

Brief Report

Effect of Testosterone Administration on Liver Fat in Older Men With Mobility Limitation: Results From a Randomized Controlled Trial

Grace Huang,¹ Shalender Bhasin,¹ Elizabeth R. Tang,² Adam Aakil,² Stephan W. Anderson,² Hernan Jara,² Maithili Davda,¹ Thomas G. Travison,^{1,3} and Shehzad Basaria¹

¹Section of Endocrinology, Diabetes, and Nutrition and

²Department of Radiology, Boston University School of Medicine and Boston Medical Center, Massachusetts.

³Department of Biostatistics, Boston University School of Public Health, Massachusetts.

Address correspondence to Shehzad Basaria, MD, Androgen Clinical Research Unit, Section of Endocrinology, Diabetes, and Nutrition, Boston University School of Medicine, 670 Albany Street, Boston, MA02118. Email: shehzad.basaria@bmc.org

Background. Androgen receptor (AR) knockout male mice display hepatic steatosis, suggesting that AR signaling may regulate hepatic fat. However, the effects of testosterone replacement on hepatic fat in men are unknown. The aim of this study was to determine the effects of testosterone administration on hepatic fat in older men with mobility limitation and low testosterone levels who were participating in a randomized trial (the Testosterone in Older Men trial).

Methods. Two hundred and nine men with mobility limitation and low total or free testosterone were randomized in the parent trial to either placebo or 10-g testosterone gel daily for 6 months. Hepatic fat was determined by magnetic resonance imaging in 73 men (36 in placebo and 37 in testosterone group) using the volumetric method. Insulin sensitivity (homeostatic model assessment–insulin resistance) was derived from fasting glucose and insulin.

Results. Baseline characteristics were similar between the two groups, including liver volumes (1583 ± 363 in the testosterone group vs 1522 ± 271 mL in the placebo group, $p = .42$). Testosterone concentrations increased from 250 ± 72 to 632 ± 363 ng/dL in testosterone group but did not change in placebo group. Changes in liver volume during intervention did not differ significantly between groups ($p = .5$) and were not related to on-treatment testosterone concentrations. The change in homeostatic model assessment–insulin resistance also did not differ significantly between groups and was not related to either baseline or change in liver fat.

Conclusion. Testosterone administration in older men with mobility limitation and low testosterone levels was not associated with a reduction in hepatic fat. Larger trials are needed to determine whether testosterone replacement improves liver fat in men with nonalcoholic hepatic steatosis.

Key Words: Testosterone—Older men—Liver fat—Insulin resistance.

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NONALCOHOLIC fatty liver disease (NAFLD), a common condition in middle-aged and older adults, is often considered a hepatic manifestation of metabolic syndrome and is associated with increased risk of diabetes and cardiovascular disease (1). It has been proposed that NAFLD should be included in the definition of metabolic syndrome to help identify individuals at increased risk for cardiovascular disease (2).

Low circulating testosterone levels in men have been associated with obesity, type 2 diabetes, and metabolic syndrome (3,4). Testosterone administration in hypogonadal as well as eugonadal, younger and older men has been reported to decrease whole-body and visceral fat (5–7), but the effects of testosterone on hepatic fat have not been

investigated. Male mice with genetic disruption of androgen receptor demonstrate insulin resistance and hepatic steatosis, suggesting that androgen receptor signaling is important in the regulation of liver fat (8). In a recent study, parenteral administration of testosterone undecanoate significantly reduced liver enzymes in hypogonadal men with NAFLD and metabolic syndrome (9), although changes in liver fat were not measured. Accordingly, we investigated the effects of testosterone administration on liver fat using magnetic resonance imaging (MRI) in older men with low serum testosterone levels.

The Testosterone in Older Men with Mobility Limitation trial was a randomized, double-blind placebo-controlled trial designed to determine the effect of testosterone therapy

in older men with mobility limitation and low testosterone levels. Of the 209 men randomized in the parent trial, baseline and end-of-treatment MRI scans of the liver were obtained in 73 men. We employed proton-weighted T1 and T2 MRI of the liver for volumetric assessment, which has been shown to correlate with fatty liver infiltration (10–12).

METHODS

Study Design

The eligibility criteria and design of the Testosterone in Older Men trial have been published (13–15) and are described here briefly. This parallel group, placebo-controlled, double-blind randomized trial was approved by the institutional review boards of Boston University Medical Center, New England Research Institutes, Watertown, MA, and the Boston Veterans Administration Health Care System. All participants provided written informed consent.

Eligibility

The participants were community-dwelling men, aged 65 years and older, with total testosterone of 100–350 ng/dL or free testosterone of less than 50 pg/mL and mobility limitation. We excluded men with prostate cancer, lower urinary tract symptom score of more than 21, prostate-specific antigen of more than 4 ng/mL, congestive heart failure, myocardial infarction within 3 months, uncontrolled hypertension, alanine or aspartate aminotransferase concentrations greater than three times the upper limit of normal, hemoglobin A1c greater than 8.5%, hematocrit greater than 48%, or body mass index greater than 40 kg/m². Men using testosterone, growth hormone, or any anabolic therapy or drugs that affect gonadal function were excluded. For this study, men with pacemakers, metallic prosthesis, and claustrophobia were also excluded.

Randomization and Study Intervention

Eligible participants were randomized to either placebo or testosterone gel using a concealed computer-generated randomization table. The participants and outcome assessors were blinded to intervention.

The participants applied daily transdermal gel containing either placebo or 100-mg testosterone (Testim 1%; Auxilium Pharmaceuticals, Norristown, PA) for 6 months. Two weeks after randomization, the dose of testosterone gel was increased to 15 g or reduced to 5 g daily if the average of two testosterone levels drawn 4–6 hours after gel application was less than 500 or more than 1,000 ng/dL.

MRI Assessment of Liver Fat Volume and T1 and T2 Relaxation Times

Two hundred and nine men were randomized in the original parent trial (106 in testosterone and 103 in placebo). The study was terminated early by the Data Safety

Monitoring Board (DSMB) on December 15, 2009 because of the higher frequency of adverse events in men assigned to the testosterone arm of the trial than in those assigned to the placebo arm of the trial. At the time of trial's cessation, 138 men had completed the study (68 in testosterone and 70 in placebo group). Of these 138 completers, 73 men had baseline as well as end-of-treatment evaluable MRI scans of the liver—obtained on 36 participants in the placebo group and 37 participants in the testosterone group—using a 1.5T MRI system (Intera, Philips Medical Systems, Cleveland, OH), a quantitative technique based on the mixed-turbo spin-echo sequence. This pulse sequence allows for combined, self-coregistered, and volumetric mapping of T1 and T2 and analyzed for liver volume and hepatic T1 and T2 values. Volumetric assessment of liver has been demonstrated to directly correlate with hepatic fat (10,11). In addition, hepatic steatosis has been demonstrated to influence both T1 and T2 relaxation times of the liver (12). The images were analyzed using quantitative MRI algorithms written in Mathcad (Parametric Technology Corporation, Needham, MA), allowing for generation of proton density-weighted images as well as T1 and T2 parametric maps.

Liver Volumetry

For volumetric assessment, two investigators who were blinded to group assignments contoured the liver on all slices of the proton density-weighted images using Slicer v.2.6 (Brigham and Women's Hospital, Boston, MA). The voxels of the segmented regions of interest were counted, and the resulting total number was multiplied by the voxel volume to calculate the liver volume using Mathcad algorithms.

Hepatic T1 and T2 Values

To determine the peak T1 and T2 values from the segmented liver histograms, the images were processed using quantitative MRI algorithm written in Mathcad. T1 and T2 values for a given voxel at each position were computed using the following equation:

$$T1 = \left[-T1 / \ln \left[\left(1 + \frac{S1_{IR}}{S1} \right) / 2 \right] \right],$$

where S1 and S1_{IR} represent the pixel values of the magnitude images of the first echo acquisitions of the dual-echo spin-echo with and without inversion recovery, respectively. This equation represents an approximation of the T1 equation applied using the mixed-turbo spin-echo pulse sequence, derived by solving the Bloch equations.

$$T2 = \frac{TE_2 - TE_1}{\ln(S1/S2)},$$

where S1 and S2 represent the pixel values of the magnitude images acquired with TE₁ and TE₂, respectively. T1 and T2 parametric values were plotted against voxel frequency for each respective segmented liver volume. Modal T1 and T2 values were selected from each respective plot at the peak voxel frequency.

Hormone Measurements

Total testosterone was measured at Quest diagnostics, San Juan Capistrano, CA, using a Bayer-Advia-Centaur immunoassay that has been validated against liquid chromatography mass spectrometry and has a sensitivity of 10 ng/dL (15). Free testosterone was calculated using a published law-of-mass-action equation (15). Sex hormone-binding globulin levels were measured using an immunofluorometric assay with sensitivity 2.5 nmol/L (15).

Insulin Sensitivity

Fasting insulin was measured using a radioimmunoassay and glucose by a glucose oxidase method. Insulin resistance was calculated using the homeostatic model assessment (HOMA) index (6).

Statistical Considerations

This analysis involved 73 participants who had baseline and end of study MRIs. Baseline characteristics were compared between the placebo and testosterone groups using Student's *t* test, chi-square test, or Fisher's exact test, as appropriate. Change in each outcome measure was calculated for each participant and compared across treatment arms by Student's *t* test. No randomized trial to date has reported the quantitative effect of testosterone therapy on liver volume in older men. Under reasonable assumptions about the variation in change in liver volume, a sample of 73 men is sufficient to provide more than 90% power to detect a between-group difference in change in liver volume similar to that observed in other intervention trials (16). Sensitivity analyses utilized Wilcoxon rank-sum tests. The association between total testosterone concentration with change in liver volume and insulin resistance were evaluated by the Spearman correlation statistics. Covariable-adjusted analyses were used with multiple linear regression.

RESULTS

Baseline Characteristics

The two groups were similar in their baseline characteristics (Table 1). Men were predominantly Caucasian with mean age of 74 years. Age, body mass index, homeostatic model assessment-insulin resistance (HOMA_{IR}), fasting lipids, and liver function tests were similar in the two arms. Baseline liver volumes were 1583 ± 363 and 1522 ± 271 mL

in the testosterone and placebo groups, respectively. Rigorously obtained data on the distribution of liver volumes in a random sample of age-matched, community-dwelling men are not available, but these liver volume measurements are slightly higher than those reported for adults with no known history of NAFLD (17) (1036–1531 mL), comparable with the values reported in overweight adults and less than those in persons with NAFLD and obesity whose liver volumes typically exceed 2000 mL (16).

Hormone Levels

Serum testosterone concentrations increased from 250 ± 72 to 632 ± 363 ng/dL in the testosterone group (*p* < .01) but did not change significantly in the placebo group (Figure 1). Estrone and estradiol levels also increased significantly in men assigned to the testosterone arm but not in those assigned to the placebo arm (Figure 1). There was no change in sex hormone-binding globulin in either treatment group.

Table 1. Baseline Characteristics by Treatment Group

Characteristics	Testosterone (<i>n</i> = 37)	Placebo (<i>n</i> = 36)	<i>p</i> Value*
Age (y)	74.3 ± 6.4	73.5 ± 5.5	.57
BMI (kg/m ²)	29.7 ± 4.9	30.8 ± 4.4	.34
Race			.20 [†]
White	30 (83)	33 (94)	
Black	5 (13.9)	1 (2.9)	
Other	1 (2.8)	1 (2.9)	
Type 2 diabetes	9 (24)	8 (22)	.83
Statin use	22 (61)	20 (56)	.63
Metabolic parameters			
Total cholesterol (mg/dL)	170 ± 37	171 ± 34	.90
LDL (mg/dL)	91 ± 32	94 ± 30	.64
HDL (mg/dL)	48 ± 14	48 ± 12	.80
Triglycerides (mg/dL)	160 ± 105	143 ± 72	.43
HbA1c (%)	6.2 ± 0.7	6.0 ± 0.5	.20
Fasting insulin (μIU/mL)	12 ± 22	6 ± 5	.16
Fasting glucose (mg/dL)	105 ± 20	103 ± 18	.60
HOMA _{IR}	3.1 ± 5.0	1.5 ± 1.3	.11
Hormones			
Total testosterone (ng/dL)	250 ± 72	229 ± 58	.17
Free testosterone (pg/mL)	49 ± 14	44 ± 13	.09
SHBG (nmol/L)	35 ± 17	36 ± 15	.67
Estrone (pg/mL)	37 ± 20	30 ± 10	.07
Estradiol (pg/mL)	21 ± 10	20 ± 6	.67
Free estrone (pg/mL)	1.41 ± 0.75	1.12 ± 0.38	.06
Free estradiol (pg/mL)	0.49 ± 0.23	0.47 ± 0.16	.57
Liver parameters			
Aspartate aminotransferase (IU/L)	22 ± 6	23 ± 14	.59
Alanine aminotransferase (IU/L)	22 ± 9	23 ± 16	.72
Liver volume (mL)	1583 ± 363	1522 ± 271	.42
T1 (ms)	410 ± 48	425 ± 45	.17
T2 (ms)	45 ± 7	44 ± 7	.59

Notes: Data represent mean ± SD or *N* (%). BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein; HbA1c = glycosylated hemoglobin; HOMA_{IR} = homeostatic model assessment for insulin resistance; SHBG = sex hormone-binding globulin.

*Student's *t* tests of means or chi-square tests of proportions.

[†]Fisher's exact test.

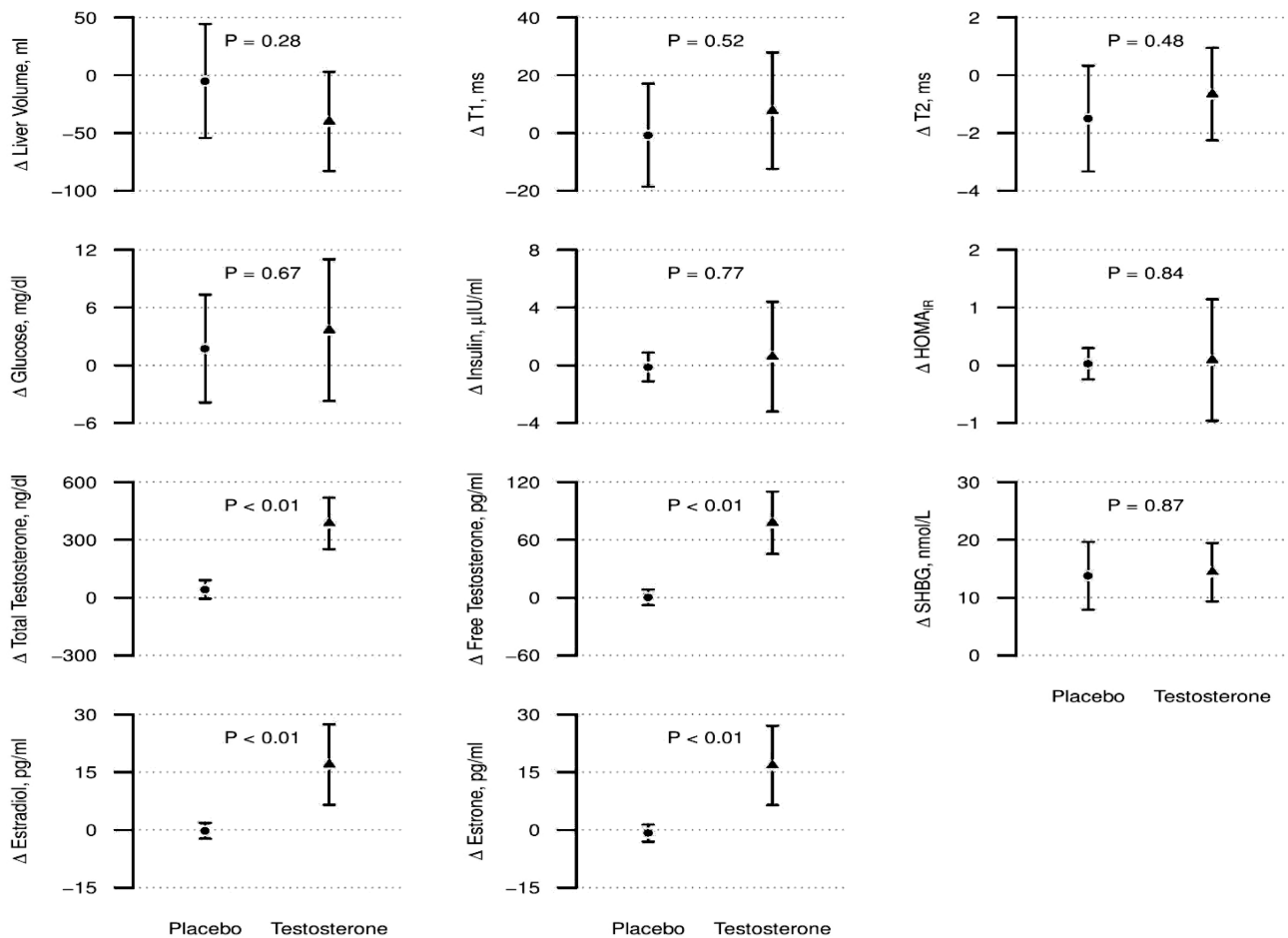


Figure 1. Mean (95% confidence interval) change from baseline in liver volume, T1 and T2 relaxation times, fasting insulin, fasting glucose, homeostatic model assessment–insulin resistance (HOMA_{IR}), total and free testosterone, sex hormone–binding globulin (SHBG), and estrone and estradiol at 6 months. *p* values are reported for comparisons between testosterone and placebo groups.

Hepatic Volume and MRI Relaxometry

Baseline liver volumes were 1583 ± 363 and 1522 ± 271 mL in the testosterone and placebo groups, respectively. There was no significant difference in the change from baseline in liver volume between the testosterone and placebo groups (mean change: -40 vs -4.9 mL, $p = .28$; Figure 1). Similarly, no significant differences were seen in the change in T1 and T2 values between the groups. Multiple regression analyses did not show a significant relationship between the change in testosterone or sex hormone–binding globulin concentrations and the change in liver volume. The liver function tests also did not change in either group.

Insulin Resistance

Neither fasting glucose nor insulin concentrations changed significantly in either group. The change in HOMA_{IR} was not significantly different between the two groups ($p = .84$; Figure 1). Linear regression analyses did not show a significant relationship between change in HOMA_{IR}

and change in liver volume, even after controlling for baseline HOMA_{IR}, body mass index, and diabetes.

Sensitivity Analyses

Stratification by diabetes status or by insulin resistance status (above or below the median for baseline HOMA_{IR}) did not affect the results. Analysis of interaction effects revealed no significant difference in the change in liver volume between men with insulin resistance and in those without insulin resistance, dichotomized as HOMA values greater than or less than 2.6 (18). Also, there was no significant difference in the change in liver volume between men with high and low baseline liver volume, dichotomized as values above or below the median.

DISCUSSION

Testosterone administration for 6 months in older men with mobility limitation was not associated with greater improvements in hepatic fat than those associated with

placebo administration. Furthermore, fasting glucose, insulin levels, and insulin resistance did not improve with testosterone administration in this population. A number of recent observations have led to speculation that androgens may reduce hepatic fat accumulation. Male mice with liver-specific inactivation of the androgen receptor gene exhibit greater liver fat accumulation than wild-type controls (8). Similarly, severe androgen deficiency, induced by surgical orchiectomy, is associated with the development of hepatic steatosis in male mice fed a high-fat diet (19). Testosterone replacement in hypogonadal men has been reported to reduce whole-body and visceral fat and some components of the metabolic syndrome (5–7). An open-label cross-over study reported that transdermal testosterone administration enhanced fat oxidation suggesting direct regulation of hepatic fat oxidation by testosterone (20). One trial has reported improvements with testosterone administration in liver enzymes in hypogonadal men with metabolic syndrome and NAFLD (9). However, our data do not support the notion that testosterone administration reduces hepatic fat in older men with low testosterone levels.

Our study has some notable strengths and some limitations. The trial had many features of a good trial design: concealed randomization, placebo-control, blinding, parallel group design, and oversight by an independent DSMB. Testosterone dose was adjusted in a blinded manner to maintain testosterone levels in the target range. However, liver fat was not the primary outcome of the trial, and the trial was not initially designed to provide adequate power to estimate the effect of testosterone on changes in liver fat. By analogy to other treatments in populations diagnosed with NAFLD, we might speculate that the sample size available in the Testosterone in Older Men trial provides reasonable power to detect clinically significant differences between arms, but this cannot be definitively shown given available data. Furthermore, the 6-month intervention duration may not have been long enough to demonstrate a significant change in hepatic fat content. Liver fat changes were measured using MRI by assessing liver volume as well as liver T1 and T2 relaxation times. Although hepatic steatosis has been shown to affect liver volume as well as liver T1 and T2 relaxation times, chemical shift imaging-based approaches and proton magnetic resonance spectroscopy are more accurate for this assessment and offer the capability of quantifying the degree of steatosis (10).

It is possible that testosterone may reduce hepatic fat in hypogonadal men with hepatic steatosis. The participants in this study did not have a known diagnosis of nonalcoholic hepatic steatosis, but sensitivity analyses did not reveal a significant difference between those with higher or lower baseline levels of liver fat. It is also possible that testosterone administration may reduce liver fat in men with a greater degree of insulin resistance than that noted in our cohort. Several studies have reported that reversing insulin resistance

prevents hepatic lipid synthesis. However, the effects of testosterone replacement on insulin resistance have been inconsistent across trials. Although states of severe androgen deficiency have been associated with the development of insulin resistance (21,22), testosterone administration has not improved insulin sensitivity in trials in community-dwelling men in which insulin sensitivity has been measured using rigorous techniques (23,24). Furthermore, our sensitivity analyses did not reveal any differences in the change in liver fat between those with higher or lower baseline levels of insulin resistance or between those who had diabetes and those who did not. Similarly, data on the role of estrogens in the pathogenesis of hepatic steatosis remain unclear. Both mice and men with CYP₁₉ (aromatase) deficiency exhibit hepatic steatosis and liver enzyme elevations (despite normal serum testosterone levels), which are reversed by estradiol replacement (25–27). On the contrary, higher estrogen levels have been associated with increased risk for diabetes and metabolic syndrome in men (28,29). Hence, the role of estrogens in the development of fatty liver disease needs to be further elucidated.

In conclusion, testosterone administration in older men with mobility limitation and low testosterone levels did not improve liver fat or insulin sensitivity to a greater extent than placebo. Larger clinical trials of longer duration are needed to determine whether testosterone replacement improves liver fat and insulin sensitivity in patients with NAFLD.

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