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MR spectroscopy of hepatic fat and adiponectin and leptin levels during testosterone therapy in type 2 diabetes: a randomized, double-blinded, placebo-controlled trial

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Abstract

Background: Men with type 2 diabetes mellitus (T2D) often have lowered testosterone levels and an increased risk of cardiovascular disease (CVD). Ectopic fat increases the risk of CVD, whereas subcutaneous gluteofemoral fat protects against CVD and has a beneficial adipokine-secreting profile.
Hypothesis: Testosterone replacement therapy (TRT) may reduce the content of ectopic fat and improve the adipokine profile in men with T2D.
Design and methods: A randomized, double-blinded, placebo-controlled study in 39 men aged 50–70 years with T2D and bioavailable testosterone levels <7.3 nmol/L. Patients were randomized to TRT ($n=20$) or placebo gel ($n=19$) for 24 weeks. Thigh subcutaneous fat area (TFA, %fat of total thigh volume), subcutaneous abdominal adipose tissue (SAT, % fat of total abdominal volume) and visceral adipose tissue (VAT, % fat of total abdominal volume) were measured by magnetic resonance (MR) imaging. Hepatic fat content was estimated by single-voxel MR spectroscopy. Adiponectin and leptin levels were measured by in-house immunofluorometric assay. Coefficients (b) represent the placebo-controlled mean effect of intervention.
Results: TFA ($b=-3.3$ percentage points (pp), $P=0.009$), SAT ($b=-3.0$ pp, $P=0.006$), levels of adiponectin ($b=-0.4$ mg/L, $P=0.045$), leptin ($b=-4.3$ µg/mL, $P<0.001$), leptin:adiponectin ratio ($b=-0.53$, $P=0.001$) and HDL cholesterol ($b=-0.11$ mmol/L, $P=0.009$) decreased during TRT compared with placebo. Hepatic fat content and VAT were unchanged.
Conclusions: The effects of TRT on cardiovascular risk markers were ambiguous. We observed potentially harmful changes in cardiovascular risk parameters, markedly reduced subcutaneous fat and unchanged ectopic fat during TRT and a reduction in adiponectin levels. On the other hand, the decrease in leptin and leptin:adiponectin ratio assessments could reflect an amelioration of the cardiovascular risk profile linked to hyperleptinaemia in ageing men with T2D.

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Introduction

The safety of long-term testosterone replacement therapy (TRT) has not been clarified regarding the risk of cardiovascular disease (CVD) (1, 2, 3). Nonetheless, the usage of TRT has escalated in the Western countries during the past decades especially in ageing men without clear organic indication for TRT (4). Ageing men with type 2 diabetes mellitus (T2D) often have lowered testosterone levels (5), ectopic fat depots (6), a deranged adipokine profile with e.g. low adiponectin levels (7), hyperleptinaemia (8) and an increased risk of CVD (5, 9). However, the causal relations are unclear, and lowered testosterone levels could simply be a marker of illness, i.e. T2D and obesity (9). Non-obese, hypogonadal men may have more subcutaneous fat compared with eugonadal men, whereas no difference is found regarding visceral adipose tissue (VAT) (10). Regional fat distribution affects the CVD risk of which lower body subcutaneous fat storage protects against CVD (11) and represents a normal physiological expansion of nonpathogenic adipocytes (12). On the other hand, ectopic fat such as VAT (11) and non-alcoholic fatty liver disease (NAFLD) (13, 14) are associated with increased CVD risk (11, 13) and might contribute to the pathogenesis of insulin resistance (13, 15, 16). Irrespective of testosterone levels, ectopic fat storage occurs when continuous nutritional overload leads to adipocyte expansion failure in the subcutaneous fat compartments, resulting in the redistribution of fat to the liver, muscle and VAT (17). This fat storage at ectopic sites is further promoted by aging in itself (18). Theoretically, TFA is more protective against CVD risk compared to SAT (19, 20) possibly due to a long-term entrapment of excess free fatty acids (FFA) (11, 21) and a more favourable adipokine-secreting profile. There is evidence of a primary secretion of leptin from the subcutaneous compartments (20, 22) though leptin levels might be associated with VAT in obese men (20). A deranged adipokine secretion contributes to CVD (23) as elevated adiponectin levels may inhibit inflammation (24) and atherosclerosis (25), whereas the role of leptin is a double-edged sword. Healthy lean individuals are in a low leptin state where leptin regulates food intake and has insulin-sensitizing effects (23, 26), whereas hyperleptinaemia in obesity and/or T2D is associated with an increased risk of CVD (27). Hyperleptinaemia without a concomitant increase in leptin activity is a state called leptin resistance (18). The index of leptin levels corrected by adiponectin concentrations (leptin:adiponectin ratio) could be a better cardio-metabolic marker than levels of adiponectin and leptin alone (28, 29).

There is evidence that TRT may reduce ectopic fat deposition and improve the adipokine profile in ageing men with and without T2D (30, 31, 32, 33, 34, 35, 36) possibly through the inhibition of the adipogenic lineage (37, 38). A beneficial effect on ectopic fat deposition is consistent with reports showing that TRT increases lipid oxidation in hypopituitary men (39, 40) and in ageing men without T2D (41), but could also simply reflect an overall effect of TRT on total fat mass (35, 41, 42). In ageing men with T2D, regional abdominal adipose tissue during TRT has only been assessed in one former study by a validated tool i.e. magnetic resonance imaging (MRI) reporting unchanged VAT and decreased SAT, whereas TFA was not reported (31). One previous study has evaluated the efficacy of TRT on the content of hepatic fat in ageing men with T2D (30) and the sparse reports on the content of hepatic fat in men without T2D during TRT are conflicting probably due to different assessment methods, inhomogeneous groups of included patients e.g. large age spans and varying biochemical cutoffs for hypogonadism (32, 33, 34, 36). In theory, a reduction of SAT may result in a beneficial change in the adipokine profile with an increase in adiponectin levels and a decrease in leptin concentrations (43, 44). Previous papers evaluating levels of adiponectin and leptin during TRT are however inconsistent in aging men with T2D (30, 31, 45, 46).

In the present study, we hypothesized that TRT reduces the content of ectopic fat and improves the adipokine profile in men with T2D during TRT in ageing men with T2D and lowered bioavailable testosterone (BioT) levels.

Subjects and methods

This 24-week, randomized, double-blinded, placebo-controlled trial was conducted at Odense University Hospital (Denmark) from April 2012 to November 2013. The study was approved by the Local Ethics Committee and the Danish Health and Medicines Authority. The trial was declared in ClinicalTrials.gov (identifier: NCT01560546), and all patients gave written informed consent at the screening visit. To find eligible patients, we used advertisement at general practitioners, in magazines, newspapers, and through written invitations to patients with newly diagnosed T2D, who were referred to the Department of Endocrinology at Odense University

Hospital. The inclusion criteria included men, aged 50–70 years, T2D diagnosed within the last 10 years, metformin treated for at least 3 months and a bioavailable testosterone level <7.3 nmol/L. The main exclusion criteria were BMI ≥40 kg/m^2, haematocrit >50%, known malignant disease, PSA >3 µg/L, nycturia >3 times, clinically significant disease of the heart, lung or kidneys, abnormal routine blood samples, severe untreatable hypertension, former or present abuse of alcohol or medicine/drugs within a year, primary or secondary hypogonadism, ongoing severe mental illness, wish of fatherhood and treatment with morphine, 5α reductase inhibitors, oral glucocorticoid steroids or antidiabetic medications apart from metformin. In all, 59 patients attended a screening visit, 43 patients were eligible and included and 39 patients completed the study.

Patients were randomly assigned to 5 g gel daily containing placebo (n=21) or 50 mg testosterone, Testim (n=22). In total, 19 patients in the placebo group and 20 patients in the TRT group completed the study. Due to obesity, claustrophobia and failure in the acquisition of the MRI/MRS scans, nine patients in the placebo group and 10 patients in the TRT group failed MRI/MRS scans. Patients were informed not to change their diet, and they were allowed to continue habitual activities throughout the study. Two non-testosterone-related serious adverse events occurred in the study as reported previously (35). The study design, population, assays along with data and methods of evaluation on testosterone levels, lean body mass, total fat mass, lipids and insulin sensitivity are reported in further detail elsewhere (35).

All patients were on a stable antidiabetic treatment regimen throughout the entire study and only metformin was allowed. Concomitant medication, i.e. antihypertensive, cholesterol lowering and anti-thrombotic drugs, was equally distributed between the placebo and testosterone groups, which was also the case regarding the subgroups undergoing MRI and/or MRS. We examined the patients on two consecutive days before and after 24 weeks of TRT. Dose titration was performed after three weeks treatment with an increase to 10 g gel daily if BioT levels <7.3 nmol/L. Safety monitoring was externally handled to ensure continued blinding and included evaluation of PSA, haematocrit and haemoglobin after 3, 12 and 24 weeks of treatment.

Magnetic resonance imaging (MRI)

MRI was performed with a 3.0-T high-field MR Unit (Phillips Achieva, Phillips Healthcare). Three abdominal slices (10 mm thick, 20 mm apart, lower slice at the dorsal, intervertebral space of L4/L5) and one femoral slice (15 cm from the major trochanter and perpendicular to subcutaneous fat) were recorded using an axial, T1-weighted gradient-echo sequence. In-house developed software using MATLAB (MathWorks, Natick, Massachusetts, United States) was applied for automatic segmentation of the images, yielding SAT (% fat of total abdominal volume), VAT (% fat of total abdominal volume) and TFA (% fat of total thigh volume). First, the images were bias-corrected (47). The different compartments were then automatically delineated by unrolling the images and using the graph-cut method (48). The threshold for fat was determined as the 4th cluster using a k-means clustering with 5 groups.

Magnetic resonance spectroscopy (MRS)

Single-voxel liver 1H MRS was performed to measure the hepatic fat content. MRS measurements were performed using a Philips Achieva 3.0-T MR scanner (Philips Healthcare). Liver MR images in all three planes were used to localize voxels for MRS. The MR spectroscopic data were acquired using a SENSE XL torso coil with 16 channels, following shimming, with volumes of interest (30×30×30 mm^3) manually placed within the right lobe of the liver (segment six or seven), avoiding major blood vessels, intrahepatic bile ducts and the lateral margins of the liver in all dimensions. The point-resolved spectroscopy (PRESS) technique was performed without water suppression (repetition time ms/echo time ms, 2000/35). We collected spectra during a single breath-hold (17.5 s). The water peak and the major fat peak of methylene, located at 4.7 and 1.3 ppm respectively, were automatically fitted by using a spectroscopic analysis package included in the Philips workstation. Area ratios (hepatic fat/water ratio) were calculated for each patient (Supplementary Fig. 1, see section on supplementary data given at the end of this article). Automated spectral results were reviewed by an experienced MR spectroscopist who was blinded to the treatment allocation.

Euglycemic–hyperinsulinemic clamp

After an overnight fast, a 2-h basal tracer equilibration period was followed by a 4-h period with insulin infusion at a rate of 40 U/m^2/min. A [3-^{3}H]-glucose infusion was used throughout the 6-h study, and [3-^{3}H]-glucose was added to the glucose infusates to maintain plasma-specific activity constant at baseline levels during the 4-h clamp

period. By varying the infusion of 20% glucose based on bedside plasma glucose measurements every 10–20 min, plasma glucose was kept constant at approximately 5.5 mmol/L. Steele's non-steady-state formulas were used to calculate the rates of total glucose appearance (Ra) and glucose disposal (Rd). Insulin-stimulated Rd was taken as an estimate of whole-body insulin sensitivity.

Biochemical assays

Adiponectin (mg/L) was determined by a validated in-house time-resolved immunofluorometric assay based on two monoclonal antibodies and recombinant human adiponectin (obtained from R&D Systems) (49). The intra-assay coefficient of variation averaged <10%. Leptin (µg/L) was determined by a validated in-house time-resolved immunofluorometric assay based on commercial reagents (from R&D Systems: two monoclonal antibodies (cat. no. MAb 398 for coating and BAM 398 for detection) and recombinant human leptin as standard (cat. no. 398-LP)) and carried out essentially as the adiponectin time-resolved immunofluorometric assay (49). The recovery of exogenous leptin added to serum averaged 96.8±0.2% (the intra-assay CV averaged 50 assay setups).

Analysis of ALAT and GGT were performed in a Modular System (Roche Diagnostics) with dedicated reagents.

Statistical methods

The sample size of the study was determined by the anticipated effect of TRT on lean body mass (42) with an assumption of type 1 error (α)=0.05, type 2 error (β)=0.1, S.D.=1.3 kg, along with a 25% drop-out rate, resulting in 20 patients in each group. In the present study, the primary outcome measures included changes in SAT, TFA, VAT, hepatic fat content and levels of adiponectin and leptin. The changes in SAT, TFA and VAT are given as percentage points (pp). Per-protocol analyses were performed. Differences in baseline values were analysed using unpaired *t*-test on normally distributed data. Wilcoxon rank-sum tests were conducted at baseline and on delta values if data could not be transformed to normally distributed data using natural logarithm. Outcome measurements were assessed by multiple linear regression analyses controlled for baseline values on normally distributed data for the placebo-controlled mean effect of intervention between groups (*b*). The models were checked with residual plots and Box–Cox analysis. Absolute changes during 24 weeks from baseline are given as delta values. We used non-parametric Spearman's rank correlation to analyse the correlations between delta values. All tests were done two-sided and results of $P<0.05$ were considered statistically significant. Results are expressed as arithmetic mean±S.D., geometric mean (95% CI) or median (interquartile range) as appropriate. Statistical analyses were performed with STATA, version 13.

Results

The testosterone and placebo groups were comparable regarding all baseline measurements (Tables 1 and 2).

Total fat mass (TFM), SAT, TFA, VAT, hepatic fat content and body composition

As reported, TFM ($b=-1.3$ kg, $P=0.009$) was reduced during TRT compared with placebo ($n=39$) (35) and TFM was also reduced ($b=-1.1$ kg, $P=0.045$) during TRT compared with placebo in the subgroups undergoing MRI and/or MRS ($n=27$) (Table 2). This was accompanied by reductions in SAT ($b=-3.0$ pp) and TFA ($b=-3.3$ pp) during TRT compared with placebo. There was no change in hepatic fat content or VAT during TRT compared with placebo (Fig. 1 and Table 1). Total lean body mass was increased, while body weight, BMI and waist circumference (WC) were unaltered (35).

Adipokines, lipids, liver enzymes and insulin sensitivity

Adiponectin ($b=-0.4$ mg/L), leptin ($b=-4.3$ µg/L), leptin:adiponectin ratio ($b=-0.53$) and GGT ($b=-7.8$ U/L) levels decreased, while ALAT was unchanged during TRT compared with placebo (Fig. 2 and Table 1). As reported, HDL cholesterol ($b=-0.11$ mmol/L) was decreased while total cholesterol, LDL cholesterol, TG, HOMA-IR and insulin-stimulated glucose disposal rate (Rd) were unchanged (35).

Correlations

At baseline in all patients

We examined the correlations between leptin, adiponectin and CVD risk modifiers.

Adiponectin was positively associated with age and HDL cholesterol, while adiponectin was negatively associated with TG and HOMA-IR (Table 3).

Leptin was positively associated with BMI, WC, TFM, VAT, hepatic fat content and HOMA-IR, whereas leptin

Table 1 Clinical and para clinical characteristics. Data are presented as arithmetic mean ± S.D. Bold type indicates statistically significant differences.

	Testosterone		Placebo		
	n	Values	*n*	Values	***P* value**
TFA/TTA (%)[#]	12		13		
Baseline		30.7 (26.1–36.1)		27.9 (24.0–32.3)	
24 weeks		27.3 (22.8–32.7)		28.0 (24.2–32.4)	
Δ 24-week baseline[†]		–3.7 (–4.9; –2.4)		–0.8 (–1.7; 1.2)	**0.009**
TMA/TTA (%)[#]	12		13		
Baseline		8.4 (7.5–9.4)		8.6 (7.4–9.9)	
24 weeks		8.0 (7.1–9.0)		8.3 (6.8–10.1)	
Δ 24-week baseline[†]		–0.4 (–1.2; 0.1)		–0.0 (–0.8; 0.2)	0.79
VAT/TAT (%)[#]	12		13		
Baseline		22.0 (18.4–26.3)		23.2 (20.8–25.8)	
24 weeks		21.4 (17.1–26.6)		22.6 (20.3–25.1)	
Δ 24-week-baseline[†]		–0.1 (–2.3; 1.5)		–0.4 (–1.4; 0.2)	0.91
SAT/TAT (%)[#]	12		13		
Baseline		32.1 (28.0–36.7)		31.4 (28.7–34.5)	
24 weeks		28.7 (24.7–33.5)		31.2 (28.7–33.8)	
Δ 24-week baseline[†]		–2.6 (–4.9; –1.2)		–0.8 (–1.9; 1.0)	**0.006**
Hepatic fat/water ratio[†]	11		11		
Baseline		0.39 (0.10; 0.59)		0.40 (0.16; 0.73)	
24 weeks		0.25 (0.05; 0.66)		0.24 (0.15; 0.53)	
Δ 24-week-baseline		0.00 (–0.05; 0.04)		–0.10 (–0.31; 0.01)	0.12
ALAT (U/L)[#]	20		19		
Baseline		31.8 (25.2–40.2)		33.9 (27.3–42.0)	
24 weeks		29.5 (22.5–38.5)		34.8 (27.1–44.5)	
Δ 24-week baseline[†]		–1.5 (–7.0; 4.5)		–1.0 (–2.0; 8.0)	0.27
GGT (U/L)[#]	20		19		
Baseline		33.5 (25.4–44.3)		39.7 (32.7–48.2)	
24 weeks		29.3 (22.1–38.7)		42.4 (34.4–52.2)	
Δ 24-week baseline[†]		–2.0 (–10.0; 0.5)		0.0 (–6.0; 8.0)	**0.019**
Adiponectin (mg/L)[#]	20		19		
Baseline		7.5 (6.1–9.3)		6.2 (5.1–7.6)	
24 weeks		6.8 (5.5–8.4)		6.1 (5.2–7.2)	
Δ 24-week baseline[†]		–0.7 (–1.2; –0.2)		–0.2 (–0.8; 0.4)	**0.045**
Leptin (μg/L)[#]	20		19		
Baseline		13.2 (10.0–17.5)		11.6 (9.0–14.9)	
24 weeks		9.5 (6.8–13.2)		12.4 (9.6–16.0)	
Δ 24-week-baseline[†]		–3.7 (–5.4; –1.3)		0.4 (–1.8; 3.6)	**<0.001**
LAR[#]	20		19		
Baseline		1.8 (1.2–2.6)		1.9 (1.4–2.5)	
24 weeks		1.4 (0.9–2.2)		2.0 (1.5–2.7)	
Δ 24-week baseline[†]		–0.3 (–0.4; –0.1)		0.1 (–0.2; 0.6)	**0.001**

P value refers to the placebo-controlled mean effect of intervention between groups.
[#]Geometric mean (95% CI) or [†]median (interquartile range).
LAR, leptin:adiponectin ratio; SAT, subcutaneous adipose abdominal tissue; TAT, total abdominal tissue; TFA, total fat area thigh; TMA, total muscle area thigh; TTA, total thigh area; VAT, visceral adipose tissue.

was negatively associated with testosterone levels (TT, BT, FT and DHT), SHBG and insulin-stimulated Rd (Table 3).

Leptin:adiponectin ratio was positively correlated to BMI, WC, TFM, VAT, hepatic fat content, TG and HOMA-IR, whereas leptin:adiponectin ratio was negatively correlated to HDL, TT, DHT, SHBG and insulin-stimulated Rd (Table 3).

Hepatic fat content was positively associated with BMI, WC, TFM, ALAT and HOMA-IR, whereas hepatic fat content was negatively associated with insulin-stimulated Rd (Table 4).

Δ24 weeks-baseline in the testosterone group

We performed correlations between the changes of possible CVD risk modifiers and changes in levels of adiponectin and leptin after TRT. Δ-Adiponectin levels were positively associated with Δ-HDL cholesterol

Table 2 Clinical and para-clinical characteristics for the patients undergoing MRI and/or MRS.

	Testosterone ($n=13$)			**Placebo** ($n=14$)			
	Baseline	24 weeks	Δ	Baseline	24 weeks	Δ	***P* value**
Age (years)*	61.6±5.7			59.4±6.6			
Duration of T2D (years)*	4.3±3.0			3.1±2.3			
BMI (kg/m^2)*	29.6±3.0	29.8±3.2	0.1 (−0.2; 0.7)	30.1±2.6	30.0±2.6	−0.1 (−0.5; 0.6)	0.48
Total lean body mass (kg)*	59.0±6.8	60.7±6.3	2.1 (1.1; 2.5)	60.2±4.8	60.0±5.2	−0.1 (−0.8; 0.5)	0.02
Total fat mass (kg)	25.8 (22.4–29.8)	24.7 (21.0–28.9)	−1.3 (−1.7; −0.5)	27.1 (24.4–30.2)	26.6 (24.0–29.4)	0.1 (−1.0; 0.7)	0.048
Total testosterone (nmol/L)†	7.2 (6.9; 11.6)	26.6 (11.5; 36.4)	15.2 (0.4; 23.3)	9.6 (8.1; 12.5)	9.4 (8.4; 11.7)	0.8 (−0.4; 1.9)	0.04
Bio testosterone (nmol/L)†	4.2 (3.3; 4.5)	11.1 (4.8; 24.7)	6.9 (0.3; 18.7)	4.7 (4.0; 5.4)	4.8 (4.2; 6.0)	0.4 (−0.2; 0.9)	0.04
Free testosterone (nmol/L)†	0.21 (0.17; 0.24)	0.61 (0.25; 1.12)	0.39 (0.01; 0.85)	0.24 (0.21; 0.28)	0.25 (0.21; 0.30)	0.02 (−0.01; 0.05)	0.04
SHBG (nmol/L)	32 (26–39)	25 (20–31)	−5 (−10; −3)	27 (21–35)	26 (20–35)	−2 (−4; 3)	0.004
DHT (nmol/L)	0.60 (0.41–0.86)	3.33 (1.83–6.06)	3.38 (1.87; 5.49)	0.56 (0.38–0.82)	0.56 (0.38–0.81)	−0.02 (−0.21; 0.23)	<0.001
Total cholesterol (mmol/L)*	4.0±0.8	3.7±1.1	−0.1 (−0.3 to 0.1)	3.8±1.0	3.7±0.9	−0.1 (−0.3; 0.4)	0.31
LDL (mmol/L)*	2.1±0.2	2.3±0.2	0.0 (−0.2; 0.3)	2.2±0.2	2.3±0.2	−0.1 (−0.3; 0.1)	0.72
HDL (mmol/L)*	1.0±0.1	1.0±0.1	0.0 (−0.1; 0.1)	0.9±0.0	1.0±0.0	0.1 (0.0; 0.2)	0.13
Triglycerides (mmol/L)	1.5 (1.1–2.2)	1.4 (1.0–1.9)	0.2 (−0.8; 0.3)	1.5 (1.3–1.9)	1.4 (1.1–1.6)	−0.1 (−0.2; 0.1)	0.67
Haemoglobin (mmol/L)†	8.6 (8.5; 9.1)	9.2 (9.1; 9.6)	0.5 (−0.1; 0.8)	9.0 (8.6; 9.3)	8.9 (8.4; 9.1)	−0.2 (−0.6; −0.1)	0.01
Haematocrit (%)*	42.5±0.0	45.0±0.0	0.04 (−0.01; 0.04)	43.0±0.0	42.4±0.0	−0.01 (−0.02; 0.01)	0.006
PSA (μg/L)	0.6 (0.4–1.1)	0.7 (0.5–1.2)	0.1 (0.0; 0.3)	1.0 (0.6–1.6)	1.0 (0.6–1.6)	0.0 (−0.1; 0.3)	0.34
HOMA-IR	3.7 (2.4–5.6)	3.9 (2.6–5.9)	0.0 (−0.3; 0.4)	3.9 (2.8–5.5)	4.3 (3.2–5.9)	0.5 (−0.2; 1.0)	0.5
Insulin-stim Rd (mg/min/m^2)	171.3 (146.3–200.5)	179.6 (150.7–213.9)	3.4 (−23.4; 40.2)	170.2 (148.1–195.6)	163.0 (136.1–195.2)	−6.2 (−26.2; 10.8)	0.28
Adiponectin (mg/L)	6.9 (5.3–9.0)	6.3 (4.8–8.2)	−0.5 (−1.1; −0.1)	5.8 (4.6–7.4)	5.9 (4.8–7.2)	−0.0 (−0.3; 0.4)	0.03
Leptin (μg/L)	11.4 (8.3–15.8)	8.6 (5.5–13.5)	−2.4 (−3.9; −1.2)	12.2 (8.8–16.9)	12.9 (9.5–17.5)	−0.51 (−2.2; 3.6)	0.01
LAR	1.6 (1.0–2.8)	1.4 (0.7–2.6)	−0.2 (−0.4; −0.1)	2.1 (1.5–3.0)	2.2 (1.6–3.0)	−0.0 (−0.5; 0.6)	0.067

Data presented as geometric mean (95% CI). All Δ values are presented as median (interquartile range).
*Arithmetic mean±S.D. or †median (interquartile range).

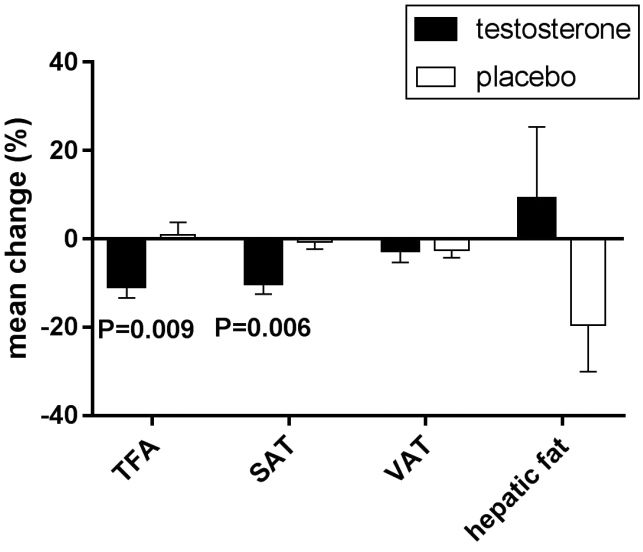

Figure 1
Mean change (%) in subcutaneous thigh fat area (TFA), subcutaneous abdominal adipose tissue (SAT), visceral adipose tissue (VAT) and hepatic fat content. Data are presented as mean ± S.E.M.

(r_s=0.51, P=0.04) and Δ-leptin (r_s=0.62, P=0.003), whereas Δ-adiponectin was negatively associated with Δ-SHBG (r_s=–0.53, P=0.02). Δ-Leptin levels showed a trend towards negative correlation with Δ-insulin-stimulated Rd (r_s=–0.44, P=0.051).

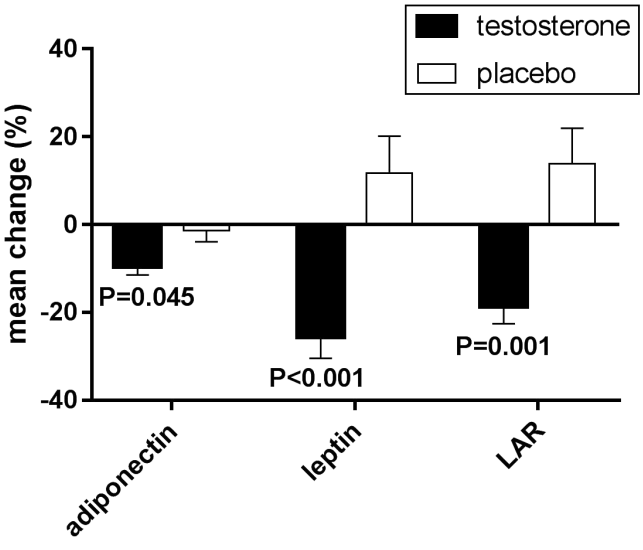

Figure 2
Mean change (%) in adiponectin, leptin and leptin:adiponectin ratio (LAR). Data are presented as mean ± S.E.M.

No other significant correlations were observed between Δ-adiponectin levels, Δ-leptin levels, Δ-leptin:adiponectin ratio, Δ-hepatic fat content and changes in clinical/biochemical parameters during TRT.

Discussion

We are the first to evaluate hepatic fat content during testosterone therapy or placebo in men with T2D using the currently most accurate imaging technique, MRS. Furthermore, we contribute data on regional fat deposits and assessments of leptin and adiponectin levels as well as the leptin:adiponectin ratio. In the present study, we showed that subcutaneous fat was reduced during TRT, whereas ectopic visceral and hepatic fat depots were unaltered. In addition to the observed decrease in adiponectin and HDL levels, this might suggest an increased risk of CVD during TRT, whereas the diminished hyperleptinaemia and leptin:adiponectin ratio could be beneficial regarding the risk of CVD.

Thigh and regional abdominal fat

We found a reduction in TFA and SAT during TRT, whereas VAT was unaltered. Reports on the effect of TRT on regional abdominal adipose tissue in ageing men without T2D have been inconsistent possibly due to the application of different methods for assessment of regional abdominal adipose tissue (MRI, CT or ultrasound), inclusion of various patient cohorts and the use of a variety of testosterone doses and administration forms (oral, patch, gel or injections) (19). Our results are in accordance with results from studies using a sufficient testosterone dose in ageing men without T2D reporting a decrease in SAT (19, 50 51, 52) and TFA (19, 51), but no change in VAT (19, 50 51, 52) during TRT. In contrast, an older study by Marin *et al*. (54) in 31 middle-aged obese men with higher baseline testosterone levels reported unchanged SAT and a decrease in VAT assessed by CT despite no change in TFM and lean body mass (53). The lack of change in TFM in that study suggests that the decrease in VAT during TRT might be attributed to other changes during the experiment. In ageing men with T2D, two previous studies have evaluated regional abdominal fat by MRI during TRT and correspondingly reported a reduction in total body subcutaneous fat mass (30), a reduced amount of SAT (31), unchanged VAT (30, 31), while TFA was not reported (30, 31). There is evidence that lower body subcutaneous fat protects against CVD (11), and this

Table 3 Association analyses for adiponectin, leptin and leptin:adiponectin ratio (LAR): univariate correlations with clinical/biochemical parameters at baseline.

Parameter	*n*	Adiponectin	*P* value	Leptin	*P* value	LAR	*P* value
Age (years)	39	0.39	**0.01**	−0.05	0.78	−0.27	0.1
BMI (kg/m^2)	39	−0.09	0.6	0.7	**<0.001**	0.58	**<0.001**
Waist circumference (cm)	39	−0.15	0.37	0.74	**<0.001**	0.68	**<0.001**
Total fat mass (kg)	38	−0.09	0.57	0.74	**<0.001**	0.62	**<0.001**
TFA/TTA (%)	25	0.11	0.6	0.14	0.52	0.02	0.92
VAT/TAT (%)	25	−0.12	0.57	0.45	**0.03**	0.42	**0.04**
SAT/TAT (%)	25	0.05	0.83	0.24	0.25	0.1	0.65
Hepatic fat/water ratio	22	−0.4	0.07	0.64	**0.001**	0.65	**0.001**
HDL cholesterol (mmol/L)	37	0.64	**<0.001**	−0.18	0.29	−0.55	**<0.001**
Triglycerides (mmol/L)	37	−0.55	**<0.001**	0.08	0.65	0.4	**0.01**
Total testosterone (nmol/L)	39	0.05	0.77	−0.47	**0.002**	−0.42	**<0.001**
Bio testosterone (nmol/L)	39	−0.06	0.71	−0.37	**0.02**	−0.28	0.09
Free testosterone (nmol/L)	39	−0.06	0.73	−0.38	**0.02**	−0.29	0.08
SHBG (nmol/L)	39	0.27	0.09	−0.4	**0.01**	−0.47	**0.003**
DHT (nmol/L)	39	0.12	0.47	−0.72	**<0.001**	−0.59	**<0.001**
HOMA-IR	38	−0.35	**0.03**	0.7	**<0.001**	0.76	**<0.001**
Insulin-stimulated Rd (mg/min/m^2)	39	0.09	0.6	−0.56	**<0.001**	−0.52	**<0.001**

Spearman's rank correlation.
SAT, subcutaneous adipose abdominal tissue; TAT, total abdominal tissue; TFA, total fat area thigh; TMA, total muscle area thigh; TTA, total thigh area; VAT, visceral adipose tissue.

could be mediated by higher adiponectin levels. Thus, TFA was positively associated with serum adiponectin in a population-based study in young (20–29 years) healthy men (44). We observed a reduction in TFA accompanied by a decrease in adiponectin levels; however, there was no correlation between these variables. Whether the reduction in adiponectin levels observed in response to TRT in our study represents an unhealthy effect of TRT or simply reflects the overall reduction in TFM remains to be established.

Table 4 Association analyses for hepatic fat content: univariate correlations with clinical/biochemical parameters at baseline.

Parameter	*N*	Hepatic fat content	*P* value
Age (years)	22	−0.31	0.16
BMI (kg/m^2)	22	0.61	**0.002**
Waist circumference (cm)	22	0.53	**0.01**
Total fat mass (kg)	21	0.49	**0.02**
TFA/TTA (%)	20	−0.08	0.73
VAT/TAT (%)	20	0.42	0.07
SAT/TAT (%)	20	0.34	0.15
ALAT (U/L)	22	0.63	**0.002**
GGT (U/L)	22	0.4	0.07
HDL-cholesterol (mmol/L)	20	−0.29	0.21
Triglycerides (mmol/L)	20	0.21	0.37
Total testosterone (nmol/L)	22	−0.1	0.64
Bio testosterone (nmol/L)	22	−0.11	0.63
Free testosterone (nmol/L)	22	−0.09	0.68
SHBG (nmol/L)	22	−0.21	0.36
DHT (nmol/L)	22	−0.22	0.33
HOMA-IR	22	0.55	**0.01**
Insulin-stimulated Rd (mg/min/m^2)	22	−0.69	**<0.001**

Spearman's rank correlation.
SAT, subcutaneous adipose abdominal tissue; TAT, total abdominal tissue; TFA, total fat area thigh; TMA, total muscle area thigh; TTA, total thigh area; VAT, visceral adipose tissue.

Hepatic fat content

To our knowledge, we are the first to assess the effect of TRT on hepatic fat content with the 'gold-standard' imaging method MRS (54) in a homogenous cohort of aging men with T2D. We hypothesized that hepatic fat content would decrease in response to TRT either as a consequence of reduced TFM (31, 35) or due to increased lipid oxidation (39, 40, 41). However, we observed no change in hepatic fat content measured by MRS. Stable VAT and hepatic fat content during TRT are consistent with the lack of change in insulin sensitivity evaluated by euglycemic–hyperinsulinemic clamp in our study as previously reported (35). In our study, overnight fasting FFA levels were not changed during TRT (35). This is in line with unchanged hepatic fat content during TRT according to the portal/visceral hypothesis stating that an increase in VAT is associated with rise in portal vein plasma FFAs (12). In support of our results, a recent study in men with T2D showed unchanged hepatic fat content during TRT assessed by MRI (30). Similarly, in men without T2D, two previous studies showed unchanged hepatic fat content during TRT (34, 36), whereas one study reported

reduced hepatic fat content (33). However, the reduction of hepatic fat content during TRT was assessed by the less precise CT in the RCT by Hoyos *et al.* (33). Besides being more obese, younger, and without T2D, the patients underwent a weight loss programme in addition to the TRT thus attenuating the conclusions that can be drawn from the study by Hoyos *et al.* (33).

Adiponectin and CVD risk

We observed a potentially unhealthy reduction in adiponectin levels during TRT even though the study cohort already at baseline had adiponectin levels comparable to adiponectin levels in the lowest quartile of men with T2D ($n = 741$) (55). A population-based study in healthy young men reported that adiponectin levels were inversely associated with SAT rather than VAT, whereas adiponectin levels were positively associated with TFA (44). However, in the present study, we could not demonstrate similar correlations at baseline, and we found no relationships between the change in adiponectin levels during TRT and changes in TFM, TFA, SAT and VAT. Our data suggest that TRT reduced adiponectin levels independent of changes in fat distribution, and this supports the view that TRT suppresses adiponectin levels through either a direct inhibition of the production or secretion and/or an increased breakdown of adiponectin (43). Previous studies in ageing men with T2D have reported that adiponectin levels were decreased (46), unchanged (30, 31) or increased (45) during TRT. However, in the study by Heufelder *et al.* (45), the modest elevation in adiponectin levels during TRT was quantitatively difficult to distinguish from the effect of diet and exercise in both the TRT and placebo group (45). In RCT's reporting unchanged adiponectin levels during TRT (30, 31), included patients were more obese compared to our study. Thus, a larger decrease in TFM, as normally seen (15), could have contributed to an increase in adiponectin, which then would hide the decrease in adiponectin levels likely caused by a direct effect of TRT. Our baseline data support a link between high adiponectin levels and healthier cardiometabolic profile as adiponectin levels were positively associated with HDL cholesterol and negatively associated with levels of TG and HOMA-IR, respectively; TRT aggravated this profile. Thus, the decrease in both levels of adiponectin and HDL cholesterol during TRT in our study might suggest a worsened cardiometabolic profile. However, the clinical impact may be minor considering the low adiponectin levels at baseline.

Leptin, leptin:adiponectin ratio and CVD risk

We confirmed that obesity assessed by TFM, BMI and WC was associated with higher levels of leptin and leptin:adiponectin ratio at baseline (56), and our data also suggested that levels of leptin and leptin:adiponectin ratio were closer associated with hepatic fat content and VAT than the subcutaneous fat compartments (SAT, TFA). Previous studies reported that leptin levels were more strongly associated with SAT than VAT (20, 22). However, in the study by Neeland *et al.* (20), leptin levels were actually associated with VAT in obese men but not in women (20). Although baseline leptin levels correlated inversely with measures of circulating testosterone in our study, the 20–30% reduction in levels of leptin and leptin:adiponectin ratio during TRT did not correlate with changes in any of the regional fat compartments (TFA, SAT, VAT, hepatic fat content). Together these findings might imply a potential direct suppressive effect of TRT on leptin levels, which may be independent of the reduction in the regional fat deposits. No other study has reported levels of leptin and leptin:adiponectin ratio in relation to regional fat compartments assessed by MRI, CT and/or MRS in ageing men with T2D. In accordance with our findings, other RCTs have reported decreased leptin levels during TRT in men with T2D (30, 46, 57).

Overall, the findings that levels of leptin and leptin:adiponectin ratio were related to markers of increased CVD risk such as increased ectopic fat, a poorer lipid profile and lower insulin sensitivity and that levels of leptin and leptin:adiponectin ratio were reduced in response to TRT, suggest that the amelioration in hyperleptinaemia during TRT even when adjusted for adiponectin may be beneficial regarding the CVD risk (27, 29).

Strengths and limitations

We have used the 'gold-standard' imaging method MRS in evaluating hepatic fat content in a homogenous patient cohort in which all patients were diagnosed with T2D. Gold-standard method was applied for testosterone measurement. We performed per-protocol analyses with a low drop-out rate, and no drop-outs were due to lack or adverse effects of the gel. The average duration

of T2D was relatively short (3–4 years). The included patients with T2D were relatively well controlled on stable antidiabetic treatment with metformin alone and had fasting insulin levels demonstrating the absence of marked beta-cell failure. This design was chosen to exclude T2D patients with increasing pancreatic beta-cell failure, and hence, poorer and more variable HbA1c levels, which would increase the need for change in antidiabetic medication. To our knowledge, there is no reason to believe that TRT improves beta-cell function, and therefore, no reason to include T2D patients with marked beta-cell failure, poorer glycemic control and need for further antidiabetic drugs. Furthermore, no changes of the antidiabetic treatment (metformin) or the cholesterol-lowering drugs were allowed throughout the study as any change would have compromised the achievement of valid results. Thus, it is not possible to generalize the observed effects of TRT in our study to all men with T2D.

Unfortunately, we were not able to establish a quantitative measure for hepatic fat content, only a change. Thus, we cannot determine whether the content of hepatic fat was actually different between groups at baseline. However, our results on hepatic fat content are not influenced by this limitation. Regrettably, the patients had difficulties in completing the MRI and MRS scans due to obesity, claustrophobia and failure in the acquisition of the MRI/MRS scans, which resulted in a considerable drop-out rate. We acknowledge the limitations of our results regarding hepatic, visceral and subcutaneous fat deposits by these drop-outs of patients from MRI/MRS scans, and we cannot exclude a type II error. However, the data reported on hepatic fat are obtained using the currently most accurate method.

In conclusion, the effects of TRT on cardiovascular risk markers were ambiguous. On one hand, we observed potentially harmful changes in cardiovascular risk parameters, markedly reduced subcutaneous fat (TFA and SAT), unchanged ectopic fat (VAT and hepatic) during TRT and a reduction in adiponectin levels. On the other hand, the decrease in leptin levels and leptin:adiponectin ratio could reflect an amelioration of the cardiovascular risk profile linked to hyperleptinaemia in ageing men with T2D.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-17-0071.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

M A conceived and designed the study protocol. D M H was responsible for testosterone analyses. P E A, A D and J O were responsible for MRI and MRS collection and MRS analysis. T L N and A N C were responsible for MRI analysis. L V M was responsible for data collection, analysis and writing. K H contributed to interpretation of data and editing of the manuscript. M A contributed to data analysis, interpretation, writing and editing of the manuscript. All authors had access to data and had final responsibility for the manuscript content. M A and L V M had final responsibility for the decision to submit for publication.

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References

1 Xu L, Freeman G, Cowling BJ & Schooling CM. Testosterone therapy and cardiovascular events among men: a systematic review and meta-analysis of placebo-controlled randomized trials. *BMC Medicine* 2013 **11** 108. (doi:10.1186/1741-7015-11-108)

2 Tanna MS, Schwartzbard A, Berger JS, Alukal J & Weintraub H. The role of testosterone therapy in cardiovascular mortality: culprit or innocent bystander? *Current Atherosclerosis Reports* 2015 **17** 490. (doi:10.1007/s11883-015-0490-0)

3 Hwang K & Miner M. Controversies in testosterone replacement therapy: testosterone and cardiovascular disease. *Asian Journal of Andrology* 2015 **17** 187–191. (doi:10.4103/1008-682X.146968)

4 Nguyen CP, Hirsch MS, Moeny D, Kaul S, Mohamoud M & Joffe HV. Testosterone and 'age-related hypogonadism' – FDA concerns. *New England Journal of Medicine* 2015 **373** 689–691. (doi:10.1056/NEJMp1506632)

5 Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, Macisaac RJ, Clarke S, Zajac JD & Jerums G. Low testosterone levels are common and associated with insulin resistance in men with diabetes. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 1834–1840. (doi:10.1210/jc.2007-2177)

6 Kotronen A, Juurinen L, Hakkarainen A, Westerbacka J, Corner A, Bergholm R & Yki-Jarvinen H. Liver fat is increased in type 2 diabetic patients and underestimated by serum alanine aminotransferase compared with equally obese nondiabetic subjects. *Diabetes Care* 2008 **31** 165–169. (doi:10.2337/dc07-1463)

7 Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE & Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes:

close association with insulin resistance and hyperinsulinemia. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1930–1935. (doi:10.1210/jc.86.5.1930)
8 Fischer S, Hanefeld M, Haffner SM, Fusch C, Schwanebeck U, Kohler C, Fucker K & Julius U. Insulin-resistant patients with type 2 diabetes mellitus have higher serum leptin levels independently of body fat mass. *Acta Diabetologica* 2002 **39** 105–110. (doi:10.1007/s005920200027)
9 Araujo AB, Dixon JM, Suarez EA, Murad MH, Guey LT & Wittert GA. Clinical review: endogenous testosterone and mortality in men: a systematic review and meta-analysis. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 3007–3019. (doi:10.1210/jc.2011-1137)
10 Katznelson L, Rosenthal DI, Rosol MS, Anderson EJ, Hayden DL, Schoenfeld DA & Klibanski A. Using quantitative CT to assess adipose distribution in adult men with acquired hypogonadism. *American Journal of Roentgenology* 1998 **170** 423–427. (doi:10.2214/ajr.170.2.9456958)
11 Neeland IJ, Turer AT, Ayers CR, Berry JD, Rohatgi A, Das SR, Khera A, Vega GL, McGuire DK, Grundy SM *et al*. Body fat distribution and incident cardiovascular disease in obese adults. *Journal of the American College of Cardiology* 2015 **65** 2150–2151. (doi:10.1016/j.jacc.2015.01.061)
12 Heilbronn L, Smith SR & Ravussin E. Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. *International Journal of Obesity and Related Metabolic Disorders* 2004 **28** (Supplement 4) S12–S21. (doi:10.1038/sj.ijo.0802853)
13 Targher G, Day CP & Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *New England Journal of Medicine* 2010 **363** 1341–1350. (doi:10.1056/NEJMra0912063)
14 Stefan N, Fritsche A, Schick F & Haring HU. Phenotypes of prediabetes and stratification of cardiometabolic risk. *Lancet Diabetes and Endocrinology* 2016 **4** 789–798. (doi:10.1016/S2213-8587(16)00082-6)
15 Stern JH, Rutkowski JM & Scherer PE. Adiponectin, leptin, and fatty acids in the maintenance of metabolic homeostasis through adipose tissue crosstalk. *Cell Metabolism* 2016 **23** 770–784. (doi:10.1016/j.cmet.2016.04.011)
16 Stefan N & Haring HU. The role of hepatokines in metabolism. *Nature Reviews Endocrinology* 2013 **9** 144–152. (doi:10.1038/nrendo.2012.258)
17 Unger RH. The physiology of cellular liporegulation. *Annual Review of Physiology* 2003 **65** 333–347. (doi:10.1146/annurev.physiol.65.092101.142622)
18 Carter S, Caron A, Richard D & Picard F. Role of leptin resistance in the development of obesity in older patients. *Clinical Interventions in Aging* 2013 **8** 829–844. (doi:10.2147/CIA.S36367)
19 Frederiksen L, Hojlund K, Hougaard DM, Mosbech TH, Larsen R, Flyvbjerg A, Frystyk J, Brixen K & Andersen M. Testosterone therapy decreases subcutaneous fat and adiponectin in aging men. *European Journal of Endocrinology* 2012 **166** 469–476. (doi:10.1530/EJE-11-0565)
20 Neeland IJ, Ayers CR, Rohatgi AK, Turer AT, Berry JD, Das SR, Vega GL, Khera A, McGuire DK, Grundy SM *et al*. Associations of visceral and abdominal subcutaneous adipose tissue with markers of cardiac and metabolic risk in obese adults. *Obesity* 2013 **21** E439–E447. (doi:10.1002/oby.20135)
21 Manolopoulos KN, Karpe F & Frayn KN. Gluteofemoral body fat as a determinant of metabolic health. *International Journal of Obesity (2005)* 2010 **34** 949–959. (doi:10.1038/ijo.2009.286)
22 Wium C, Eggesbo HB, Ueland T, Michelsen AE, Torjesen PA, Aukrust P & Birkeland K. Adipose tissue distribution in relation to insulin sensitivity and inflammation in Pakistani and Norwegian subjects with type 2 diabetes. *Scandinavian Journal of Clinical and Laboratory Investigation* 2014 **74** 700–707. (doi:10.3109/00365513.2014.953571)
23 Fasshauer M & Bluher M. Adipokines in health and disease. *Trends in Pharmacological Sciences* 2015 **36** 461–470. (doi:10.1016/j.tips.2015.04.014)
24 Bays HE, Gonzalez-Campoy JM, Bray GA, Kitabchi AE, Bergman DA, Schorr AB, Rodbard HW & Henry RR. Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. *Expert Review of Cardiovascular Therapy* 2008 **6** 343–368. (doi:10.1586/14779072.6.3.343)
25 Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K & Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *Journal of Clinical Investigation* 2006 **116** 1784–1792. (doi:10.1172/jci29126)
26 Bluher M & Mantzoros CS. From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. *Metabolism* 2015 **64** 131–145. (doi:10.1016/j.metabol.2014.10.016)
27 Hou N & Luo JD. Leptin and cardiovascular diseases. *Clinical and Experimental Pharmacology and Physiology* 2011 **38** 905–913. (doi:10.1111/j.1440-1681.2011.05619.x)
28 Al-Hamodi Z, Al-Habori M, Al-Meeri A & Saif-Ali R. Association of adipokines, leptin/adiponectin ratio and C-reactive protein with obesity and type 2 diabetes mellitus. *Diabetology and Metabolic Syndrome* 2014 **6** 99. (doi:10.1186/1758-5996-6-99)
29 Satoh N, Naruse M, Usui T, Tagami T, Suganami T, Yamada K, Kuzuya H, Shimatsu A & Ogawa Y. Leptin-to-adiponectin ratio as a potential atherogenic index in obese type 2 diabetic patients. *Diabetes Care* 2004 **27** 2488–2490. (doi:10.2337/diacare.27.10.2488)
30 Dhindsa S, Ghanim H, Batra M, Kuhadiya ND, Abuaysheh S, Sandhu S, Green K, Makdissi A, Hejna J, Chaudhuri A *et al*. Insulin resistance and inflammation in hypogonadotropic hypogonadism and their reduction after testosterone replacement in men with type 2 diabetes. *Diabetes Care* 2016 **39** 82–91. (doi:10.2337/dc15-1518)
31 Gianatti EJ, Dupuis P, Hoermann R, Strauss BJ, Wentworth JM, Zajac JD & Grossmann M. Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: a randomized controlled trial. *Diabetes Care* 2014 **37** 2098–2107. (doi:10.2337/dc13-2845)
32 Haider A, Gooren LJ, Padungtod P & Saad F. Improvement of the metabolic syndrome and of non-alcoholic liver steatosis upon treatment of hypogonadal elderly men with parenteral testosterone undecanoate. *Experimental and Clinical Endocrinology and Diabetes* 2010 **118** 167–171. (doi:10.1055/s-0029-1202774)
33 Hoyos CM, Yee BJ, Phillips CL, Machan EA, Grunstein RR & Liu PY. Body compositional and cardiometabolic effects of testosterone therapy in obese men with severe obstructive sleep apnoea: a randomised placebo-controlled trial. *European Journal of Endocrinology* 2012 **167** 531–541. (doi:10.1530/EJE-12-0525)
34 Huang G, Bhasin S, Tang ER, Aakil A, Anderson SW, Jara H, Davda M, Travison TG & Basaria S. Effect of testosterone administration on liver fat in older men with mobility limitation: results from a randomized controlled trial. *Journal of Gerontology: Series A, Biological Sciences and Medical Sciences* 2013 **68** 954–959. (doi:10.1093/gerona/gls259)
35 Magnussen LV, Glintborg D, Hermann P, Hougaard DM, Hojlund K & Andersen M. Effect of testosterone on insulin sensitivity, oxidative metabolism, and body-composition in aging men with type 2 diabetes on metformin monotherapy. *Diabetes, Obesity and Metabolism* 2016 **18** 980–989. (doi:10.1111/dom.12701)
36 Sattler F, He J, Chukwuneke J, Kim H, Stewart Y, Colletti P, Yarasheski K & Buchanan T. Testosterone supplementation improves carbohydrate and lipid metabolism in some older men with abdominal obesity. *Journal of Gerontology and Geriatric Research* 2014 **3** 1000159. (doi:10.4172/2167-7182.1000159)
37 Singh R, Artaza JN, Taylor WE, Gonzalez-Cadavid NF & Bhasin S. Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. *Endocrinology* 2003 **144** 5081–5088. (doi:10.1210/en.2003-0741)
38 Xu XF, De Pergola G & Bjorntorp P. Testosterone increases lipolysis and the number of beta-adrenoceptors in male rat adipocytes. *Endocrinology* 1991 **128** 379–382. (doi:10.1210/endo-128-1-379)

39 Birzniece V, Meinhardt UJ, Handelsman DJ & Ho KK. Testosterone stimulates extra-hepatic but not hepatic fat oxidation (Fox): comparison of oral and transdermal testosterone administration in hypopituitary men. *Clinical Endocrinology* 2009 **71** 715–721. (doi:10.1111/j.1365-2265.2009.03524.x)

40 Gibney J, Wolthers T, Johannsson G, Umpleby AM & Ho KK. Growth hormone and testosterone interact positively to enhance protein and energy metabolism in hypopituitary men. *American Journal of Physiology: Endocrinology and Metabolism* 2005 **289** E266–E271. (doi:10.1152/ajpendo.00483.2004)

41 Frederiksen L, Hojlund K, Hougaard DM, Brixen K & Andersen M. Testosterone therapy increased muscle mass and lipid oxidation in aging men. *Age* 2012 **34** 145–156. (doi:10.1007/s11357-011-9213-9)

42 Isidori AM, Giannetta E, Greco EA, Gianfrilli D, Bonifacio V, Isidori A, Lenzi A & Fabbri A. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. *Clinical Endocrinology* 2005 **63** 280–293. (doi:10.1111/j.1365-2265.2005.02339.x)

43 De Maddalena C, Vodo S, Petroni A & Aloisi AM. Impact of testosterone on body fat composition. *Journal of Cellular Physiology* 2012 **227** 3744–3748. (doi:10.1002/jcp.24096)

44 Frederiksen L, Nielsen TL, Wraae K, Hagen C, Frystyk J, Flyvbjerg A, Brixen K & Andersen M. Subcutaneous rather than visceral adipose tissue is associated with adiponectin levels and insulin resistance in young men. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 4010–4015. (doi:10.1210/jc.2009-0980)

45 Heufelder AE, Saad F, Bunck MC & Gooren L. Fifty-two-week treatment with diet and exercise plus transdermal testosterone reverses the metabolic syndrome and improves glycemic control in men with newly diagnosed type 2 diabetes and subnormal plasma testosterone. *Journal of Andrology* 2009 **30** 726–733. (doi:10.2164/jandrol.108.007005)

46 Kapoor D, Clarke S, Stanworth R, Channer KS & Jones TH. The effect of testosterone replacement therapy on adipocytokines and C-reactive protein in hypogonadal men with type 2 diabetes. *European Journal of Endocrinology* 2007 **156** 595–602. (doi:10.1530/EJE-06-0737)

47 Christian Thode L, Eugenio Iglesias J & Leemput KV. N3 bias field correction explained as a bayesian modeling method. In *Bayesian and Graphical Models for Biomedical Imaging. Lecture Notes in Computer Science*. Eds MJ Cardoso, I Simpson, T Arbel, D Precup & A Ribbens. Cham: Springer, 2014.

48 Li K, Wu X, Chen DZ & Sonka M. Optimal surface segmentation in volumetric images – a graph-theoretic approach. *IEEE Transactions on Pattern Analysis and Machine Intelligence* 2006 **28** 119–134. (doi:10.1109/TPAMI.2006.19)

49 Frystyk J, Tarnow L, Hansen TK, Parving HH & Flyvbjerg A. Increased serum adiponectin levels in type 1 diabetic patients with microvascular complications. *Diabetologia* 2005 **48** 1911–1918. (doi:10.1007/s00125-005-1850-z)

50 Munzer T, Harman SM, Hees P, Shapiro E, Christmas C, Bellantoni MF, Stevens TE, O'Connor KG, Pabst KM, St Clair C *et al*. Effects of GH and/or sex steroid administration on abdominal subcutaneous and visceral fat in healthy aged women and men. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 3604–3610. (doi:10.1210/jcem.86.8.7773)

51 Schroeder ET, Zheng L, Ong MD, Martinez C, Flores C, Stewart Y, Azen C & Sattler FR. Effects of androgen therapy on adipose tissue and metabolism in older men. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 4863–4872. (doi:10.1210/jc.2004-0784)

52 Svartberg J, Agledahl I, Figenschau Y, Sildnes T, Waterloo K & Jorde R. Testosterone treatment in elderly men with subnormal testosterone levels improves body composition and BMD in the hip. *International Journal of Impotence Research* 2008 **20** 378–387. (doi:10.1038/ijir.2008.19)

53 Marin P, Holmang S, Gustafsson C, Jonsson L, Kvist H, Elander A, Eldh J, Sjostrom L, Holm G & Bjorntorp P. Androgen treatment of abdominally obese men. *Obesity Research* 1993 **1** 245–251. (doi:10.1002/j.1550-8528.1993.tb00618.x)

54 Koplay M, Sivri M, Erdogan H & Nayman A. Importance of imaging and recent developments in diagnosis of nonalcoholic fatty liver disease. *World Journal of Hepatology* 2015 **7** 769–776. (doi:10.4254/wjh.v7.i5.769)

55 Schulze MB, Rimm EB, Shai I, Rifai N & Hu FB. Relationship between adiponectin and glycemic control, blood lipids, and inflammatory markers in men with type 2 diabetes. *Diabetes Care* 2004 **27** 1680–1687. (doi:10.2337/diacare.27.7.1680)

56 Stanley S, Wynne K, McGowan B & Bloom S. Hormonal regulation of food intake. *Physiological Reviews* 2005 **85** 1131–1158. (doi:10.1152/physrev.00015.2004)

57 Kalinchenko SY, Tishova YA, Mskhalaya GJ, Gooren LJ, Giltay EJ & Saad F. Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. *Clinical Endocrinology* 2010 **73** 602–612. (doi:10.1111/j.1365-2265.2010.03845.x)

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