Testosterone Supplementation Improves Carbohydrate and Lipid Metabolism in Some Older Men with Abdominal Obesity

Sattler FR1*, He J2, Chukwuneke J3, Kim H3, Stewart Y1, Colletti P1, Yarasheski KE3 and Buchanan TA1

1Department of Medicine, University of Southern California, Los Angeles, CA, USA
2Department of Preventive Medicine, University of Southern California, Los Angeles, CA, USA
3Department of Radiology, University of Southern California, Los Angeles, CA, USA

*Corresponding author: Fred R Sattler, MD, Professor of Medicine and Biokinesiology, Chief of Infectious Diseases, USC Keck School of Medicine, 2020 Zonal Avenue (IRD Building, Room 436), Los Angeles, CA 90089, USA, Tel: 323-226-4635; Fax: 323-226-7726; E-mail: fsattler@usc.edu

Abstract

Background/Objectives: The effects of testosterone supplementation on carbohydrate and lipid metabolism in obese older men are uncertain. We conducted a single-arm open-label prospective pilot study to investigate the effects of testosterone supplementation on central and peripheral insulin sensitivity in older men with upper body obesity and insulin resistance.

Subjects/Methods: Twenty men (62-78 years-old) with morning testosterone levels <13.9 nmol/L (400 ng/dL), waist circumference ≥ 102 cm, and HOMA-IR ≥ 4.0 or HgbA1C 5.7-6.4% applied transdermal testosterone (10 mg) daily for 20 weeks. Insulin sensitivity (Si) was determined by a 2-stage glucose clamp, liver and intramyocellular lipid by 1H-MR spectroscopy and body composition by DEXA.

Results: Testosterone supplementation significantly reduced total fat (-1.9 ± 2.4 kg, p=0.002), trunk fat (-1.3 ± 1.4 kg, p=0.0007) and extremity fat (-0.7 ± 1.1 kg, p=0.01), and increased extremely lean tissue (+1.3 ± 1.4 kg, p=0.0006). Whole body (WB) Si improved by 21% (0.76 ± 1.57 dL/min per μU/mL, p=0.04) and insulin-stimulated glucose uptake (Rd) by 24% (0.91 ± 1.74 dL/min per μU/mL, p=0.03). Improvements in glucose kinetics were limited to men with reductions in trunk and extremity fat greater than median declines for the entire group. Reductions in intramyocellular lipid were associated with improvements in WB Si (p=0.04) and Rd (p=0.03). Change in Rd accounted for 90% of the change in WB Si. Hepatic glucose output and liver lipid/H2O were unchanged (p=0.05). Multivariable analyses revealed that reductions in extremity fat, trunk fat, and FFA levels during the clamp accounted for 45% (p=0.004), 31% (p=0.002) and 8% (p=0.04) of respective changes in Rd. Triglycerides decreased by -0.40 ± 0.67 mmol/L (p=0.02), LDL-C by 0.35 ± 0.57 mmol/L (p=0.02), and HDL-C by -0.14 ± 0.19 mmol/L (p=0.004).

Conclusions: Testosterone supplementation that resulted in greater reductions in regional adiposity was associated with improved insulin sensitivity, lower LDL-C and fasting triglycerides, but lower HDL-C. Placebo controlled trials need to further examine the potential cardiometabolic risks/benefits of androgen supplementation for older men with low testosterone levels, central obesity, and insulin resistance.

Keywords: Obesity; Insulin resistance; Testosterone; Aging; Metabolism

Introduction

Guidelines for testosterone supplementation therapy in older men with biochemical and clinical manifestations of hypogonadism are available in the United States and Europe [1,2]. Many of these men also have upper body obesity, as nearly 70% of Americans are obese or overweight [3], the prevalence of pre-diabetes has doubled [4] and these increase with aging. Obesity and its associated insulin resistance are components of the metabolic syndrome [5], which are associated with heart attack, stroke, peripheral vascular disease and amputation, and risk for type 2 diabetes. Obesity and insulin resistance, as well as the full metabolic syndrome, are associated with lower testosterone levels [6-9]. Low testosterone levels correlate inversely with the magnitude of abdominal obesity [10-12]. Further, hypogonadal men have an increased risk for metabolic syndrome [13]. Thus, it is important to understand how testosterone supplementation when it is administered according to treatment guidelines affects components of
the metabolic syndrome in older hygonadal men with upper body obesity and insulin resistance.

The effects of testosterone administration on upper body obesity have been mixed [14], as have the effects of testosterone treatment on insulin sensitivity [15-17]. Those differences may be related to the dose of testosterone, changes in blood and tissue androgen levels, or duration of therapy [16,18]. In castrated rats, restoration of testosterone to physiologic levels improves insulin sensitivity, but supraphysiologic levels worsen insulin resistance [19]. Therefore, we administered transdermal testosterone to older men with low morning testosterone levels and components of the metabolic syndrome, and investigated the effects of treatment on upper body obesity, insulin resistance and serum lipids. We determined which participants were most likely to respond favorably to the intervention and whether the effects were largely central (affecting hepatic glucose output and hepatic lipid content) or peripheral (affecting glucose disposal and intramyocellular lipid content [IMCL]). We postulated that men with large declines in trunk or extremity fat would have the greatest improvements in insulin sensitivity. To achieve these aims, men were phenotyped in a clinical trials research unit using a two-stage hyperinsulinemic-euglycemic clamp and proton magnetic resonance spectroscopy.

Subjects and Methods

Study design

Older men with low morning total testosterone levels typical of their age, upper body obesity, and evidence of insulin resistance applied 10g of 1% transdermal testosterone gel (Androgel, Solvay Pharmaceuticals Inc.) daily for 20 weeks (open label). This was a pilot project that utilized complex and intensive research measurements, so that each subject served as his own control with the expectation that outcomes would be sizable and beyond what would occur by chance. Three-day food diaries and exercise questionnaires were collected at baseline, week 10 and week 20 to confirm that subjects did not change their total energy or macronutrient intake and activity levels by more than 10% during the study. Blood tests for complete cell counts, chemistries, prostate specific antigen (PSA), and testosterone were done at baseline, weeks 10 and 20 and 3-months after completion of study therapy to monitor safety.

Study participants

Participants provided written informed consent approved by the USC Institutional Review Board. Eligible men were 60 years or older, with morning total testosterone concentrations <13.9 nmol/L (400 ng/dL) typical of older men with low levels and often with symptoms of hyponadism, waist circumference ≥ 102 cm (average of 3 measurements by a bionutritionist), and evidence consistent with insulin resistance; HgbA1c of 5.7-6.4% [20] or HOMA-IR ≥ 4.0, based on values above the 50th percentile in our prior studies of obese men ≥ 60 years old. Exclusion criteria included fasting plasma glucose ≥ 6.93 mmol/L (126 mg/dL) or history of diabetes, PSA ≥ 4.0 μg/L, hematocrit ≥ 50%, AST ≥ 2X the upper-limit-of-normal, clinical evidence of a concurrent inflammatory process (rheumatoid arthritis, active infection, etc), vigorous exercise >30 min/week (existing stable walking programs were allowed) or plans to reduce energy intake.

Two stage hyperinsulinemic-euglycemic clamp

Clamp procedure: Two stage hyperinsulinemic-euglycemic clamp studies were conducted to determine whole body (WB) insulin sensitivity (SI) and effects of insulin on the rates of peripheral glucose disposal (Rd) and hepatic glucose output (HGO) [21]. Briefly, regular insulin was infused continuously in a right forearm vein at 4 ml/hr (0.3 mUnits/kg/min) during minutes 0-120; this rate was increased to 13.3 ml/hr (1.0 mUnits/kg/min) during minutes 120-240. During the clamp, blood glucose was maintained at 4.72-5.00 mmol/L (85-90 mg/dL) by a variable infusion of 20% dextrose, based on bedside glucose levels (YSI analyzer) determined every 5 min using specimens collected from a dorsal left hand vein heated by a Gaymar T-pump (95-100°F). At -30, -20, -10, +100, +110, +120 and +220, +230, and +240 minutes, samples were collected from the left hand vein, processed immediately for plasma and stored at -80°C for batch analysis.

Calculation of insulin sensitivity: Clamp insulin sensitivity index (SI) was determined as reported previously [21]. Whole-body (WB) SI was calculated as ΔGinf / (ΔIns X Glu-ss), where ΔGinf and ΔIns are the respective increments in the dextrose infusion rate and plasma insulin concentrations between basal (average at -30, -20, -10 min) and steady state (average of +220, +230, +240 min); Glu-ss is the steady-state glucose concentration. Peripheral insulin sensitivity was determined by the rate of glucose disposal (Rd); calculated as ARd / (ΔIns X Glu-ss), during hours three and four of the clamp. Hepatic insulin sensitivity was calculated to determine the effects of insulin on hepatic glucose output (HGO), where SI HGO = ΔHGO / (ΔIns X Glu-ss), from basal to steady state at the end of hour 2.

Hormone Assays: Screening testosterone levels were quantified by liquid chromatography-tandem mass spectrometry (Quest Diagnostics, San Juan Capistrano). Samples for insulin, glucose, and free fatty acids (FFAs) collected during the glucose clamps were batch tested at the USC Clinical Trials Unit (CTU) Core Laboratory. Plasma glucose levels were determined using an YSI glucose analyzer (Yellow Springs, OH) with a coefficient of variation (CV) = 2% and detection range=0-50.0 mmol/L (0-900 mg/dL). Insulin levels were determined by an automated enzyme immunoassay ( Tosoh AIA 600 II analyzer, Tosoh Bioscience, Inc., South San Francisco, CA; sensitivity=0.31 μU/mL, inter-assay CV=6.1% and intra-assay CV=4.8%). HOMA-IR was calculated as fasting insulin (μU/mL) X fasting glucose (mM)/22.5. FFA levels were determined in duplicate at the CTU Core Laboratory by an automated immunoassay method (Wako Diagnostics, Richmond, Virginia); inter- and intra-assay CVs were 0.75% with a sensitivity of 0.0014 mEq/L, respectively.

Body composition

Dual Energy X-ray Absorptiometry (DEXA): Total, trunk, and appendicular lean tissue and fat mass were quantified using a Hologic QDR 4500 Discovery Scanner (Hologic Inc, Bedford, MA). Pre and post-treatment scans were blindly analyzed by a single DEXA-certified technician using software pre-installed on the machine.

Proton magnetic resonance spectroscopy: Proton (1H) MR spectroscopy was performed with either 1.5T or 3T General Electric scanners. For evaluation of the liver fat fraction, the proton signal was obtained using the torso coil with the 8cm PRESS (point resolved spectroscopy) voxel in the right posterior superior segment of the liver (32 average, 35 ms echo time, 2s repetition time). For assessment of the intramyocellular lipid (IMCL), the proton signal was obtained...
Results

At baseline, the participants were representative of older men with upper body obesity (Table 1). After testosterone supplementation, the median change in trunk fat was -0.86 kg; 10 men lost ~1.0 kg or more, and 10 men lost <0.8 kg. Baseline characteristics for the two groups were generally similar, but the group with larger reductions in trunk fat had higher baseline HOMA-IR, an index of insulin resistance. Morning serum testosterone levels increased from baseline (10.1 ± 2.78 nmol/L; 292 ± 80 ng/dL) to week 20 (21.5 ± 11.1 nmol/L; 619 ± 320 ng/dL; p=0.0002). Week 20 testosterone levels are typical of those for young men.

Changes in insulin sensitivity index

During the glucose clamp, whole body (WB) Si increased 0.76 ± 1.57 dL/min per μU/mL (p=0.04) and Rd increased 0.91 ± 1.74 dL/min per μU/mL (p=0.03, Table 2). However, HGO did not change significantly. HOMA-IR declined marginally (p=0.06).

Fasting lipids and free fatty acid levels

Significant reductions in fasting triglycerides, total cholesterol, and LDL-C (p ≤ 0.02, Table 2) levels occurred, but HDL-C also declined, albeit modestly by 0.14 ± 0.19 mmol/L (-5.3 ± 7.2 mg/dL; p=0.004). Basal free fatty acid levels (FFA) did not change over the 20 week treatment period (Table 2). Levels of FFA did correlate negatively with baseline WB Si, HGO, and Rd (p= -0.63, -0.58, -0.59, respectively, p ≤ 0.02 for each).

Relationship of change between Si and other parameters

Changes in total, trunk and extremity fat mass as well as percentage of total fat correlated with changes in WB Si (rho = -0.45 to -0.52; p ≤ 0.05) and for Rd (rho = -0.46 to -0.59, p ≤ 0.02) but not with HGO (data not shown). Changes in lean tissue mass and hepatic lipid were not related to any Si measure. In 12 of 15 participants, IMCL/Cr declined (mean = -41%) and corresponded with mean increases in WB Si of 0.82 (p=0.06) and Rd of 0.93 dL/min per μU/mL (p=0.04).

Factors associated with change in whole body Si and Rd

WB Si and Rd each improved significantly for men with reductions in trunk and extremity fat greater than the respective median changes (Figure 1). The effects of change in total fat on WB Si and Rd were largely due to the summation of changes in trunk plus extremity fat since together they consisted of almost 100% of the changes in total fat.
Univariable analyses were conducted to assess potential contributors to change in WB Si that included baseline HOMA-IR, Si WB, and fat mass (total and regional) as well as changes in Si Rd, Si HGO, total and regional fat and lean tissue, IMCL/Cr, hepatic lipid/H\textsubscript{2}O, FFAs, and fasting lipids. Those with \( p \leq 0.30 \) were included in multivariable models. Two models best described change in WB Si (Table 3). In one model, Rd accounted for 90% (\( p<0.0001 \)) and Si HGO accounted for 9% (\( p<0.0001 \)) of the change in WB Si. If only Rd and HDL-C were in the model, Rd still accounted for 90% (\( p<0.0001 \)) and HDL-C 3% (\( p=0.01 \)) of the change in WB Si. Thus, Rd accounted for most of the improvements in WB Si. To evaluate contributing factors for Rd, we used the same variables for univariable analyses to investigate potential contributors to change in WB Si. The multivariable model that included change in extremity fat, trunk fat, and FFAs, accounted for 45% (\( p=0.004 \)), 31% (\( p=0.002 \)) and 8% (\( p=0.04 \)) of the respective change in Rd (Table 3).

### Table 1: Study Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total Subjects</th>
<th>( \Delta ) inTrunk fat &gt;0.86 kg (^a)</th>
<th>( \Delta ) inTrunk fat &lt;0.86 kg</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>n = 20</td>
<td>67.5 (62.0, 78.0)</td>
<td>67.0 (62.0, 78.0)</td>
<td>68.0 (63.0, 77.0)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>n = 10</td>
<td>105 (85, 137)</td>
<td>113 (89, 137)</td>
<td>100 (85, 127)</td>
</tr>
<tr>
<td>Family history of diabetes</td>
<td>n = 10</td>
<td>4 (20%)</td>
<td>1 (10%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Smoking ever/currently</td>
<td>n = 10</td>
<td>14 (70%), 0 (0%)</td>
<td>9 (90%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>On treatment for lipid disorder</td>
<td>n = 10</td>
<td>12 (60%)</td>
<td>7 (70%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>On treatment for hypertension</td>
<td>n = 10</td>
<td>10 (50%)</td>
<td>6 (60%)</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>n = 20</td>
<td>32.0 (27.3, 41.7)</td>
<td>34.8 (29.7, 41.7)</td>
<td>30.6 (27.3, 39.9)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>n = 10</td>
<td>114 (104, 122)</td>
<td>117 (104, 122)</td>
<td>109 (104, 121)</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>n = 10</td>
<td>130 (110, 173)</td>
<td>136 (115, 156)</td>
<td>129 (110, 173)</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>n = 10</td>
<td>5.86 (4.94, 6.66)</td>
<td>6.24 (4.94, 6.65)</td>
<td>5.83 (5.05, 6.66)</td>
</tr>
<tr>
<td>Fasting triglycerides, mmol/L</td>
<td>n = 10</td>
<td>1.59 (0.72, 4.87)</td>
<td>1.60 (0.92, 4.87)</td>
<td>1.52 (0.72, 2.45)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>n = 10</td>
<td>4.61 (3.00, 6.73)</td>
<td>4.92 (3.47, 5.57)</td>
<td>4.40 (3.00, 6.73)</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>n = 10</td>
<td>3.11 (0.98, 5.00)</td>
<td>3.13 (1.61, 3.76)</td>
<td>2.73 (0.98, 5.00)</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>n = 10</td>
<td>1.06 (0.62, 1.76)</td>
<td>1.09 (0.78, 1.76)</td>
<td>0.98 (0.62, 1.42)</td>
</tr>
<tr>
<td>HgbA1C, %</td>
<td>n = 10</td>
<td>5.9 (5.3, 6.3)</td>
<td>6.0 (5.3, 6.3)</td>
<td>5.9 (5.5, 6.1)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>n = 10</td>
<td>4.81 (1.44, 9.32)</td>
<td>6.47 (4.35, 9.32)</td>
<td>4.13 (1.44, 8.06)</td>
</tr>
<tr>
<td>AM total testosterone, nmol/L</td>
<td>n = 10</td>
<td>10.6 (5.2, 13.9)</td>
<td>9.6 (5.2, 13.4)</td>
<td>11.0 (5.9, 13.9)</td>
</tr>
</tbody>
</table>

\( a \). Median change in trunk fat = -0.86 kg; b. median (minimum, maximum); c. for mg/dL, divide by 0.0555; d. for mg/dL, divide by 0.0113; e. for mg/dL, divide by 0.0259; f. for ng/dL, divide by 0.0347

**Changes in body composition**

After 20 weeks of testosterone replacement average total body mass was unchanged (Table 2). Total, trunk and extremity fat mass declined 1.9 ± 2.4 kg (maximum=-7.4 kg), 1.3 ± 1.4 kg (maximum=4.6 kg) and 0.7 ± 1.1 kg (maximum=3.0 kg), respectively (\( p \leq 0.01 \) for each). IMCL/Cr ratios decreased 28 ± 35% (maximum=-85%; \( p=0.008 \)) but hepatic lipid/H\textsubscript{2}O was unchanged. Total LBM increased 2.1 ± 2.0 kg (maximum=7.1 kg, \( p=0.0002 \)) as did extremity LBM by 1.3 ± 1.4 kg (maximum=4.8 kg, \( p=0.0006 \)).

**Adverse effects from testosterone therapy**

Treatment was generally well-tolerated and there were no serious adverse events. Hematocrit and PSA did not increase significantly whereas American Urological Association urinary obstruction scores declined (ANOVA \( p=0.04 \)). Framingham 10-year cardiovascular risk scores at baseline (18.7 ± 6.01%) remained similar over the ensuing 32 weeks (ANOVA \( p=0.90 \)).

**Discussion**

In this mechanistic 20 week pilot study, transdermal testosterone (10 g of a 1% gel daily) supplementation in older obese men achieved serum testosterone levels typical of men in their 3rd and 4th decades of...
life. This regimen reduced total fat mass by an average of 1.9 kg and reciprocally increased total lean mass by 2.1 kg, similar to changes reported in treatment studies [25,26].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Change at Week 20</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Composition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body mass, kg</td>
<td>104.5 (84.4, 133.6) a</td>
<td>0.3 (-4.8, 4.1)</td>
<td>0.77</td>
</tr>
<tr>
<td>Total fat mass, kg</td>
<td>34.6 (24.3, 57.1)</td>
<td>-1.4 (-7.4, 2.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Trunk fat mass, kg</td>
<td>19.3 (13.6, 30.0)</td>
<td>-0.9 (-4.6, 0.2)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Extremity fat mass, kg</td>
<td>12.9 (9.1, 27.6)</td>
<td>-0.7 (-3.0, 2.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>% total body fat</td>
<td>31.5 (23.9, 42.7)</td>
<td>-1.8 (-6.5, 0.1)</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>Hepatic Lipid/H2O (n=19) b</strong></td>
<td>0.11 (0.01, 1.13)</td>
<td>-36% (-84, 127)</td>
<td>0.12</td>
</tr>
<tr>
<td>IMCL/Cr (n=15) c</td>
<td>5.8 (0.2, 30)</td>
<td>-30% (-85, 43)</td>
<td>0.008</td>
</tr>
<tr>
<td>Extremity lean mass, kg</td>
<td>29.1 (21.2, 34.1)</td>
<td>1.2 (-1.1, 4.8)</td>
<td>0.0006</td>
</tr>
<tr>
<td><strong>Total lean body mass, kg</strong></td>
<td>64.3 (47.1, 75.0)</td>
<td>1.4 (-0.8, 7.1)</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>Insulin Sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole body (WB) d</td>
<td>2.93 (1.07, 8.51)</td>
<td>1.05 (-2.44, 3.73)</td>
<td>0.04</td>
</tr>
<tr>
<td>Hepatic glucose output (HGO) d</td>
<td>2.89 (0.03, 7.83)</td>
<td>-0.16 (-2.58, 7.49)</td>
<td>0.59</td>
</tr>
<tr>
<td>Rate of disposal (Rd) d</td>
<td>2.93 (1.07, 8.51)</td>
<td>1.16 (-3.08, 3.85)</td>
<td>0.03</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.55 (0.64, 11.4)</td>
<td>-0.75 (-6.94, 4.18)</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Plasma Lipids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting triglycerides, mmol/L e</td>
<td>1.59 (0.72, 4.87)</td>
<td>-0.33 (-2.70, 0.43)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L f</td>
<td>4.61 (3.00, 8.73)</td>
<td>-0.52 (-1.74, 0.88)</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L f</td>
<td>3.11 (0.98, 5.00)</td>
<td>-0.23 (-1.45, 0.70)</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L f</td>
<td>1.06 (0.62, 1.76)</td>
<td>-0.10 (-0.60, 0.05)</td>
<td>0.004</td>
</tr>
<tr>
<td>Basal free fatty acids, mEq/</td>
<td>0.35 (0.24, 0.53)</td>
<td>-0.01 (-0.22, 0.21)</td>
<td>0.97</td>
</tr>
<tr>
<td>Δ in Clamp FFA g (0-4 hours)</td>
<td>0.30 (0.17, 0.41)</td>
<td>-0.01 (-0.12, 0.17)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

a. Median (minimum, maximum). b. Only 19 had proton spectrometry at baseline and week 20. c. In four participants, IMCL and EMCL could not be clearly separated at either baseline or week 20. d. units are dL/min per μU/mL; e. for mg/dL, divide by 0.0113; f. for mg/dL, divide by 0.0259; g. free fatty acids

Table 2: Change in Cardiometabolic Variables during Study Treatment

Insulin sensitivity for whole body and peripheral glucose disposal improved significantly, but only in men that lost more than the median reduction of adipose tissue (>0.86 kg trunk fat or >0.67 kg extremity fat). Total cholesterol, LDL-C and fasting triglycerides decreased across the study population, although HDL-C declined modestly by 0.1 mmol/l (3.9 mg/dL). Decreases in HDL-C or adiponectin as reported could be associated with increases in inflammation [27]. Yet, on balance testosterone supplementation had favorable effects on three of the major components of the metabolic syndrome including LDL-C and triglycerides in most of the older men.

A primary goal was to identify the principal target tissue (central versus peripheral) of the insulin sensitizing actions of testosterone in these older, centrally obese men. Improvements in whole body Si were driven largely by enhanced peripheral glucose disposal (~90%), and 45% of the improvements in Rd were associated with reductions in extremity fat mass. This suggests that testosterone primarily affects peripheral insulin sensitivity. Improvements in whole body Si and Rd were not related to enhancements in muscle mass despite increases in extremity lean mass (~1.2 kg), which is largely muscle. However, testosterone treatment was associated with reductions in IMCL. This is consistent with reports indicating that IMCL is more tightly associated with insulin resistance than BMI, waist-to-hip ratio or total body fat [28,29]. Elevated IMCL probably reflects accumulation of long chain acyl-CoA. This can activate protein kinase C, lead to serine (rather than tyrosine) phosphorylation on insulin response substrate-1 (IRS-1), and limit PI-3 kinase activation which is necessary for translocation of GLUT4 to the cell membrane for intracellular transport of glucose [30]. Thus, reducing IMCL should improve...

muscle glucose disposal. Further, in vitro data indicate that testosterone directly up-regulates IRS-1 signaling and GLUT4 transporter activity, as expected to occur in muscle. Finally, testosterone increases mitochondrial function and oxidative phosphorylation capacity in muscle, which may result in improved insulin sensitivity [31,32]. Thus, testosterone may regulate muscle metabolism by several different mechanisms that improve whole body insulin sensitivity and the peripheral rate of glucose disposal.

Table 3: Predictors of Change in Whole Body Si and Rd

<table>
<thead>
<tr>
<th>Variable</th>
<th>R² (p-value)</th>
<th>R² (p-value)</th>
<th>R² (p-value)</th>
<th>R² (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Si Rd</td>
<td>90% (0.002)</td>
<td>90% (&lt;0.0001)</td>
<td>90% (&lt;0.0001)</td>
<td>45% (0.004)</td>
</tr>
<tr>
<td>Δ Si HGO</td>
<td>42% (&lt;0.0001)</td>
<td>9% (&lt;0.0001)</td>
<td>3% (0.01)</td>
<td>31% (0.002)</td>
</tr>
<tr>
<td>Δ Total Fat Mass</td>
<td>7% (0.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ Extremity Fat</td>
<td>6% (0.27)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ Triglycerides</td>
<td>8% (0.24)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ HDL-cholesterol</td>
<td>9% (0.21)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Other variables included baseline total and regional lean and fat mass, HOMA-IR, and WB Si, and change in trunk fat, total lean body mass (LBM), extremity LBM, hepatic lipid by magnetic resonance spectroscopy (MRS), intramyocellular lipid (IMCL) by MRS, free fatty acids (4h minus basal), and LDL-cholesterol.

b. Variables with p≤0.3 from univariable regression analysis are shown.
c. All variables are change from baseline to week 20.
d. Model contains only change in Si for Rd and HGO. Other variables were not significant.
e. Model contains only change Si Rd and HDL-C. Other variables were not significant.
f. Model contains only change extremity fat, trunk fat, and free fatty acids at 4 hours minus basal in the clamp. Other variables were not significant.

Total body mass did not change due to off-setting increases in lean mass and reductions in fat mass. At least in vitro, testosterone can direct pluripotent stem cells down a myogenic lineage, inhibit adipogenesis [39], and inhibit differentiation of pre-adipocytes into mature fat cells; this occurs by inhibition of β-catenin nuclear translocation and its downstream effects on Wnt signaling necessary for differentiation of adipocytes [40]. These mechanisms support our observed in vivo actions of testosterone—comparable alterations in lean versus fat mass as reported by others [18,41]. Taken together, it appears that testosterone supplementation improved peripheral insulin sensitivity through multiple biochemical and cell-signaling pathways that regulate muscle and adipose tissue metabolism.

The goal of this study was not to investigate the effects of testosterone for treatment of Metabolic Syndrome but to determine the effects of treatment, when used according to published guidelines, on components of the Syndrome in older men with upper body obesity. In these men, baseline morning testosterone levels were 13.9 nmol/L (<400 ng/dL) which is higher than the commonly used breakpoint for treatment of symptomatic hyponogadism (10.4 nmol/L [<300 ng/dL]). This threshold was determined with older platform immunoassays that were regularly used when treatment guidelines were established. However, with newer analytical platforms, like LC-MS/MS used in this study, the normal reference range is >12.08 nmol/L (>349 ng/dL) [42], and using GC-MS, the threshold at which...
symptoms associated with low testosterone are more common is <11 nmol/L (<320 ng/dL) [43]. Most participants in our study had levels below these thresholds and, thus, were generally similar to many men who would be candidates to receive clinically indicated testosterone replacement, if they are symptomatic.

The treatment dose and duration achieved levels typical of young men [of at least 20.8 nmol/L (600 ng/dL)] in 17 (85%) participants and provided improvements in components of the Metabolic Syndrome. Further, there were no important adverse events and the 10 year Framingham cardiovascular disease risk scores, which were just under the high risk category of 20%, remained unchanged after 20 weeks of testosterone replacement and for the next three months after treatment was discontinued.

This study is unique with respect to phenotyping methods (glycemic clamp, IMCL, hepatic lipid) and the eligibility criteria: older men with abdominal obesity and components of the Metabolic Syndrome. Four studies of testosterone supplementation in older men found no beneficial effects on surrogate measures of SI, but none used the glycemic clamp and older men were not selected for upper body obesity [44-47]. Whereas, two studies have shown improvements in SI by glycemic clamps in obese middle aged men (younger than our men) [15,48]. One recent 6-month study using a different 50-100 mg testosterone gel in 62-72-year-old men with “obesity” showed no improvement in Rd versus placebo [35]. However, some of their men did not have upper body obesity (waist circumference 94-101 centimeters) by metabolic syndrome criteria. Importantly, trunk and lower extremity fat did not decrease significantly, which may explain the lack of improvement in Rd [49]. Whereas, in our study significant improvements in WB SI and Rd occurred only in centrally obese men who had sizeable reductions in trunk, extremity, and intramuscular fat content. Thus, relatively large reductions in these fat compartments may be needed to achieve improvements in insulin sensitivity in older men with upper body obesity and components of the Metabolic Syndrome.

There were several limitations. First, the number of participants (N=20) was relatively small and there was no control group. The significant reciprocal increases in lean tissue, including extremity muscle mass, and significant decreases in total and trunk fat would not have occurred with diet alone or with aerobic exercise, and none of our participants were doing resistance exercise to increase lean mass. Three-day food diaries and exercise questionnaires (data not shown) did not change during or for the three months after therapy. Thus, changes in body composition that affected SI can be reasonably attributed to treatment with testosterone. Second, we did not quantify changes in abdominal fat compartments (VAT and SAT), which presumably decreased based on reductions in trunk fat by DEXA. We recognize that reductions in VAT may decrease release of pro-inflammatory cytokines, which should facilitate pathways enhancing insulin sensitivity. Finally, our study was relatively short with treatment lasting only 20 weeks. We can only conjecture as to whether longer therapy (e.g. 1-3 years) would result in greater reductions in fat mass and IMCL and whether this would be associated with even more favorable effects on clinically important measures of metabolism. Regardless, we believe that our results using the two stage glucose clamp for HGO and Rd along with MRS for quantifying hepatic lipid and IMCL provide compelling direct and inferential evidence that testosterone improved insulin sensitivity by largely peripheral effects, but changes in VAT per se and IL-6 and TNFα levels should be quantified in future studies.

The long term safety of testosterone replacement remains uncertain as adverse cardiovascular events have been reported in some high risk populations [50-53]. Results of this study are not intended to support or promote use of testosterone for treatment of aging or other perceived benefits but to understand the cardiometabolic effects of testosterone if it is prescribed according to standardized guidelines. Further, the long term safety of testosterone supplementation for low levels in symptomatic persons has not been ascertained [54-56]. Thus, before prescribing testosterone for symptoms of hypogonadism in older men, the full spectrum of potential risks and benefits should be considered for each patient. As part of these considerations, our results suggest that treatment may beneficially affect important components of metabolism in some patients, especially in those with sizable amounts of upper body fat mass. Based on results of our prior studies and those of others [18,25,26], it is unclear why testosterone supplementation produces larger reductions in fat mass up to 6-7 kg in some men with very little change in other men. Regardless, larger, placebo controlled studies are needed to confirm these promising preliminary findings and the long term safety of testosterone supplementation. Finally, it will be important to determine why certain men may have substantial reductions in upper body fat and muscle lipid along with improvements in insulin sensitivity and serum lipids, while these are not affected in other obese older men receiving testosterone.

Acknowledgements

The authors gratefully acknowledge the contributions of the study participants and research staff of the USC Clinical Trials Unit without whom this project would not have been successful.

Funding for Study: Financial support for this study was provided from R01 AG18169 (Sattler, FR; Principal Investigator) and NCCR GCRC M01 RR00043. KEY was supported by P41 GM103422, P30 DK056341, and P30 DK020579.

Conflict of Interest: Dr. Sattler received Androlog from Solvay Pharmaceuticals Inc. which is now a component of Abbott/Abbvie. Solvay/Abbott/Abbvie provided no input for study design, had no access to the data/outcomes, and have not read or reviewed this manuscript. The authors have no other conflicts of interest.

References


