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Article type : 3 Original Article - Australia, Japan, SE Asia

The effects of testosterone on body composition in obese men are not sustained after cessation of testosterone treatment

Short title: Loss of treatment effect after T cessation

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Key words: Testosterone, obesity, fat mass, lean mass

Word Count: Abstract 250; Main text 3,243; Tables: 2; Figures 3;
Supplementary table: 1; Supplementary Figure: 1.

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Disclosure statement: Bayer Pharma AG (Berlin, Germany) provided testosterone, placebo and financial support to conduct investigations, but had no role in trial design, data analysis or writing the manuscript.

Trial Registration: clinicaltrials.gov Identifier NCT01616732

Abstract

Background: Testosterone treatment in obese dieting men augments the diet-associated loss of fat mass, but protects against loss of lean mass. We assessed whether body composition changes are maintained following withdrawal of testosterone treatment.

Methods: We conducted a pre-specified double-blind randomised placebo-controlled observational follow-up study of a randomized controlled trial (RCT). Participants were men with baseline obesity (body mass index ≥30kg/m^2) and a repeated total testosterone level ≤12nmol/L, previously enrolled in a 56-week testosterone-treatment trial combined with a weight loss program. Main outcome measures were mean adjusted differences (MAD) (95% confidence interval), in body composition between testosterone and placebo-treated men at the end of the observation period.

Results: Of the 100 randomised men, 82 completed the RCT, and 64 the subsequent observational study. Median [IQR] observation time after completion of the RCT was 82 weeks [74; 90] in men previously receiving testosterone (cases) and 81 weeks [67; 91] in men previously receiving placebo (controls), p=0.51. At the end of the RCT, while losing similar amounts of weight, cases had, compared to controls, lost more fat mass, MAD -2.9kg (-5.7, -0.2), p=0.04, but had lost less lean mass MAD 3.4kg (1.3, 5.5), p=0.002. At end of the observation period, the former between group differences in fat mass, MAD -0.8kg, (-3.6, 2.0), p=1.0, in lean mass, MAD -1.3kg (-3.0, 0.5), p=0.39, and in appendicular lean mass, MAD -0.1kg/m^2 (-0.3, 0.1), p=0.45, were no longer apparent. During observation, cases lost more lean mass, MAD -3.7kg (-5.5, -1.9), p=0.0005 and appendicular lean mass, MAD -0.5kg/m^2 (-0.8, -0.3), p<0.0001 compared to controls.

Conclusions: The favourable effects of testosterone on body composition in men subjected to a concomitant weight loss program were not maintained at 82 weeks after testosterone treatment cessation.

Introduction

While dietary measures are first line treatments of obesity, weight loss achieved with caloric restriction is modest, and difficult to sustain in the long term [1]. In addition, the beneficial effects of dieting on reducing body fat are mitigated by a concomitant loss of muscle mass. Exercise interventions attenuate, but do not fully prevent this diet-associated loss of muscle mass: a systematic review of interventional studies concluded that the addition of exercise to caloric restriction reduced the loss of muscle mass by only 50% [2].

In men, testosterone treatment increases lean body mass and reduces fat mass [3, 4]. We recently conducted a 56 week placebo controlled randomised controlled trial (RCT) of testosterone treatment among obese men with a lowered testosterone level but no evidence of pathological androgen deficiency due to pituitary or testicular disease [5]. All participants were subjected to a rigorous weight loss program. As expected, men receiving placebo lost both fat mass and lean mass during diet. In contrast, testosterone treatment prevented the diet-associated loss of lean mass, and the weight loss among testosterone treated men was almost exclusively due to loss of fat mass [5].

The long term risks of testosterone treatment in men without recognisable medical disease of the hypothalamic-pituitary-gonadal axis are unknown. While not powered for safety, short term (≤ 12 months) testosterone treatment trials have generally not demonstrated a consistent increase in clinically important adverse events. In the 12-month Testosterone Trials, the rates of adverse events in carefully selected men were similar in the testosterone and placebo-treated men [6]. A meta-analysis of smaller mostly short term testosterone RCTs of medium to low quality reported an increase in haemoglobin and haematocrit and a small decrease in high-density lipoprotein cholesterol with testosterone treatment [7]. These findings were considered to be of unknown clinical significance. Similarly, although controversial due to lack of definitive data, there is no evidence that short term testosterone treatment, especially when administered by the intramuscular or transdermal route, increases cardiovascular risk [8-10].

Given the current evidence supporting potential benefits of short term testosterone treatment on some outcome measures such as sexual function [6] body composition and bone mineral density [3, 4], yet the absence of long term safety data, the question arises whether benefits of testosterone treatment might be sustained after stopping testosterone administration. In addition, it is not well established whether cessation of physiologically-dosed testosterone treatment is associated with long term iatrogenic suppression of the hypothalamic-pituitary-testicular (HPT) axis, which could potentially be harmful. We therefore conducted an observational follow-up study of a recently completed RCT [5] to investigate whether the effects of testosterone treatment on body composition in obese men were sustained after at least 12 months of treatment cessation. We also evaluated whether testosterone treatment is associated with prolonged iatrogenic suppression of the HPT axis, and whether potentially adverse effects of testosterone treatment are maintained.

Materials and Methods

Design Overview

This study reports the post-trial follow-up phase of a 56-week RCT designed to assess the effects of testosterone treatment on body composition added to a rigorous weight loss program, reported in detail previously [5]. Briefly, 100 obese (body mass index (BMI) >30kg/m^2) men with a repeated fasting morning total testosterone level of <12 nmol/L confirmed by liquid chromatography/mass spectrometry were 1:1 randomized to 1000mg intramuscular testosterone undecanoate treatment or matching placebo, administered for 56 weeks according to standard schedule. In addition, all subjects received a very low energy diet for 10 weeks followed by a 46 weeks weight maintenance diet [5]. At the 56-week final study visit of the RCT phase, whilst both study investigators and participants remained blinded to treatment allocation, all men were encouraged to continue with dieting and exercise and were invited to attend for a single follow-up appointment at the Austin Hospital Andrology Research Unit after at least 12 months following completion of the RCT for assessment of body composition and hormonal studies.

The original RCT protocol (HREC 2012/04495) and the observational follow-up study (LNR/16/Austin/61) reported here were approved by the local ethics committee. Each participant provided written informed consent.

Main Outcome Measures

The primary outcome of the RCT was the change in fat mass [5]. The main pre-specified exploratory outcome measures of this observational follow-up study were the changes in fat mass, lean mass, appendicular lean mass and body weight after at least 12 months from testosterone treatment cessation. Other outcome measures included laboratory studies, drawn in the morning (8am-10am), in the fasted state. All follow-up measurements were conducted at the study center with the same methodology used during the RCT phase of the study. Briefly, levels of haemoglobin, haematocrit, prostate specific antigen (PSA), luteinizing hormone (LH), sex hormone binding globulin and total testosterone were determined by electrochemiluminescence immunoassay and free testosterone calculated as described previously [5]. Body composition was quantified by dual energy absorptiometry (Prodigy, Version 13.60; GE Lunar, Madison, WI, USA) [5].

Statistical Methods

Data shown are mean (standard deviation) or median [IQR], according to normality testing using the Kolmogorov-Smirnov test with Lilliefors correction. Comparison of baseline characteristics was based on Welch's t-test for normally distributed parameters. Alternatively, Wilcoxon rank-sum test was used in case of non-normal distribution. Correlations were based on Kendall's Tau rank test.

Changes in the outcomes between cases and controls over the follow-up visits were analysed using a linear mixed model with restricted maximum likelihood estimator. The model included random intercepts per subject, and as fixed variables the baseline levels of the respective outcome variable, four time points (at 0, 10 and 56 weeks and at the end of follow-up), group (placebo and testosterone), and the interaction of time point x group. The latter represents the measure of interest (between-group change over time), which was quantitatively expressed as mean adjusted difference (MAD) surrounded by the profiled 95% confidence interval. P values for the change from baseline to end of observation and week 56 week to end of observation were conventionally adjusted for multiple testing by Holm-Bonferroni method. In addition to the main analysis following the intention-to-treat (ITT) principle, we performed a sensitivity analysis per protocol including only those participants who completed the observation follow-up period. All tests were two-tailed with $p<0.05$ denoting statistical significance. Analyses were conducted using R version 3.3.2 for Mac, effects 3.1-2, phia 0.2-1 and lme4 package 1.1-12 [11, 12].

Role of the Funding Sources

MNTF was supported by a postgraduate scholarship (1055305) and MG by a Career Development Fellowship (1024139) both from the National Health and Medical Research Council (Australia). Bayer Pharma AG (Berlin, Germany) provided testosterone, placebo and financial support to conduct investigations during the RCT phase, but did not provide funding for this observational follow-up study. Bayer Pharma AG had no role in trial design, data analysis or writing the manuscript.

Results

Study Subjects

Patient recruitment and retention during the 56 week of this RCT has been prescribed previously and is shown in supplementary Figure 1. During the follow-up phase (from week 56 to the end of the observation period), 18 of the 82 RCT completers withdrew from the study, including one participant receiving testosterone treatment. The low number of testosterone initiation during the follow-up phase is expected given restrictions on testosterone prescribing for men without pathological androgen deficiency in Australia [13]. Thus 64 of the 100 participants originally randomised were available for a follow-up visit (Supplementary Figure 1). No participant received weight altering medications including glucocorticoids, insulin, glucacon like peptide-1 analogs during the follow-up phase or was subjected to bariatric surgery. Baseline characteristics were comparable between the groups [5], and did not differ in participants completing the trial compared to the entire randomised cohort Table 1.

The median [IQR] post-RCT observation time in those who completed the observational follow-up phase of the study was 82 [74; 90] weeks in cases and 81 [67; 91] weeks in controls, p=0.51.

Hormone Levels

By the end of the observational follow-up phase, the testosterone levels that had been increased in cases during the RCT phase decreased to levels similar to baseline, and the previous significant difference in total testosterone levels in cases compared to controls was no longer apparent at trial end, MAD -0.4 nmol/L (-2.5, 1.7), p=0.71 (Figure 1a, Table 2). Findings for calculated free testosterone were similar (Figure 1b, Table 2). LH levels, which had been suppressed in cases during the RCT phase, had, at the end of the observation phase, increased to levels similar to baseline and slightly higher than those of controls, MAD 1.4 IU/L (0.3, 2.4), p=0.026 (Figure 1c, Table 2).

Body composition

During the RCT phase, both cases (-11.4 kg (-13.9, -8.8)) and controls (-10.9kg (-13.6, -8.1) had lost significant amounts of body weight, with no difference between cases and controls at the end of the 56-week RCT, MAD -0.5 kg (-4.3, 3.3), p=0.80 [5]. However, whilst controls lost both lean mass (-4.0kg (-5.5, -2.5)) and fat mass (-2.9 kg (-4.3, -1.6)), cases lost almost exclusively fat mass (-5.7kg (-6.9, -4.5)), and lean mass loss was prevented (-0.6kg (-2.0, 0.8)) [5]. At the end of the observational follow-up phase, both cases and controls had regained body weight, with no between group difference, MAD -1.5 kg (-5.3, 2.3), p=1.0 (Figure 2a, Table 2). While cases had lost more fat mass during the RCT compared to controls, MAD -2.8kg (-4.6, -1.0), p=0.003 at RCT end [5], both cases and controls regained fat mass during the observation period. At the end of the observation period, the former difference in fat mass between cases and controls was no longer apparent, MAD -0.8 kg (-3.6, 2.0), p=1.0 (Figure 2b, Table 2). Similarly, although controls had lost more lean mass during the RCT (MAD at 56 weeks 3.4kg (1.3, 5.5), p=0.002) [5], during follow-up cases lost more lean mass than controls, MAD -3.7 kg (-5.5, -1.9), p=0.0005, and at the end of the observational follow-up period, the difference in lean mass was no longer significant MAD -1.3 kg, (-3.0, 0.5), p=0.39 (Figure 2c, Table 2). Findings were similar for appendicular lean mass (Figure 2d, Table 2). Figure 2e shows the lean mass to fat mass ratio as an integrated parameter of the body composition changes during the RCT and the observational follow-up phase. While this ratio had increased in cases at the end of the RCT (p<0.0007), compared to controls, this difference was not sustained, and at the end of the observation period, it had declined significantly (p=0.0012), and was no longer increased (p=0.82). By contrast, the lean mass to fat mass ratio remained similar in controls throughout the study (Figure 2e).

<u>Safety outcomes</u>

During the RCT phase, both haemoglobin (MAD 15 g/L (12-19), p<0.001) and haematocrit (MAD 0.04 (0.03-0.05), p<0.001) increased significantly following 56 weeks of testosterone treatment [5]. During the observation period both parameters declined in cases, (MAD -12 g/L (-16, -9), p<0.0001 for haemoglobin and MAD -0.04 (-0.05, -0.03) p<0.0001 for haematocrit) to levels similar to baseline, with no difference compared to controls (MAD 3 g/L (0, 7), p=0.09 for haemoglobin and MAD 0.01 (0, 0.02) p=0.16 for haematocrit) (Figure 3a and 3b, Table 2). During the RCT phase, there were no differences in PSA levels between cases and controls. At the end of the observation period, one case and one control patient had a PSA level increase by 1.0 μg/L or more compared to prerandomisation levels, but no participant had a PSA level above the upper limit of the reference range. PSA levels were similar between groups at study end (Figure 3c, Table 2).

<u>Sensitivity analysis</u>

Outcomes were unchanged after performing a per protocol analysis (Supplementary Table 1), including only those men who completed the observation period, except for LH which was of borderline significance (p=0.026) at the end of the follow-up period compared to baseline, was no longer significant (p=0.13) in the per protocol analysis.

Discussion

In this observational double blind and placebo-controlled follow-up study of a 56 week RCT of testosterone treatment in dieting obese men, the benefits of testosterone on body composition were not maintained when assessed after at least 12 months of testosterone treatment cessation. In addition, there was no evidence that previous testosterone treatment led to sustained iatrogenic suppression of the HPT axis, nor was it associated with persisting changes in haematological and prostate safety parameters.

Whether the effects of testosterone treatment persist after treatment cessation has not been well documented previously. Most data come from uncontrolled observational studies and the findings have been equivocal. A small survey in 33 Japanese men reported that subjective quality of live benefits were maintained several years after cessation of testosterone treatment [14]. An observational study in 24 obese men receiving testosterone treatment for 54 weeks reported that 24 weeks after testosterone withdrawal, testosterone effects on fat mass were maintained, but effects on lean mass were not [15]. In a larger uncontrolled observational cohort of 147 middle aged and older men previously treated with long term testosterone for a mean duration of more than 5 years, cessation of testosterone treatment for a mean of 16.9 months was associated with weight gain, increased waist circumference and an increase in glucose and lipid parameters [16]. There has been, to our knowledge, only one controlled study that has assessed the durability of testosterone treatment effects. That study assessed the durability of shorter term (6 months) testosterone treatment effects 6 months after treatment cessation, in different population compared to our cohort [17]. In that study of intermediate-frail or frail men older than 65 years with a baseline circulating total testosterone of <12 nmol/L, the effects of testosterone treatment on the main RCT outcomes of muscle strength, lean mass and quality of life were not maintained at reassessment 6 months after completion of the RCT [17]. Our study is consistent with these findings, and extends the evidence to middle-aged obese men with a substantially lower baseline testosterone of 7 nmol/L who were additionally subjected to a rigorous weight loss program, with a longer observational follow-up.

The mechanisms by which testosterone treatment improves body composition parameters are not fully clarified. The current evidence is consistent with both direct effects on adipocyte and myocyte differentiation and function [18], as well as indirect effects on motivation and mood [6] that may promote adherence to healthy lifestyle measures. Indeed, at the end of RCT phase of our study [5], men randomised to testosterone but not placebo maintained increased spontaneous activity levels, suggesting that during treatment, increased physical activity may have contributed to the observed changes in body composition in testosterone-treated men. Overall the current study suggests that whatever the underlying mechanisms promoting body composition changes during testosterone treatment are, they require continuous exposure to testosterone treatment to remain operational.

During the observation period, men regained a substantial proportion of the weight loss achieved during the RCT. This is consistent with previous studies demonstrating that weight loss, even if successful, is difficult to maintain [1]. Weight regain during the observational follow-up period occurred irrespective whether men had received testosterone treatment or placebo during the RCT phase. Interestingly during the RCT phase, placebo treated men demonstrated a modest (+2.9 nmol/L) increase in endogenous testosterone levels in association with the 11% loss of body weight, similar in magnitude to the increase in endogenous testosterone with weight loss reported previously [19]. However, this increase in endogenous testosterone in placebo-treated men was not sustained at the end of the observational follow-up phase, likely because of weight regain. Based on mechanistic and clinical studies, low circulating testosterone and obesity have been proposed to interact in a mutually reinforcing relationship [20]. However our findings suggest that the modest increases in endogenous circulating testosterone during weight loss are not to overcome this bidirectional low testosterone-obesity cycle.

This study demonstrates that 56 weeks of testosterone treatment does not cause persisting iatrogenic HPT axis suppression after 12 months or more after testosterone treatment cessation, even in obese men who might be more vulnerable to this phenomenon given adipose tissue-mediated functional HPT axis suppression [21, 22]. Indeed, although at the end of the follow-up phase the weight of the participants was still lower than at randomisation, 97% of men that had received testosterone treatment during the RCT phase were still obese (BMI$\geq$30 kg/m^2) at the end of the observation period. However, it is possible that the modest weight loss maintained to the end of the follow-up may have facilitated the endogenous HPT axis recovery after the RCT phase. The fact that at the end of the observation phase, LH levels were higher in men that had received testosterone during the RCT phase compared to those receiving placebo is further evidence of a compensatory central gonadal axis response driving endogenous HPT axis recovery. Importantly, the absence of HPT axis suppression at the end of the observation period is not due to ineffective testosterone treatment during the RCT: testosterone treatment was clearly effective, as evidenced by the marked reduction in fat mass and LH levels, and the increases in lean mass and haematocrit during the RCT phase.

From a practical clinical perspective, these results indicate that if a short term (up to 56 weeks) trial of testosterone treatment is considered in a man with non-classical hypogonadism, the risk of long term persisting iatrogenic HPT axis suppression is low. We cannot exclude the possibility that a more short lived period of HPT axis suppression that had resolved at the follow-up visit may have occurred. However, at the follow-up visit, no participant reported symptoms consistent with temporary HPT axis suppression during the follow-up phase. Therefore, even if transient HPT axis suppression occurred, this would appear unlikely to be of clinical significance.

The strengths of this follow-up study include its double blind, randomised placebo-controlled parallel group design, with sufficient length of follow-up to demonstrate changes in body composition after testosterone withdrawal. We exclusively focussed on obese men with testosterone levels, measured by validated mass spectrometry, that were substantially lower than levels reported in unselected community-dwelling obese men [23, 24]. The administration of effective intramuscular testosterone performed by medical staff at the study centre resulted in a 100 % medication adherence, and the trial was notable for the successful implementation of a rigorous weight loss program with a relatively low drop out rate.

The main limitation of the study was that measurements during the follow-up phase were limited to a single time point. Hence, it was not possible to evaluate the time course of the loss of testosterone treatment effects. In addition, we cannot exclude the possibility that a period of transient HPT axis suppression may have accelerated the loss of the beneficial body composition effects accrued during testosterone treatment. Indeed, induced severe hypogonadism, either experimentally in healthy young men [25] or therapeutically as androgen deprivation treatment given to men with prostate cancer [26] has been associated with increased fat mass and loss of muscle mass. When asked at the follow-up visit, no participant recalled symptoms consistent with testosterone withdrawal following treatment cessation. However, this issue and especially the potential for more subtle transient HPT axis suppression contributing to accelerated fat gain and muscle loss after testosterone treatment is stopped can only be resolved in a study with assessments at multiple time points.

In conclusion, the beneficial effects of 56 weeks of testosterone treatment on body composition in dieting obese men did not persist 82 weeks after cessation of testosterone treatment. Given that testosterone treatment may need to be maintained for longer durations, and possibly indefinitely, to maintain treatment-associated benefits, longer term controlled studies will be necessary to more precisely define the benefits and potential risks of testosterone treatment.

Acknowledgements

MNTF was supported by a postgraduate scholarship (1055305) and MG by a Career Development Fellowship (1024139) both from the National Health and Medical Research Council (Australia).

Conflicts of Interest

MG has received research funding from Bayer Pharma, Novartis, Weight Watchers, Lilly and speaker's honoraria from Besins Healthcare. MNTF has received research funding from Bayer Pharma. The other authors have no conflicts of interest to declare.

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Tables

Table 1. Baseline characteristics of randomly assigned study participants

	All randomised (N=100)	Non-completers (N=36)	Completers (N=64)	p
Age (y)	53.2 [47.4; 59.9]	51.7 [43.0; 55.2]	56.9 [49.3; 61.2]	0.014
Weight (kg)	120 (17.7)	125 (19.9)	116 (15.6)	0.019
BMI (kg/m^2)	37.4 [34.7; 41.2]	37.7 [35.4; 41.9]	37.0 [34.6; 40.7]	0.25
Waist circumference (cm)	123 [117; 133]	127 [119; 136]	122 [117; 131]	0.12
Fat mass (kg)	45.4 (10.3)	48.3 (11.5)	43.9 (9.2)	0.04
Lean mass (kg)	67.7 [62.8; 72.4]	69.2 [64.5; 73.2]	66.4 [61.3; 72.2]	0.07
ALM/height^2 (kg/m^2)	9.6 (0.9)	9.7 (0.8)	9.6 (1.0)	0.72
TT (nmol/L)	8.3 (2.4)	8.4 (2.6)	8.3 (2.2)	0.90
cFT (pmol/L)	166 (45)	168 (53)	165 (41)	0.74
LH (IU/L)	4.2 [3.2; 5.4]	4.5 [3.6; 5.3]	3.8 [3.0, 5.4]	0.15
Haemoglobin (g/L)	150 (8.4)	150 (6.9)	149 (9.2)	0.56
Haematocrit	0.43 (0.02)	0.44 (0.02)	0.43 (0.02)	0.49
PSA (µg/L)	0.7 [0.5; 1.2]	0.8 [0.5; 1.2]	0.7 [0.5; 1.1]	0.60

Data are mean (SD), median [IQR] based on normality testing, using the Kolmogorov-Smirnov test with Lilliefors correction. p values were calculated for the difference between groups using

Welch's t-test for normally distributed parameters or otherwise Wilcoxon rank-sum test, p<0.05 was considered significant. BMI, body mass index; ALM, appendicular lean mass; TT, total testosterone; cFT, calculated free testosterone; LH, luteinizing hormone; PSA, prostate specific antigen.

Table 2: Change in main outcomes, intention to treat analysis

	Testosterone group N=49	Placebo group N=51	Mean Adjusted Difference week 0 vs. follow-up[1]	Mean Adjusted Difference week 56 vs. follow-up[2]
Fat mass (kg)				
Week 0	46.1 [35.6; 50.9]	44.1 [38.4; 53.0]		
Week 56	30.7 [23.5; 44.8]	36.3 [28.7; 46.1]		
End of follow-up	37.7 [32.9; 46.1]	45.2 [34.4, 48.9]	-0.8 (-3.6, 2.0), p=1.0	2.3 (-0.6, 5.1), p=0.48
Lean mass (kg)				
Week 0	66.6 [63.5; 72.1]	69.1 [60.8; 72.5]		
Week 56	67.2 [63.8; 71.8]	61.8 [56.8, 70.4]		
End of follow-up	64.5 [60.4; 69.2]	61.2 [56.3; 70.9]	-1.3 (-3.0, 0.5), p=0.39	-3.7 (-5.5, -1.9), p=0.0005
Body weight (kg)				
Week 0	116.0 [108.8, 129.0]	118.4 [103.8; 135.6]		
Week 56	101.1 [93.5; 119.0]	101.7 [93.4; 117.8]		
End of follow-up	106.6 [99.8; 117.4]	112.6 [94.2, 128.7]	-1.5 (-5.3, 2.3), p=1.0	-0.6 (-4.5, 3.3), p=1.0
ALM/height^2 (kg/m^2)				
Week 0	9.7 [9.1; 10.4]	9.4 [9.0, 10.1]		
Week 56	9.8 [9.5; 10.4]	9.2, [8.6; 9.6]		
End of follow-up	9.6 [8.8; 10.3]	9.2 [8.5; 9.8]	-0.1 (-0.3, 0.1), p=0.45	-0.5 (-0.8, -0.3), p<0.0001
TT (nmol/L)				
Week 0	8.5 [6.6; 10.4]	8.4 [7.1; 10.0]		

Week 56	14.1 [11.9; 18.7]	10.0 [8.5, 11.6]		
End of follow-up	9.1 [6.4; 10.4]	8.8 [7.4; 10.5]	-0.4 (-2.5, 1.7), p=0.71	-6.0 (-8.1, -3.8), p<0.0001
cFT (pmol/L)				
Week 0	200 [164, 239]	202 [172; 238]		
Week 56	352 [268; 464]	205 [174; 242]		
End of follow-up	176 [148; 206]	189 [155; 230]	-14 (-63, 34), p=0.56	-173 (-223, -122), p<0.0001
LH (IU/L)				
Week 0	4.5 [3.3; 5.6]	4.2 [3.1; 5.2]		
Week 56	0.1 [0.1; 0.1]	4.1 [2.8; 5.6]		
End of follow-up	5.0 [3.4; 7.3]	4.1 [3.0; 5.5]	1.4 (0.3, 2.4), p=0.026	5.7 (4.5, 6.8), p<0.0001
Haemoglobin (g/L)				
Week 0	148 [142; 152]	151 [144; 158]		
Week 56	161 [154; 170]	149 [144; 157]		
End of follow-up	148 [145; 151]	150 [144; 154]	3 (0, 7), p=0.09	-12 (-16, -9), p<0.0001
Haematocrit				
Week 0	0.43 [0.41; 0.44]	0.44 [0.42; 0.45]		
Week 56	0.46 [0.44; 0.49]	0.44 [0.42; 0.45]		
End of follow-up	0.42 [0.42; 0.44]	0.44 [0.42; 0.45]	0.01 (0, 0.02), p=0.16	-0.04 (-0.05, -0.03), p<0.0001
PSA (ug/L)				
Week 0	0.72 [0.54; 1.10]	0.71 [0.48; 1.15]		
Week 56	0.86 [0.59;1.40]	0.66 [0.56; 1.45]		
End of follow-up	0.66 [0.44; 1.07]	0.77 [0.42; 1.23]	0.15 (-0.07, 0.38) p=0.75	-0.04 (-0.27, 0.20) p=1.0

Data are median [IQR] for testosterone and placebo groups.

[1]Mean adjusted difference (MAD) refers to the between-group change at week 0 (commencement of the RCT) and the end of the follow-up observation, or [2]the change from week 56 to the end of the follow-up observation using a mixed model and intention to treat

analysis. ALM, appendicular lean mass; TT, total testosterone; cFT, calculated free testosterone; LH, luteinizing hormone; PSA, prostate specific antigen.

Figure Legends

Figure 1. Changes in circulating total testosterone, calculated free testosterone and LH levels.

Shown are circulating levels of total testosterone (TT) (a), calculated free testosterone (cFT) (b) and luteinizing hormone (LH) (c) in men receiving testosterone or placebo during the RCT phase, depicted as adjusted means surrounded by 95% CI at 0,10, 56 weeks and at the end of the follow-up observation period of a median [IQR] of 81 weeks [69; 91]. P, placebo group; T, testosterone group. Corresponding mean adjusted differences (MAD) are reported in Table 2.

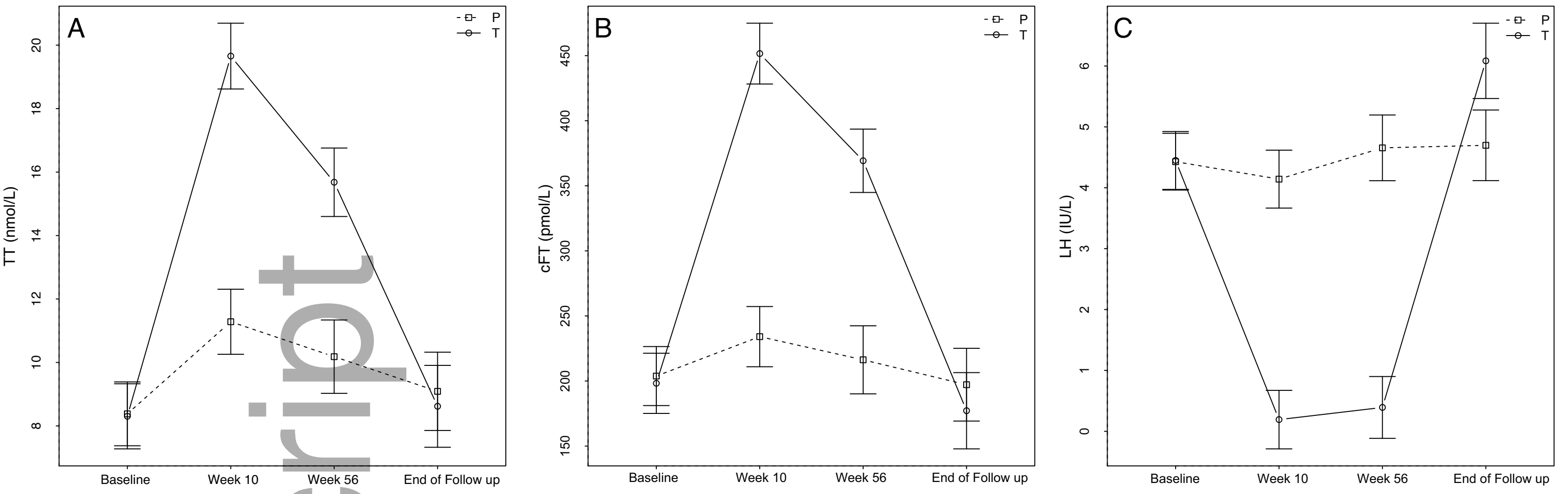
Figure 2. Changes in body composition parameters.

Shown are body weight (a), fat mass (b), lean mass (c) appendicular lean mass (ALM) corrected for height squared (d) and lean to fat mass ratio (e) in men receiving testosterone or placebo during the RCT phase, depicted as adjusted means surrounded by 95% CI at 0,10, 56 weeks and at the end of the follow-up observation period of a median [IQR] of 81 weeks [69;

91]. P, placebo group; T, testosterone group. Corresponding mean adjusted differences (MAD) are reported in Table 2.

Figure 3. Changes in safety parameters.

Shown are haemoglobin (a), haematocrit (b), and prostate specific antigen (PSA) levels (c) in men receiving testosterone or placebo during the RCT phase, depicted as adjusted means surrounded by 95% CI at 0,10, 56 weeks and at the end of the follow-up observation period of a median [IQR] of 81 weeks [69; 91]. P, placebo group; T, testosterone group. Corresponding mean adjusted differences (MAD) are reported in Table 2.



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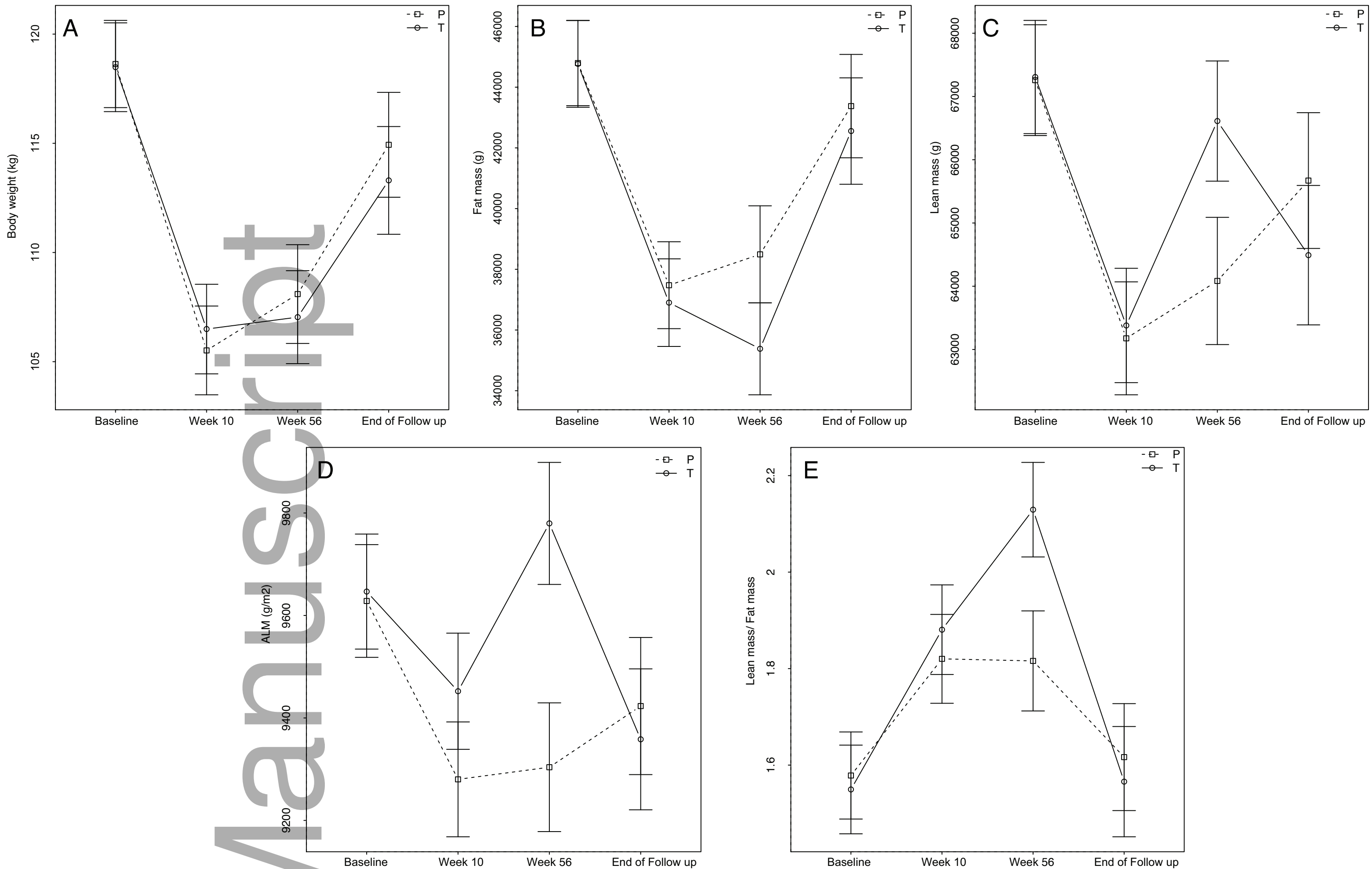

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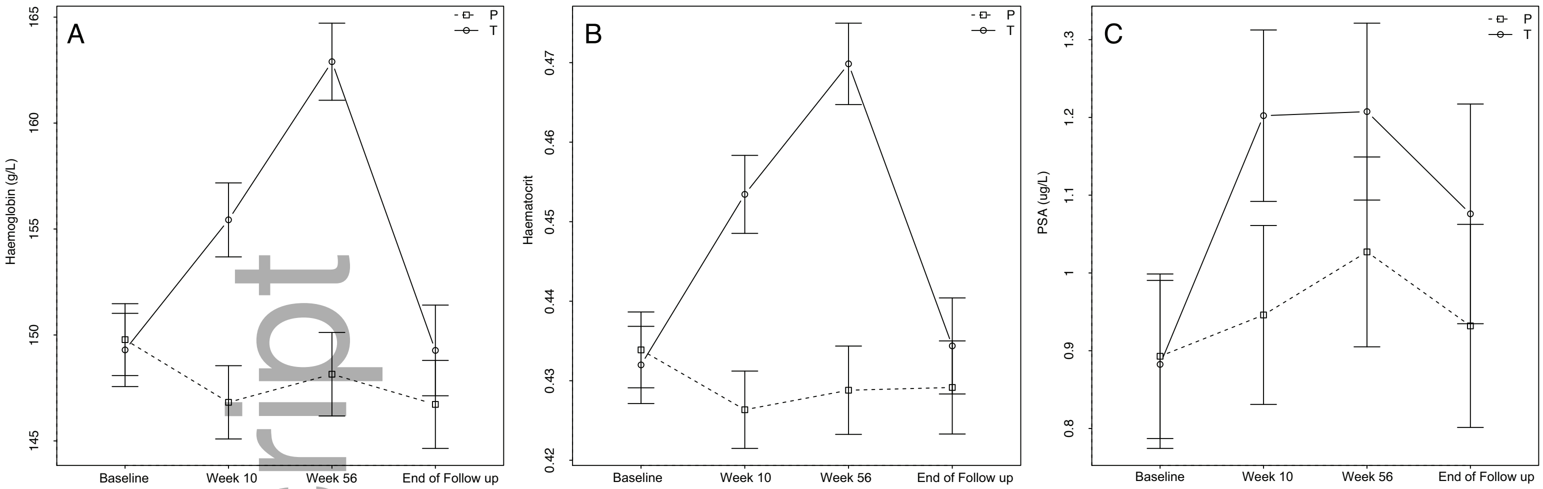

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