or placebo (n = 23). Randomization numbers were assigned to the participants in order of enrollment into the study. The randomization list, medicine labeling, randomization, and code break envelopes were generated by Ipsen Scandinavia (Kista, Stockholm, Sweden) to ensure double blinding.

In the testosterone group, participants initially received 5 g gel (50 mg testosterone, Testim; Ipsen, France), or 5 g gel (placebo). After 3 weeks of treatment, safety parameters, and testosterone levels were evaluated. If bioavailable testosterone levels were <7.3 nmol/l, the dose was increased by 10 g gel (100 mg testosterone). In the placebo group, the dose was increased in all participants, and in the testosterone group, the dose was increased in 8/20 participants. Compliance was monitored by participants self-reporting at each visit. Three subjects in the testosterone group and five subjects in the placebo group prematurely discontinued treatment as described elsewhere (2).

In total, 18 participants in the placebo group and 20 in the testosterone group completed the study. Two participants in the placebo group and one participant in the testosterone group did not complete the MRI scans due to claustrophobia. Measurements of prostate-specific antigen, hemoglobin, and hematocrit were conducted at baseline, after 3 weeks, 3 months, and 6 months of therapy. Safety monitoring was externally handled to ensure continued blinding. Digital rectal examination of the prostate was performed to record changes at baseline and after 3 and 6 months. Subjects were advised to refrain from all self-initiated resistance exercise training and intense endurance training but were allowed to continue other habitual activities throughout the study. Subjects were informed not to change their diet. The study outcomes were evaluated at baseline and 6 months.

There was no significant difference between participants in the placebo group and the testosterone group at baseline regarding age, body mass index (BMI), waist circumference (WC), LBM, and TFM (Table 1) (2). There was no significant difference between participants in the placebo group and the testosterone group at baseline regarding total testosterone (TT), free testosterone (FT) and bioavailable testosterone (BT), LH, fasting insulin, or insulin sensitivity measured by the euglycemic hyperinsulinemic clamp (Rd; Table 2) (2).

Dual X-ray absorptiometry
TFM, central fat mass (CFM), lower extremity fat mass (LEFM), and LBM were measured by DXA using a Hologic Discovery Device (Waltham, MA, USA). The coefficient of variation (CV) was 0.8% for TFM and 0.6% for LBM.

Magnetic resonance imaging
MRI was performed using a 3.0 Tesla High field MR Unit (Philips Achieva, Phillips Healthcare, Best, The Netherlands). One abdominal slice (10 mm-thick, intervertebral space of L4/L5, perpendicular to subcutaneous fat) was recorded using an axial, T1-weighted gradient-echo sequence (repetition time (TR) 150 ms, echo time (TE) 2.3 ms, acquisition matrix 328×254, field of view 450×450 mm). Computer software (16)
Table 2 Total testosterone, bioavailable testosterone, free testosterone, SHBG, LH, fasting insulin, and insulin sensitivity at baseline and after 6 months of testosterone therapy (2). Values are given as median (interquartile range).

<table>
<thead>
<tr>
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<tr>
<td>Total testosterone (nmol/l)</td>
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<tr>
<td>Bioavailable testosterone (nmol/l)</td>
<td>5.1</td>
<td>(4.3–6.0)</td>
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<tr>
<td>Free testosterone (nmol/l)</td>
<td>0.27</td>
<td>(0.22–0.32)</td>
</tr>
<tr>
<td>LH (IE/l)</td>
<td>4.6</td>
<td>(3.1–7.6)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>52</td>
<td>(36–96)</td>
</tr>
<tr>
<td>Insulin-stimulated Rd (mg/min per m²)</td>
<td>207.0</td>
<td>(149.5–279.0)</td>
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Insulin-stimulated Rd, insulin-stimulated whole body glucose utilization. †P<0.01 compared with placebo.

was used to trace the different compartments of fat on the abdomen and for assessment of the areas of SAT and VAT. The thigh fat area (TFA) was determined on one femoral slice (15 cm from the major trochanter and perpendicular to subcutaneous fat) using a T1-weighted gradient-echo sequence (TR 400 ms, TE 18 ms, acquisition matrix 376 × 335, field of view 230 × 230 mm). Computer software (16) was used to trace fat and muscle compartments on the thigh to assess TFA and thigh muscle area (TMA).

**Hormones and SHBG**

Testosterone was measured between 0800 and 0900 h after an overnight fast. Serum TT was measured by liquid chromatography tandem mass spectrometry after ether extraction.

For testosterone measurements, the intra-assay CV was <10% for TT > 0.2 nmol/l and CV was <30% in the range between 0.1 and 0.2 nmol/l.

SHBG was measured by auto-DELFIA, and BT and FT were calculated according to the formulas of Vermeulen et al. (17). A single measurement of testosterone was performed to determine eligibility. BT levels were below 7.3 nmol/l (15) at re-evaluation after 3 weeks on placebo (n = 18).

Adiponectin was determined by an in-house time-resolved immunofluorometric assay as described elsewhere (18), with intra- and interassay CV averaging <5 and 10% respectively: Serum levels of LH were analyzed by time-resolved fluoroimmunoassay using commercial kits (auto-DELFIA; PerkinElmer Life Sciences, Oy, Turku, Finland). Intra-assay CV was 1.0–9.3% and interassay CV was 2.3–3.9%.

Serum levels of insulin were analyzed by time-resolved immunofluorometric assay (auto-DELFIA; PerkinElmer Life Sciences). The intra-assay CV was 2.1–3.7% and the interassay CV was 3.4–4.0%.

**Euglycemic hyperinsulinemic clamp**

The clamp protocol has been described in detail elsewhere (2). After a 120 min basal tracer equilibration period, insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was infused at a rate of 40 mU/m² per min for 180 min. A primed constant [3-³H]-glucose infusion was used throughout the 300 min study, and [3-³H]-glucose was added to the glucose infusates to maintain plasma-specific activity constant at baseline levels during the 180 min clamp period as described (19). Plasma glucose levels were clamped at ~5 mmol/l using a variable infusion rate of 20% glucose based on bedside plasma glucose measurements (ABL800 Flex; Radiometer, Copenhagen, Denmark) every 10–20 min. The basal and insulin-stimulated steady-state periods were defined as the last 20 min of the 120 basal and 180 min insulin infusion period. Tritiated glucose-specific activity was determined on samples deproteinized with barium and zinc as described elsewhere (19). Steele’s nonsteady-state formulas were used to calculate rates of glucose disposal (Rd), assuming a glucose distribution volume of 200 ml/kg body weight and a pool fraction of 0.65 (19). Insulin-stimulated whole body glucose utilization was taken as an estimate of whole-body insulin sensitivity.

**Statistical analysis**

The sample size of the study was determined by the effect of testosterone on LBM (type 1 error (α) = 0.05, type 2 error (β) = 0.1, s.d. = 1.3 kg, minimal relevant difference (MIREDF) = 1.3 kg, and power (1-β) = 90%). This calculation resulted in 15 subjects in each group. We decided to enroll 20 participants in each group. **Figure 1** Mean change in central fat mass (CFM) and lower extremity fat mass (LEFM) during testosterone therapy. **P<0.01; open circle, placebo; closed circle, testosterone. Data are presented as mean ± S.E.M.**
to allow for dropouts (2). Data were analyzed using Stata version 11. In univariate analysis and in the analysis of correlation of delta values, we used the Pearson correlation. The outcomes were compared by multiple linear regression analysis controlled for baseline values (20). Results are given as the coefficient ($b$) equivalent to the mean difference in the effect of the intervention (21). The models were checked with residual plots and Box–Cox analysis. Results were considered statistically significant at $P<0.05$. Data are given as median and interquartile range.

**Results**

TT, BT, FT, SHBG, LH, fasting insulin, and insulin-stimulated Rd at baseline and after 6-month testosterone therapy are displayed in Table 2 (2).

**Body composition**

**DXA data** At baseline, CFM was 14.0 (12.6–17.5) kg and 13.2 (9.2–18.1) kg and LEFM was 6.6 (5.7–7.9) kg and 6.9 (5.7–9.5) kg in the testosterone and placebo groups respectively. There was no significant difference between participants in the testosterone group and placebo group at baseline regarding CFM and LEFM. LEFM significantly decreased ($b=−0.47$ kg, $P=0.007$) in response to testosterone therapy whereas CFM was numerically but not statistically reduced CFM ($b=−0.66$ kg, $P=0.10$) (Fig. 1A and B).

**MRI data** At baseline, there was no significant difference between the testosterone and the placebo groups regarding VAT, SAT, TFA, and TMA. SAT significantly decreased during testosterone therapy ($b=−3.0$% fat area/total area, $P=0.018$) while VAT was unchanged ($b=1.0$% fat area/total area, $P=0.54$). TFA decreased ($b=−3.0$% fat area/total area, $P<0.001$) and TMA increased ($b=3.2$% muscle area/total area, $P<0.001$) in response to treatment with testosterone (Fig. 2).

**Adiponectin**

There was no significant difference in serum adiponectin levels between the testosterone group (7.2 (6.3–10.1) mg/l) and the placebo group (8.6 (5.2–10.9) mg/l) at baseline. During testosterone therapy, adiponectin levels decreased in the testosterone group compared with placebo group ($b=−1.3$ mg/l, $P=0.001$), corresponding to ~13% decrease in adiponectin levels (Fig. 3).

No significant univariate correlations were observed between baseline adiponectin levels in all participants and WC, TFM, CFM, SAT, or VAT or BT. Similarly, no significant correlations were observed between changes in adiponectin levels and changes in WC, TFM, CFM, SAT, VAT, or testosterone levels in participants treated with testosterone.

**Discussion**

We conducted a randomized, double-blinded, placebo-controlled study on the effect of testosterone therapy in aging men with a WC $>94$ cm and defined low normal bioavailable testosterone levels (15), assessed using the gold standard testosterone analysis (22). We have previously reported that TFM decreased during testosterone therapy (2). This study extends this finding by showing that the testosterone-induced reduction in fat mass is tissue/area specific. Thus, testosterone therapy significantly decreased subcutaneous fat on the abdomen and on the lower extremities, whereas the amount of VAT remained unchanged. Our data were in agreement with a previous meta-analysis (23) and in agreement with studies on testosterone therapy in aging obese men (6, 8). However, no change in TFM was
observed in nonobese aging men during testosterone therapy (24). Data from randomized placebo-controlled studies on the effect of testosterone treatment on the regional distribution of fat are conflicting (Table 3). In accordance with our results, Svarthberg et al. (10) reported a significant decrease in SAT and no change in VAT in abdominally obese, aging men with low normal testosterone measuring regional body composition by computed tomography (CT). Five studies (6, 7, 8, 11, 24) have reported results that contradicted our data. Two studies on middle-aged obese men reported a significant decrease in VAT during testosterone therapy (7, 11). In one of the studies, the authors used an oral preparation of testosterone and reported no significant change in testosterone levels or LBM (7). In the other study, an increase in testosterone levels was reported; however, no change was found in fat mass or LBM (11), suggesting that the testosterone dose had been insufficient to obtain the expected increase in LBM during testosterone therapy (23). Hence, it could be speculated that the decrease in VAT during testosterone therapy reported by Marin et al. (7, 11) may be related to other changes during the experiment. A study on nonobese aging men (24) reported a significant increase in VAT in the placebo group during testosterone therapy. VAT, however, did not decrease in the testosterone-treated group. No change was reported in TFM, SAT, or TFA and the lack of change may be attributable to the fact that the participants in this study were nonobese (24). A decrease in VAT, SAT, and TFA was reported in overweight, aging men during testosterone therapy (8) but the decrease in VAT and SAT was, however, not significant when compared with placebo. The lack of significant effect on SAT and VAT compared with placebo may be due to the relatively short duration of the study, i.e. MRI scans were performed after 12 weeks of treatment, and a relatively low dose of testosterone was used. Finally, a large study found no change in SAT or VAT during testosterone therapy measured by ultrasound; however, ultrasound is relatively insensitive and may fail to detect small changes in SAT and VAT (6).

Genetic studies on the androgen receptor (AR) support our findings. The number of CAG repeats (CAGn) within the CAG repeat polymorphism of the androgen receptor has been inversely correlated with the activity of the receptor (25, 26). In healthy young men, TFM increased whereas LBM decreased with increasing CAGn, suggesting a direct effect of longer CAG repeats on the testosterone-induced increase in LBM and decrease in TFM (27). Nielsen et al. (27) also reported a significant positive association between increasing CAG repeats and subcutaneous fat on the abdomen and thigh; however, no significant association with VAT was seen. Accordingly, we reported a significant decrease in SAT and TFA, whereas VAT remained unchanged.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Randomized controlled trials on the effect of testosterone therapy on body composition, adiponectin, and insulin resistance (IR).</th>
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<td>Reference</td>
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The adipocyte-derived hormone adiponectin may be a key component in the associations between excess adiposity, insulin resistance, inflammation, and cardiovascular disease (28). A decrease in the circulating levels of adiponectin has been shown to contribute to the development of type 2 diabetes mellitus and the metabolic syndrome (29). In our study, we found a 13% decrease in adiponectin levels during testosterone therapy. A few randomized double-blinded, placebo-controlled studies have investigated the effect of testosterone therapy on adiponectin levels (Table 1). In accordance with our results, two studies on testosterone therapy in aging men with diabetes (12) and in aging healthy men (13) using supraphysiological doses of testosterone reported a significant decrease in adiponectin of ~20–25%. In contrast, a study of 36 months’ duration on sedentary aging men reported no change in adiponectin levels during testosterone therapy (14).

The mechanism of the decrease in adiponectin levels during testosterone therapy is not clear. Theoretically, the negative effect of testosterone therapy on adiponectin levels may be mediated by the change in regional fat. Fat on the lower extremities has been positively associated with adiponectin levels and insulin sensitivity whereas SAT has been negatively associated with adiponectin levels in young (4) and in aging men (30, 31). In Prader–Willi syndrome, patients have increased levels of adiponectin compared with weight-matched controls due to increased peripheral distribution of fat and a relatively low amount of central fat. Hence, the decrease in adiponectin may be caused by a decrease in fat on the lower extremities (LEFM and TFA), which is not balanced by the decrease in SAT. Alternatively, testosterone may have a direct suppressive effect on adiponectin levels. Compared with eugonadal men, hypogonadal young men have higher adiponectin levels and adiponectin levels are reduced during testosterone treatment (32). Animal and human fat cell studies have reported increased lipolysis during testosterone exposure (33, 34), suggesting that the effect of testosterone therapy on adiponectin levels could be mediated through increased lipolysis (35). Accordingly, β-adrenergic induction of lipolysis decreased adiponectin secretion in adipocytes (36).

Adiponectin circulates in three different molecular forms: high molecular weight (HMW), medium molecular weight, and low molecular weight. In particular, HMW adiponectin has gained interest as some studies suggest that this molecular form exerts the strongest biological effects (37). However, we did not measure HMW adiponectin as we have previously shown that the strength of the inverse correlation between serum testosterone and the fraction of HMW was similar to that of serum testosterone and total adiponectin (r values of −0.43 and −0.47 respectively) (38). Consequently, as both measures of adiponectin action showed the same relationship with serum testosterone, we chose to document the effect of testosterone on adiponectin by measurements of total adiponectin.

Interestingly, we reported a decrease in adiponectin levels and an increased basal lipid oxidation during testosterone therapy (2). It has been suggested that adiponectin exerts its insulin-sensitizing effect in part through an increase in lipid oxidation (28). Recombinant globular adiponectin has been shown to increase muscle lipid oxidation in mice via a pathway that involves AMP-activated kinase and acetyl-CoA carboxylase (39). However, as adiponectin levels were decreased during testosterone therapy, our data did not support that the reported increase in lipid oxidation (2) was elicited by adiponectin. Hypothetically, testosterone therapy may have an independent effect on lipid oxidation in muscle; however, further studies are warranted.

In summary, we extended our previous findings by providing further support for the notion that testosterone therapy may have positive as well as negative effects on important metabolic measures. Thus, we reported previously that testosterone therapy had positive effects, e.g. increased LBM, decreased TFM, and increased basal lipid oxidation. However, HDL-cholesterol was decreased during testosterone therapy (2) in agreement with a meta-analysis (40) and a recent systematic review (41); however, in a meta-analysis, Isidori et al. (23) found no change in HDL-cholesterol. Overall insulin sensitivity was unchanged during testosterone therapy in men with low normal testosterone levels (2). However, two recent large studies on testosterone therapy in men with metabolic syndrome and type 2 diabetes mellitus reported a significant decrease in insulin resistance assessed by a surrogate marker of insulin resistance (HOMA-IR) (42, 43).

We demonstrated that although testosterone therapy decreased subcutaneous fat on the lower extremities and abdomen, no effect was observed on VAT and the insulin-sensitizing adipokine, adiponectin, was decreased. A decrease in HDL-cholesterol (2) and in adiponectin levels has been associated with increased risk of cardiovascular disease. It is important to emphasize that our findings may have been different in men with overt hypogonadism, i.e. men with low testosterone levels and symptoms of hypogonadism, and we cannot exclude a significant effect of testosterone therapy on insulin sensitivity in overt insulin resistant men, e.g. type 2 diabetes mellitus.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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