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A randomized placebo-controlled trial

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Whole-body oxidative stress reduction during testosterone therapy in aging men: A randomized placebo-controlled trial

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Abstract

Background: Testosterone replacement therapy in aging men increases lean body mass and decreases whole-body fat. The safety of testosterone replacement therapy concerning cardiovascular disease is unresolved and assessment of whole-body oxidative stress may contribute to future decision making.

Objectives: To determine whole-body oxidative stress during testosterone replacement therapy and placebo in aging men and evaluate if a change in oxidative stress was mediated by changed body composition.

Materials and methods: This was a double-blinded, randomized, placebo-controlled study for 24 weeks in 38 men aged 60–78 years with bioavailable testosterone <7.3 nmol/L and waist circumference ≥94 cm who were randomized to testosterone replacement therapy (testosterone gel) ($N = 20$) or placebo ($N = 18$). At baseline and after 24 weeks, whole-body oxidative stress was assessed by oxidized derivatives of nucleic acids, 8-oxoguanosine and 8-oxo-2′-deoxyguanosine in 24-h urine samples by ultra-performance liquid chromatography tandem mass spectrometry. Lean body mass and whole-body fat were measured by dual X-ray absorptiometry. Subcutaneous and visceral adipose tissue were estimated by magnetic resonance imaging. Testosterone replacement therapy versus placebo was compared by Mann–Whitney tests on Δ-values (24–0 weeks).

Results: Baseline age was 67 (64–72) years (median [interquartile range]), body mass index 29.8 (26.6–33.3) kg/m^2, waist 107 (99–117) cm, and bioavailable testosterone 4.7 (3.7–5.9) nmol/L. During testosterone replacement therapy, 8-oxoguanosine in 24-h urine samples decreased from 21.6 (19.8; 27.7) nM to 15.0 (12.2; 18.8) nM ($p = 0.038$ vs. placebo), lean body mass increased ($p < 0.01$) and whole-body fat ($p = 0.02$) and subcutaneous adipose tissue ($p < 0.01$) decreased. 8-Oxoguanosine

in 24-h urine samples was inversely associated with Δ-lean body mass ($\rho = -0.38$, $p = 0.03$), which remained significant after adjusting for Δ-total testosterone. 8-Oxo-2′-deoxyguanosine in 24-h urine samples was unchanged ($p = 0.06$) during testosterone replacement therapy and Δ-8-oxo-2′-deoxyguanosine in 24-h urine samples was associated with Δ-whole-body fat (kg) ($\rho = 0.47$, $p < 0.01$). Δ-Values of oxidative stress biomarkers were not associated with Δ-fasting insulin or Δ-homeostatic model assessment of insulin resistance.

Discussion: Oxidative stress decreased during testosterone replacement therapy compared to placebo, which could be mediated by changed body composition.

Conclusion: Whole-body oxidative stress decreased during 24 weeks of testosterone replacement therapy in aging men.

KEYWORDS
aging, hypogonadism, testosterone, whole-body oxidative stress

1 | INTRODUCTION

Aging is associated with increased oxidative stress and changed body composition with higher fat deposition and less lean body mass.[1] Whole-body oxidative stress is defined as higher levels of reactive oxygen species because of imbalance between production of reactive oxygen species and ability to detoxify reactive products.[2] The higher concentration of reactive oxygen species causes oxidative damage to deoxyribose nucleic acid (DNA) and ribonucleic acid (RNA).[3] In steady state, 24-h urine excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) reflects average rate of oxidative damage to DNA in the whole body as excreted 8-oxodG will equal newly formed 8-oxodG.[4] Similarly, whole-body RNA oxidation can be estimated by measurement of urinary excretion of 8-oxo-7,8-dihydroguanosine (8-oxoGuo).[5] RNA oxidation, 8-oxoGuo, is higher in obese men[6] and in type 2 diabetes[7] and levels of 8-oxodG and 8-oxoGuo are positively associated with atherosclerosis.[8] These findings suggest a link between oxidative stress markers, body composition, and risk of cardiovascular disease (CVD).[9,10]

Male hypogonadism in otherwise healthy men is linked to visceral adiposity,[11,12] but the potential harmful or beneficial effects of testosterone replacement therapy (TRT) regarding cardiovascular risk need clarification.[13–16] Two 24-week randomized placebo-controlled trials (RCT) reported beneficial effects of TRT, including increased lean body mass,[17–19] reduced whole-body fat, and subcutaneous adipose tissue (SAT) on the abdomen,[20,21] whereas visceral adipose tissue (VAT) and insulin sensitivity during euglycemic hyperinsulinemic clamp were unchanged.[17,19,21] High-density lipoprotein cholesterol and adiponectin levels decreased during TRT compared to placebo,[17,19–21] which suggested increased cardiovascular risk of TRT. A large RCT observed increased number of cardiovascular events during TRT compared to placebo,[22] and the US Food and Drug Administration required a warning of CVD risk on testosterone products.[23] However, it should be considered, that all included men were old and had mobility limitations.[22]

We are not aware of any randomized studies on changes in whole-body oxidative stress during TRT. However, observational studies have suggested a link between low testosterone levels and higher oxidative stress.[24,25] In these studies, young men with congenital hypogonadism had higher oxidative stress than healthy controls,[24] and healthy aging men with total testosterone (TT) <10.4 nmol/L had higher oxidative stress-related endothelial dysfunction than men with higher TT levels.[25] Two small uncontrolled studies in men with hypogonadotropic hypogonadism[26,27] reported decreased oxidative stress after TRT, but possible associations with changes in body composition were not investigated.[26,27]

The aim of this study was to investigate markers of whole-body oxidative stress during 24 weeks TRT or placebo in aging men. Ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) is the best available method for determination of in vivo whole-body oxidative stress.[28] We examined if changes in whole-body oxidative stress were mediated by changes in body composition determined by dual X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI).

2 | MATERIALS AND METHODS

2.1 | Study design and participants

A single-center, randomized, double-blinded, placebo-controlled 24-week study was conducted to assess the effect of testosterone gel compared to placebo on body composition, components of the metabolic syndrome, and quality of life in 38 men aged 60–78 years with bioavailable testosterone (BioT) levels below young healthy male reference range (BioT < 7.3 nmol/L) and waist circumference >94 cm. The original study included a third intervention (strength training); however,

in this paper, we report the results from the testosterone and placebo groups. Study details have been reported previously.[17] The exclusion criteria were hematocrit >50%, prostate cancer or a prostate-specific antigen (PSA) >3 ng/dL, previous or ongoing malignant disease, severe ischemic heart or respiratory disease, disability, diabetes mellitus, alcohol or drug abuse, abnormal routine blood samples (thyroid stimulating hormone, ionized calcium, hemoglobin, liver, and kidney function), and treatment with 5α reductase inhibitors, morphine, or oral glucocorticoid steroids.[17] The study was approved by the Ethics Committee of Region of Southern Denmark (protocol code S-20070051, September 12, 2007) and registered by ClinicalTrials.gov (NCT00700024).

In the testosterone group (N = 20), participants initially received 5 g gel containing 50 mg testosterone (Testim, Ipsen, France), and in the placebo group (N = 18), the participants received 5 g gel (placebo). After 3 weeks of treatment, safety parameters and testosterone levels were evaluated. If BioT levels were <7.3 nmol/L, the dose was increased to 10 g gel (100 mg testosterone). The dose was increased in all participants in the placebo group and in 8/20 participants in the testosterone group. The distribution of concomitant medication in the placebo and testosterone groups was equal; 63% of participants were on antihypertensive drugs, and 24% were on cholesterol-lowering drugs.[17] The study outcomes were evaluated at 0 and 24 weeks.

The present study includes data from the RCT study "Odense Androgen Study—The Effect of Testim and Training in Hypogonadal Men."[17,29,30] The study was conducted during 2008—2011, and results regarding changes in lean body mass, whole-body as well as regional fat, insulin sensitivity, lipid oxidation, biochemical markers, and muscle strength have previously been published.[17,20,29–31] The present paper includes data on 24-h urine samples collected at baseline and after 6 months TRT or placebo. The 24-h urine samples were frozen and stored in 100 mL containers at −20°C secured by temperature alarm system. Samples had not been defrosted before oxidative stress analysis was performed in 2020. Oxidative stress analysis in urine has been validated in samples stored for over 10 years.[28]

2.2 | Biochemical analyses

Twenty-four-hour urinary excretion of 8-oxodG and 8-oxoGuo (8-oxodG/24 h and 8-oxoGuo/24 h) was determined at the Laboratory of Clinical Pharmacology, Bispebjerg-Frederiksberg Hospital, Rigshospitalet, using UPLC–MS/MS of 1 mL urine.[32] The UPLC–MS/MS method has been described elsewhere.[33] Measurements of oxidized nucleosides were reported as 24 h excretion (nM/24 h) as preferred when determining urinary excretion of 8-oxodG and 8-oxoGuo.[5] Data on 24-h volume of urine excreted were missing in one participant in the testosterone group and three participants in the placebo group.

Serum TT was measured after an overnight fast between 0800 and 0900 h by liquid chromatography tandem mass spectrometry after ether extraction. For TT measurements, the intra-assay coefficient of variation (CV) was less than ±10% for TT >0.2 nmol/L and CV was less than ±30% for TT between 0.1 and 0.2 nmol/L. Sex hormone-binding globulin was measured by autoDELFIA assay and BioT was calculated.[34] Fasting 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 inter-assay CV was 3.4%—4.0%. Fasting glucose levels were measured by ABL800 Flex Radiometer, Copenhagen, Denmark. Homeostatic model assessment of insulin resistance (HOMA-IR)[35] was calculated according to the following formula: fasting insulin (μU/L) × fasting glucose (nmol/L)/22.5.

2.3 | DXA

Lean body mass, whole-body fat, and central fat mass (CFM) were measured by DXA using a Hologic Discovery Device (Waltham, MA, USA). CV was 0.8% for whole-body fat and 0.6% for lean body mass.

2.4 | MRI

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 150 ms, echo time 2.3 ms, acquisition matrix 328 × 254, field of view 450 × 450 mm). Computer software was used to trace compartments of fat on the abdomen for assessment of SAT and VAT.[36]

2.5 | Statistics

The sample size of the original study[17] was calculated using the effect of TRT on lean body mass based on a meta-analysis by Isidori et al.[18] Intergroup differences were compared using Mann–Whitney U statistics as described by Altman.[37] Data are presented as median (25; 75 percentiles). Δ-Values for clinical and biochemical markers were calculated as follow-up treatment level minus pretreatment level. Bivariate associations of Δ-values of clinical and biochemical data were investigated by Spearman's rho correlational analyses. Linear regression analysis (reported as β-coefficient, 95% confidence intervals) was used to adjust for changes in body composition. A p-value of <0.05 was considered significant. Data were analyzed using Stata BE version 17 (StataCorp).

3 | RESULTS

At baseline, the median (interquartile range) age of participants was 67 (64–72) years, body mass index (BMI) 29.8 (26.6–33.3) kg/m^2, and waist 107 (99–117) cm. Baseline characteristics were similar in the testosterone and placebo groups regarding all study outcomes.

TT, BioT, and lean body mass increased and whole-body fat decreased during 24 weeks TRT.[17,20]

TABLE 1 Baseline and 6 months data.

	Testosterone, $N = 20$		Placebo, $N = 18$		p-Value	
	Baseline	6 months	Baseline	6 months	Baseline	Δ
Age (years)	68 (62; 72)		67 (65; 69)		0.67	
TT (nmol/L)	12.2 (9.5; 15.6)	18.7 (14.9; 25.4)	12.6 (8.6; 17.1)	10.6 (8.2; 11.0)	0.89	**<0.01****
BioT (nmol/L)	5.1 (4.3; 6.1)	9.7 (6.6; 13.6)	4.4 (3.3; 6.0)	3.9 (3.5; 4.6)	0.16	**<0.01****
BMI (kg/m^2)	29.8 (27.7; 32.9)	30.2 (27.3; 33.7)	29.2 (26.2; 33.6)	28.9 (25.9; 33.9)	0.84	**0.04***
Waist (cm)	107 (104; 114)	108 (101; 112)	106 (98; 118)	108 (95; 117)	0.54	0.70
Lean body mass (kg)	64.6 (57.4; 71.3)	65.8 (59.3; 72.5)	64.7 (58.8; 72.7)	64.4 (59.2; 74.4)	0.45	**<0.01****
Whole-body fat (kg)	24.4 (21.6; 30.7)	23.1 (20.3; 29.6)	24.0 (18.2; 33.0)	25.2 (19.9; 31.1)	0.75	**0.02***
Central fat mass (kg)	14.0 (12.6; 17.5)	13.9 (11.3; 16.9)	13.2 (9.2; 18.2)	13.6 (9.4; 17.2)	0.50	**0.03***
SAT (%)	34.7 (30.8; 39.8)	33.0 (27.4; 38.3)	31.7 (28.3; 33.6)	32.5 (29.9; 35.5)	0.04	**<0.01****
VAT (%)	17.8 (12.7; 22.2)	17.3 (14.2; 21.1)	17.4 (15.3; 23.1)	16.8 (13.7; 22.7)	0.75	0.52
Fasting insulin (pmol/L)	61 (42; 84)	49 (34; 96)	49 (33; 84)	53 (42; 71)	0.24	0.46
HOMA-IR	14 (11; 23)	12 (10; 20)	12 (8; 24)	11 (9; 20)	0.46	0.29
8-oxodG/24 h (nM)	21.6 (19.8; 27.7)	15.0 (12.2; 18.8)	23.5 (19.8; 29.4)	19.4 (16.8; 23.0)	0.62	**0.04***
8-oxoGuo/24 h (nM)	32.5 (27.2; 37.1)	26.5 (21.0; 34.3)	32.2 (28.1; 36.4)	33.2 (27.0; 39.5)	1.00	0.06

Note: Data are presented as median (25; 75 percentiles). p-Values represent Mann–Whitney test in testosterone versus placebo group at baseline and Δ-values. Urine samples were available in $N = 38$ participants and information of 24-h urine volume was available in $N = 34$ participants (testosterone group, $N = 19$; placebo, $N = 15$).

Abbreviations: 8-oxodG, 8-oxoguanosine (whole-body DNA oxidation); 8-oxoGuo, 8-oxo-2′-deoxyguanosine (whole-body RNA oxidation); BioT, biovailable testosterone; BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; SAT, subcutanous adipose tissue; TT, total testosterone; VAT, visceral adipose tissue.

*$p < 0.05$.

**$p < 0.01$.

Levels of 8-oxodG/24 h decreased in the TRT group compared with placebo ($p = 0.038$). Levels of 8-oxoGuo/24 h ($p = 0.06$) were unchanged after 24 weeks TRT compared to placebo (Table 1).

3.1 | Bivariate correlations

Δ-Oxidative stress biomarkers and Δ-testosterone levels: Δ-8-oxoGuo/24 h was inversely associated with Δ-TT and Δ-BioT, whereas no significant association was found between Δ-8-oxodG/24 h and Δ-TT and Δ-BioT (Table 2).

Δ-Oxidative stress biomarkers and Δ-body composition: Δ-8-oxodG/24 h was inversely associated with Δ-lean body mass, and Δ-8-oxodG/24 h and Δ-8-oxoGuo/24 h were positively associated with Δ-whole-body fat. Δ-8-oxoGuo/24 h was positively associated with Δ-CFM but not Δ-SAT or Δ-VAT (Table 2).

Δ-Values of oxidative stress biomarkers and Δ-insulin resistance: Δ-values of oxidative stress biomarkers were not associated with Δ-fasting insulin or Δ-HOMA-IR (Table 2).

TABLE 2 Bivariate associations between Δ-oxidative stress markers and Δ-testosterone, body composition, and insulin resistance ($N = 38$).

	Δ-8-oxodG/24 h	Δ-8-oxoGuo/24 h
Δ-TT (nmol/L)	−0.29 (0.09)	**−0.35 (0.04)***
Δ-BioT (nmol/L)	−0.28 (0.11)	**−0.37 (0.03)***
Δ-Waist (cm)	0.14 (0.43)	0.17 (0.34)
Δ-Lean body mass (kg)	**−0.38 (0.03)***	−0.23 (0.20)
Δ-Whole-body fat (kg)	**0.40 (0.02)***	**0.47 (<0.01)****
Δ-CFM (kg)	0.33 (0.06)	**0.42 (0.01)***
Δ-SAT (%)	0.09 (0.64)	−0.16 (0.40)
Δ-VAT (%)	−0.03 (0.86)	0.16 (0.39)
Δ-Fasting insulin (pmol/L)	0.19 (0.28)	0.04 (0.80)
Δ-HOMA-IR	0.23 (0.17)	0.25 (0.15)

Note: Data are presented as Spearman's ρ (p-value).

Abbreviations: 8-oxodG, 8-oxoguanosine (whole-body DNA oxidation); 8-oxoGuo, 8-oxo-2′-deoxyguanosine (whole-body RNA oxidation); BioT, biovailable testosterone; CFM, central fat mass; HOMA-IR, homeostatic model assessment of insulin resistance; SAT, subcutanous adipose tissue; TT, total testosterone; VAT, visceral adipose tissue.

*$p < 0.05$.

**$p < 0.01$.

3.2 | Regression analyses

Lean body mass: Δ-8-oxodG/24 h was not significantly associated with Δ-TT after adjustment for Δ-lean body mass. Increased lean body mass was, however, an independent negative predictor of Δ-8-oxodG/24 h ($p = 0.03$) (Table 3).

TABLE 3 Regression model for Δ-oxidative stress markers adjusted for Δ-total testosterone (TT) and Δ-lean body mass.

	Δ8-oxodG/24 h ($N = 34$)	Δ8-oxoGuo/24 h ($N = 34$)
Δ-TT	−0.09 (−0.30; 0.11) ($p = 0.35$)	−0.31 (−0.64; 0.02) ($p = 0.07$)
Δ-Lean body mass	-0.01×10^{-1} (-0.02×10^{-1}; 0.01×10^{-1}) (**$p = 0.03$**)*	-0.01×10^{-1} (-0.03; 0.01×10^{-1}) ($p = 0.19$)
R^2	0.27 (**$p < 0.01$**)**	0.25 (**$p = 0.01$**)*

Note: Data represent β-coefficient (95% confidence interval) (p-value) and adjusted R^2 value. Model adjusted for Δ-TT and Δ-lean body mass.
Abbreviations: 8-oxodG, 8-oxoguanosine (whole-body DNA oxidation); 8-oxoGuo, 8-oxo-2′-deoxyguanosine (whole-body RNA oxidation).
*$p < 0.05$.
**$p < 0.01$.

TABLE 4 Regression model for Δ-oxidative stress markers adjusted for Δ-total testosterone (TT) and Δ-whole-body fat.

	Δ8-oxodG/24 h ($N = 34$)	Δ8-oxoGuo/24 h ($N = 34$)
Δ-TT	−0.02 (−0.28; −0.01) (**$p < 0.01$**)**	−0.29 (−0.61; 0.03) ($p = 0.07$)
Δ-Whole-body fat	0.01×10^{-3} (-0.07×10^{-3}; 0.04×10^{-3}) ($p = 0.56$)	0.02×10^{-1} (-0.01×10^{-2}; 0.003) ($p = 0.07$)
R^2	0.21 (**$p = 0.03$**)*	0.29 (**$p < 0.01$**)**

Note: Data represent β-coefficient (95% confidence interval) (p-value) and adjusted R^2 value. Models adjusted for Δ-TT and Δ-whole-body fat.
Abbreviations: 8-oxodG, 8-oxoguanosine (whole-body DNA oxidation); 8-oxoGuo, 8-oxo-2′-deoxyguanosine (whole-body RNA oxidation).
*$p < 0.05$.
**$p < 0.01$.

TABLE 5 Regression model for Δ-oxidative stress markers adjusted for Δ-total testosterone (TT) and Δ-subcutanous adipose tissue (SAT).

	Δ8-oxodG/24 h ($N = 31$)	Δ8-oxoGuo/24 h ($N = 31$)
Δ-TT	−0.19 (−0.454; 0.07) ($p = 0.16$)	−0.38 (−0.78; 0.01) ($p = 0.06$)
Δ-SAT	27.7 (−55.2; 110.7) ($p = 0.49$)	−27.7 (−123.3; 68.0) ($p = 0.56$)
R^2	0.10 ($p = 0.23$)	0.12 ($p = 0.15$)

Note: Data represent β-coefficient (95% confidence interval) (p-value) and adjusted R^2 value.
Models adjusted for Δ-TT and Δ-SAT.
Abbreviations: 8-oxodG, 8-oxoguanosine (whole-body DNA oxidation); 8-oxoGuo, 8-oxo-2′-deoxyguanosine (whole-body RNA oxidation).

Whole-body fat mass: Δ-8-oxodG/24 h remained inversely associated with Δ-TT after adjusting for Δ-whole-body fat with increased TT as an independent negative predictor of Δ-8-oxodG/24 h. For Δ-8-oxoGuo/24 h, results tended to be similar ($p = 0.07$) (Table 4).

SAT: Δ-8-oxodG/24 h or Δ-8-oxoGuo/24 h were not significantly associated with Δ-TT or Δ-SAT (Table 5).

4 | DISCUSSION

In the present study, 8-oxodG/24 h significantly decreased during 24 weeks TRT compared to placebo in aging men with central obesity. Furthermore, our data suggested that decreased oxidative stress during TRT was linked to improved body composition.

Our findings of a beneficial effect of TRT on oxidative stress are in accordance with two small uncontrolled studies.[26,27] In one of these studies, plasma Coenzyme Q10 was significantly lower in men with isolated hypogonadism after pituitary surgery and increased after 6 months TRT treatment,[26] which supported higher antioxidant capacity. In another study, levels of advanced oxidation protein products decreased and total antioxidant capacity increased during 6 months TRT in 16 men with hypogonadotropic hypogonadism.[27] However, previous human studies did not report associations between changes in body composition during TRT and changes in oxidative stress markers.[26,27] Higher lean body mass was the primary study outcome of the present study and we have previously reported that TRT increased lean mass ~1.6 kg and decreased whole-body fat ~1.3 kg.[17] In bivariate analyses, decreased 8-oxodG/24 h was significantly associated with higher lean mass and decreased 8-oxodG/24 h as well as 8-oxoGuo/24 h were positively associated with decreased whole-body fat. In multiple regression analyses, decreased Δ-8-oxodG/24 h was predicted by higher lean body mass independent of increased TT, whereas reduced whole-body fat was not an independent predictor of Δ-8-oxodG/24 h after adjustment for TT. These results suggested that decreased Δ-8-oxodG/24 h during TRT was at least partly mediated by increased lean body mass. Previously, we reported higher fraction of mitochondria during TRT compared to placebo in muscle biopsies in the present study cohort.[30] Functional mitochondria are important for the production and handling of reactive oxidative species[38] and there is a potential link between TRT, mitochondria and reduction of oxidative stress.[39] The relationship in vivo between changes in whole-body oxidative stress and reduction in body weight has been investigated in patients undergoing bariatric surgery. Oxidative stress markers decreased 4 months[40] and 2 years[41] after bariatric surgery. The change in oxidative stress markers during considerable weight loss after bariatric surgery supports that changes in body composition are related to oxidative stress; however, these studies did not include data on changes in lean mass or regional fat mass after bariatric surgery. We observed no association between insulin sensitivity and whole-body oxidative stress. Insulin sensitivity was unchanged during TRT compared to placebo assessed by euglycemic hyperinsulinemic clamp in men with[19] or without T2D,[17] which could be because of myostatin elevation during TRT.[42–44] In the present study, Δ-8-oxoGuo/24 h was

significantly inversely associated with Δ-TT and Δ-BioT, which became borderline significant (p = 0.06–0.07) after correcting for changes in whole-body fat or lean body mass in regression analyses. These findings supported that changes in whole-body RNA oxidation during TRT could also be mediated by direct action of testosterone on the androgen receptor. In vitro, testosterone supplementation reduced oxidative stress in cerebellar granule cells from rats, which suggested a neuro-protective effect mediated by the androgen receptor.[45] In Leydig cells from mice, low-dose testosterone (100 nmol/L) supplementation decreased intracellular oxidative stress, whereas high-dose (≥500 nmol/L) testosterone supplementation increased oxidative damage.[46] These findings suggested that the effect of TRT could be dose dependent with a positive effect of physiological testosterone doses on oxidative stress, whereas supra-physiological testosterone exposure could be harmful. In accordance, users of androgenic anabolic steroids had significantly higher oxidative stress biomarkers than non-users.[47] The decrease in whole-body oxidative stress indicated that TRT in aging men with higher waist circumference was potentially beneficial regarding CVD.

Strengths and limitations apply in the present study. This is the first RCT to investigate the effect of TRT on whole-body oxidative stress, including the impact of increased lean body mass and decreased whole-body fat during TRT in aging men with central obesity. The strengths of the current study are the randomized, placebo-controlled design and chromatography coupled with MS on 24-h urine, which is considered the gold standard for measurement of 8-oxodG and 8-oxoGuo.[48] Twenty-four-hour urine sampling represents whole-body oxidative stress and is best suited to situations where all tissues are assumed to be affected, for example, during TRT, which affects nearly all tissue in the body. Measurement of whole-body oxidative stress by urinary excretion of 8-oxodG and 8-oxoGuo has limitations. Urinary excretion does not allow for the identification of organ-specific increase in oxidative stress and cannot identify whether oxidation occurs in the nucleotide pool or in the nucleic acid molecule. Therefore, it is mainly a measure of generalized intracellular oxidative stress.[49] Another limitation may be concerning the reproducibility of the whole-body oxidative stress markers. Measurements of 8-oxodG and 8-oxoGuo had high intra-individual variations in spot urine samples collected over days.[50] We collected 24-h urine samples to reduce variation, but we cannot exclude day-to-day variation as a potential bias. However, the study design with inclusion of a placebo-group would to some extent overcome the study limitation of variation in oxidative stress marker measurement. The study is furthermore limited by the fact that measurement of both 8-oxoGuo and 8-oxodG were not primary study outcome pre-specified, and as such, analyses are post hoc and should be seen as hypothesis-generating only.

In conclusion, decreased biomarkers of whole-body oxidative stress during 24 weeks TRT in aging men were partly mediated by improved body composition.

AUTHOR CONTRIBUTIONS

Conceptualization and investigation: Marianne Skovsager Andersen and Louise Lehmann Christensen. *Oxo analysis and validation*: Henrik Enghusen Poulsen. *Data curation*: Louise Lehmann Christensen and Dorte Glintborg. *Writing*: Dorte Glintborg, Louise Lehmann Christensen, and Marianne Skovsager Andersen. All authors read and approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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