Testosterone therapy of men with type 2 diabetes mellitus

A randomized, double-blinded, placebo-controlled study

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The three original papers are:

1. Magnussen LV, Glintborg D, Hermann P, Hougaard DM, Højlund K, Andersen M. Insulin sensitivity assessed by clamp method during testosterone in type 2 diabetes, a randomized controlled trial. Submitted. Accepted manuscript in Diabetes Obes Metab. 2016 Oct;18(10):980-9. doi: 10.1111/dom.12701. Epub 2016 Jul 12.

2. Magnussen LV, Andersen PE, Diaz A, Ostojic J, Højlund K, Hougaard D, Nielsen TL, Andersen M. Changes in ectopic fat, adiponectin, and leptin levels during testosterone therapy in type 2 diabetes. A randomized, double-blinded, placebo-controlled trial. Ready to be submitted. Accepted manuscript in Eur J Endocrinol 2017 May 18. Epub ahead of print.

3. Magnussen LV*, Hvid LG*, Hermann P, Hougaard DM, Gram B, Caserotti P, Andersen M. Testosterone therapy increases lean leg mass and preserves muscle mechanical function in aging men with type 2 diabetes, a randomized, double-blinded, placebo-controlled trial. *contributed equally. Submitted.

ABBREVIATIONS

ALAT	Alanintransaminase
BioT	Bioavailable testosterone
BMI	Body mass index
CPM	Counts per minute
CV	Coefficient of variation
CVD	Cardiovascular disease
Dyn180	Maximal voluntary dynamic contraction
DXA	Whole-body Dual-energy X-ray Absorptiometry scan
FFA	Free fatty acid
FreeT	Free testosterone
GGT	Gamma-glutamyltransferase
GOX	Glucose oxidation rate
HbA1c	Glycated hemoglobin
HGP	Hepatic glucose production
HOMA-IR	Homeostasis assessment model insulin re sistance
LBM	Total lean body mass
LOX	Lipid oxidation rate
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MVC	Maximal voluntary isometric contraction
NAFLD	Non-alcoholic fatty liver disease
NOGD	Non-oxidative glucose disposal
PSA	Prostate specific antigen
RCT	Randomized controlled trial
Rd	Glucose disposal rate
RFD100	Rate of force development
RQ	Respiratory quotient
SAT	Subcutaneous abdominal adipose tissue
SD	Standard deviation
SHBG	Sex hormone binding globulin
T2D	Type 2 diabetes
T-levels	Testosterone levels
TAT	Total abdominal tissue
TFA	Thigh subcutaneous fat area
TFM	Total fat mass
TMA	Total muscle area thigh
TotalT	Total testosterone
TRT	Testosterone replacement therapy
TTA	Total thigh area
VAT	Visceral adipose tissue
WC	Waist circumference

INTRODUCTION

The prevalence of chronic diseases including obesity and type 2 diabetes mellitus (T2D) are increasing. In developed countries, the majority of all deaths are caused by chronic disease (4). Studies indicate that 80% of early heart disease and T2D can be prevented (5). Prevention of T2D and new treatment modalities are therefore essential (6, 7).

Testosterone replacement therapy (TRT) has escalated in the Western countries during the past decades especially in aging men without clear organic indication for TRT (8). However, who to treat has not been clarified (9, 10) and the safety of long-term TRT regarding the risk of cardiovascular disease (CVD) is unknown (11-13). Aging men with T2D often have lowered testosterone

levels (T-levels) (14), ectopic fat depots (15), a deranged adipokine profile with e.g. low adiponectin levels (16), hyperleptinaemia (17), and an increased risk of CVD (14, 18). The causal relations are unclear, and lowered T-levels could simply be a marker of illness, i.e. T2D and obesity (18).

TRT improves body composition with an increased total lean body mass (LBM) and a reduction in total fat mass (TFM) (19, 20) which may be of further benefit in aging men with T2D as this improved body composition, in theory, may ameliorate the insulin resistance in T2D, reduce ectopic fat, improve a deranged adipokine secretion, and advance physical strength and function. This project will contribute to the clarification of the beneficial and potential harmful effects of TRT in aging men with T2D and lowered T-levels. No previous randomized, double-blinded, placebo-controlled study in aging men with T2D has ever evaluated the effect of TRT applying only gold-standard methods such as mass spectrometry, whole-body Dual-energy X-ray Absorptiometry (DXA), euglycemic-hyperinsulinemic clamp, and Magnetic Resonance Spectroscopy (MRS) in patients with T2D on one stable anti-diabetic treatment (Table 1).

Table 1 Randomized, double-blinded, placebo-controlled trials in aging men with T2D during testosterone therapy

Author year	n	Age (yrs.)	BMI (kg/m²)	Testosterone treatment	Time (wks.)	Diabetic treatment	Body composition	DXA/CT/MRI	Glycemic outcome	Adiponectin and leptin	Lipids
Kapoor 2006, 2007 (21, 22)	24	>30	33	TE 200 mg im 2-weekly	12	Mixed oral and/or insulin	→ BMI ↓ WC ↓ WHR → %body fat → fat free mass	ND	↓ HOMA-IR ↓ HbA1c	↓ adiponectin ↓ leptin	↓ T-chol → LDL → HDL → TG
Gopal 2010 (23)	22	39–50	24	TE 200 mg im 2-weekly	12	Mixed oral and/or insulin	→ BMI → WC → WHR	ND	→ HOMA-IR → HbA1c	NR	→ T-chol → LDL → HDL → TG
Kalinchenko 2010 (24)	170§	35–70	35	TU 1000 mg im 6-weekly once and then 12-weekly	30	Mixed oral and/or insulin	↓ weight ↓ BMI ↓ WC ↓ WHR	ND	↓ HOMA-IR NR HbA1c	NR adiponectin ↓ leptin	→ T-chol → LDL → HDL → TG
Jones 2011 (25)	220#	≥40	32	Transdermal T 60 mg/d	26	Mixed oral and/or insulin	→ BMI → WC → %body fat	ND	↓ HOMA-IR → HbA1c	NR	↓ T-chol → LDL ↓ HDL → TG
Hackett 2014 (26)	190	18–80	33	TU 1000 mg im 6-weekly once and then 12-weekly	30	NR	→ weight → BMI ↓ WC	ND	→ HOMA-IR → HbA1c	NR	↓ T-chol → LDL → HDL → TG
Gianatti 2014 (27)	75	35–70	32	TU 1000 mg im 6-weekly once and then 12-weekly	40	Mixed oral and/or insulin	→ BMI → WC	↑ LBM ↓ TFM ↓ SAT → VAT	→ HOMA-IR → HbA1c	→ adiponectin NR leptin	↓ T-chol ↓ LDL ↓ HDL → TG
Magnussen PhD thesis (1-3)	39	50–70	31	Transdermal T 50 or 100 mg/d	24	Only metformin	→ weight → BMI → WC	↑ LBM ↓ TFM ↓ SAT → VAT → hepatic fat	→ Rd clamp → HOMA-IR → HbA1c	↓ adiponectin ↓ leptin ↓ adiponectin: leptin ratio	→ T-chol → LDL ↓ HDL → TG

§n=48 with T2D. #n=137 with T2D. WHR=Waist-Hip-Ratio, WC=Waist Circumference, T-chol=Total-Cholesterol, TG=Triglycerides, VAT=Visceral Adipose Tissue, SAT= Subcutaneous abdominal Adipose Tissue, →=unchanged, ↓=decreased, ↑=increased. NR=Not Reported, ND=Not Done

BACKGROUND

Hypogonadism in men

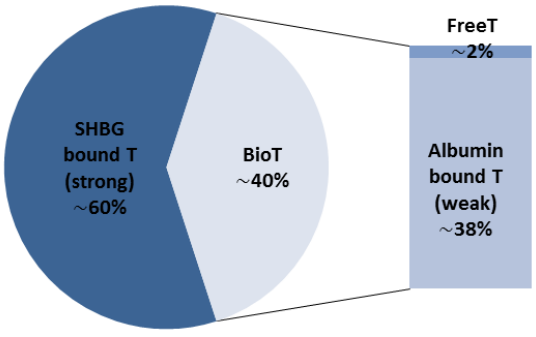
Classical hypogonadism is a clinical syndrome that results from failure of the testis to produce physiological levels of testosterone and a normal number of spermatozoa due to a disorder in the hypothalamic-pituitary-testicular axis (28). Hypogonadism can be classified as primary (testicular cause), secondary (hypothalamic/pituitary cause), or mixed hypogonadism (dual defects in the hypothalamic-pituitary axis and the testes) (29). Age-associated testosterone deficiency, also named Late Onset Hypogonadism (LOH), is the alleged clinical syndrome seen with advancing age characterized by symptoms and serum T-levels below the reference range of healthy young men (30). In aging men, several factors can result in the often mixed hypogonadism such as impaired Leydig cell function, loss of circadian rhythm of GnRH/LH/FSH-secretion in the hypothalamic-pituitary-axis, increased sex hormone binding globulin (SHBG) levels, changes in testosterone receptor sensitivity, and/or effects of altered cardiometabolic and inflammatory markers (29).

Symptoms and signs suggestive of hypogonadism are often less specific and might increase with age due to other mechanisms/diseases than lowered T-levels in itself, e.g. decreased muscle mass and strength, increased body fat, visceral obesity, diminished physical capacity, decreased energy and initiative (29). Even though a considerable portion of men with lowered T-levels can be asymptomatic (10), they may still benefit from TRT. The lack of coherence between measurements of biochemical hypogonadism and symptoms consistent with hypogonadism (9, 10) complicates the identification of the correct patients for TRT.

Biochemical hypogonadism

We applied a cut-off for lowered testosterone (bioavailable testosterone (BioT) <7.3 nmol/l) as a measurement for hypogonadism regardless of the presence of symptoms, given the lack of consistency between biochemical hypogonadism and symptoms of hypogonadism (9, 10). The cut-off levels for testosterone were determined in a previous population-based cohort of 783 men aged 20–29 years (31, 32). The cut-off value of BioT <7.3 nmol/l was defined in the n=615 non-obese, healthy men corresponding to the 2.5 percentile of the 95% confidence interval (31). The narrow age range of the young men limits the potential bias of cohort effects and limits the exposure to potential environmental confounders (32). The prevalence of lowered testosterone levels depends on the patients in question and on what basis the cut-off value for lowered testosterone is obtained in regard to health, BMI, and age. There is a consensus of using a young cohort as a reference group (14, 33, 34) because BioT/FreeT levels decline 1-2% per year in some men already from the age of 30 years (35, 36). Furthermore, in order to obtain reliable measurements of T-levels it is evident that each laboratory determines its own normal limits because of considerable variability of values obtained in different laboratories using different techniques and kits (37). Data suggest that total testosterone (TotalT) levels, which are mostly bound to the transport proteins albumin and SHBG (Fig. 1) (38), are not affected by age in lean healthy men (35). However, SHBG levels increase with age causing lowered levels of BioT and FreeT (35). Hepatic SHBG production can also differ between individuals due to genetic variations, metabolic, nutritional, and endocrine factors (39), e.g. obesity and hyperinsulinemia that are each associated with decreasing levels of TotalT and SHBG (39). Consequently, TotalT concentrations alone might sometimes be misrepresentative whereas BioT and FreeT are considered more appropriate representatives of tissue activity (19, 40). In the existing literature, the various cut-off limits for TotalT in men range from 7.2–12.1 nmol/l using various analytical procedures (31, 40) and reference groups. Although who to treat has not been clarified in general (9, 10), guidelines by the Endocrine Society suggest that clinicians should consider detection of hypogonadism in men with T2D using BioT or FreeT levels when TotalT levels are close to the lower limit of the normal range (28). Lowered BioT/FreeT levels have been observed in up to 58% of aging men with T2D compared with healthy young men (1, 14, 33).

Figure 1 Total testosterone – distribution in the blood.



The current practice guidelines recommend that serum testosterone target concentrations for older men should be within the mid-reference range of young adult men (28) which is achievable within the first day of gel application, and a steady state of T-levels is reached within a few days with once daily repeated application (41, 42). However, according to recent statements by the FDA the indication for treatment of LOH is currently not accepted because there is no definitive evidence that TRT in these men is beneficial or even safe (8). Thus, the indication to replace testosterone in older men who lack a distinct, well-recognized cause of hypogonadism remains debatable.

Muscle and fat mass

Testosterone regulates body composition by promoting the commitment of mesenchymal pluripotent cells into myogenic lineage and inhibiting their differentiation into the adipogenic lineage (43). Muscle mass is a function of the mean volume of the muscle fibres and the number of fibres present in a given skeletal muscle (44). The age-related decrease in T-levels is associated with loss of muscle mass however such a clinical feature can also occur in elderly men with sufficient endogenous T-levels (20, 45). Thus, the etiology of age-related diminished muscle mass is probably multifactorial and is due to an imbalance of protein synthesis and protein degradation (46). In general, the atrophy of the skeletal muscles is first noticeable after 40 years of age and for almost all by the age of 50 years (44). Any earlier loss in muscle mass is attributable to a loss in the volume of the muscle fibres due to a sedentary lifestyle, because no difference in the number of fibres is observed between 20–50 years of age (44).

Non-obese, hypogonadal men may have more subcutaneous fat compared with eugonadal men whereas no difference is found regarding visceral adipose tissue (VAT) (47). Irrespective of T-levels, ectopic fat storage occur when continuous nutritional overload leads to adipocyte expansion failure in the normal physiological subcutaneous fat compartments, resulting in so-called “acquired lipodystrophy” (48). This fat storage at ectopic sites is further promoted by aging in itself (49). TRT may reduce the burden of ectopic fat through the generally increased lipolysis and the inhibition of the adipogenic lineage (43, 50).

Insulin resistance in aging men with T2D

Insulin resistance can be defined as an impaired biological response to either endogenous or exogenous insulin which can be demonstrated by an impaired insulin action on whole-body glucose metabolism (51). The euglycemic-hyperinsulinemic clamp method is generally accepted as the “gold-standard” for assessing whole-body insulin sensitivity, whereas other less precise measures such as the homeostasis model of glucose tolerance (HOMA-IR) are cheaper and less time-consuming (51). Patients with T2D are in general more insulin-resistant than age- and weight-matched controls (51). Thus, the potential for detection of improvement in insulin sensitivity during TRT may be higher in men with T2D compared to men without T2D. No previous study has evaluated insulin sensitivity by the euglycemic-hyperinsulinemic clamp during TRT in aging men with T2D and lowered T-levels.

Skeletal muscle, adipose tissue, and the liver are the main target tissues of insulin action (51). Insulin resistance may be reduced during TRT because of the increase in LBM (20) with increased glucose uptake and storage (i.e. non-oxidative glucose disposal (NOGD)) as skeletal muscle is known to be the major site of glucose disposal (up to 80%) in the insulin-stimulated state (51). A reduced fat mass may also ameliorate the insulin resistance during TRT (20). Three paradigms have been used to explain the link between obesity and insulin resistance/T2D. Firstly, the portal/visceral hypothesis, secondly, the ectopic fat storage syndrome, and thirdly, the endocrine paradigm (52). The portal vein/visceral hypothesis proposes that increased VAT leads to increased FFA flux in the portal vein plasma and inhibition of insulin-action via Randle's effect in insulin-sensitive tissues (53). Hence, a decreased VAT during TRT may reduce insulin resistance. However, TRT might exacerbate insulin resistance by stimulating lipolysis (50) and inhibiting lipoprotein lipase activity in abdominal adipose tissue thus leading to increased triglyceride levels in the blood (54). Ultimately, elevated FFAs might cause an increased amount of ectopic fat in the muscles (51). Increased ectopic fat is postulated to cause defects in insulin signaling in muscle and liver, reduce insulin-stimulated muscle glucose transport activity and glycogen synthesis in muscle, and impair suppression of hepatic gluconeogenesis (52). Hyperinsulinemia in T2D might exacerbate the impaired suppression of gluconeogenesis with the continuation of lipogenesis (51) and could be responsible for the development of non-alcoholic fatty liver disease (NAFLD). NAFLD is considered an excellent surrogate indicator for hepatic insulin sensitivity (55). The endocrine hypothesis is based on the growing number of factors that are released from adipocytes with potent effects on metabolism and insulin sensitivity (52). The TRT reduced TFM may decrease the pro-inflammatory cytokines (56) associated with insulin resistance and the volume of ectopic fat. Furthermore, testosterone could have a more direct beneficial role on intracellular molecular mechanisms involved in insulin signaling, and hence the regulation of insulin sensitivity independent of changes in body composition (57-59).

Metabolic inflexibility in T2D

The euglycemic-hyperinsulinemic clamp combined with indirect calorimetry enables assessment of NOGD (reflecting glycogen synthesis) and substrate oxidation in the basal and insulin-stimulated state. Impaired insulin-stimulated NOGD is a major defect in T2D (60). Muscle glucose oxidation and lipid oxidation are abnormal in T2D and obesity during both basal and insulin-stimulated conditions (51). During fasting conditions, muscle glucose oxidation is increased and reliance on lipid oxidation is decreased in T2D (61). Moreover, patients with T2D often have an impaired ability to switch from lipid oxidation to predominantly carbohydrate oxidation during insulin-stimulated conditions, described as “metabolic inflexibility” (61). This impaired ability might play a major role in skeletal muscle insulin resistance (51). In aging men without T2D, recent studies indicate that TRT may promote a shift in substrate partitioning towards increased lipid oxidation in the basal, fasting state both in men with lowered BioT levels (19) and in men with secondary hypogonadism (62).

The adipokine burden in T2D

Adipokines and pro-inflammatory cytokines produced by white adipose tissue might be pivotal in the pathogenesis of insulin resistance (63) and NAFLD (64, 65). So far, levels of adiponectin and leptin have been assessed in this project.

Adiponectin is thought to improve insulin sensitivity by reducing gluconeogenesis in the liver (66) and by increasing glucose uptake, glycogen synthesis, and influx and combustion of FFAs in skeletal muscle (67). Adiponectin may also have anti-atherogenic and anti-inflammatory effects (67). Adiponectin levels are significantly lower in patients with insulin resistance and/or T2D (16).

Testosterone might be a negative regulator of adiponectin production in humans (68). However, considering the beneficial effect of TRT on body composition, TRT may increase adiponectin levels as several studies have reported a negative association between levels of adiponectin and TFM (69-71), insulin resistance (72), and BMI (69-71).

Leptin is a peptide hormone (73) produced predominantly in adipocytes (74). The long form of the leptin receptor, Ob-Rb, is expressed widely within the hypothalamus and also in appetite-modulating pathways in the brain stem (73). However, the mechanisms underlying the effects of energy stores and energy balance on leptin responsiveness is unclear (75). The role of leptin can be described as a double-edged sword. Healthy lean individuals are in a low leptin state where leptin regulates food intake and has insulin-sensitizing effects (76, 77) whereas in hyperleptinaemia as in obesity and/or T2D, leptin is considered a pro-inflammatory adipokine (78) associated with an increased risk of CVD (79). Hyperleptinaemia without a concomitant increase in leptin activity is a state called leptin resistance (49) and decreasing leptin levels may represent a healthier cardio-metabolic/inflammatory profile (49). Leptin levels are inversely correlated with T-levels (80). Theoretically, increasing T-levels may decrease leptin levels through a reduction in fat mass, a stimulation of the splanchnic β-adrenoceptors (81), or by a direct suppressive effect on ob gene expression (82). 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 (83, 84).

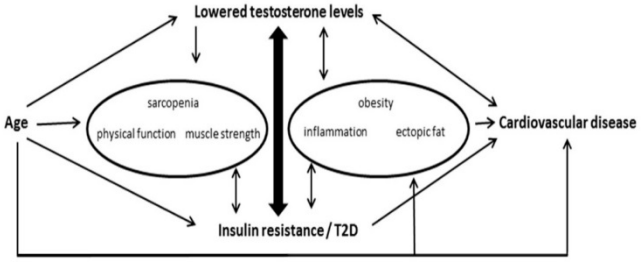
Muscle strength and physical function

Lowered T-levels, insulin resistance, and physical inactivity are each associated with loss of strength and skeletal muscle mass (20, 45). Strength and muscle mass are in general highly correlated. TRT in aging men with lowered T-levels can produce muscle hypertrophy (45) and reduce fat mass (20). Thus, TRT could be an attractive alternative or supplement to resistance training in the effort to prevent or treat age-related declines in muscle mechanical function especially in aging men who are not able to participate in high intensity training regimes (85). An excessive loss of strength and muscle mass define the sarcopenia syndrome (86), which is associated with a higher risk of adverse health outcomes such as physical disability, poor quality of life, and death (86, 87). Aging men with T2D might have an increased risk of developing sarcopenia due to the decreased T-levels and insulin resistance (87). Furthermore, the increased ectopic fat in skeletal muscles in T2D might lead to impaired protein synthesis (88), irreversible changes in skeletal muscle structure and function, and an increasing sedentary lifestyle, contributing to increased frailty (44) and risk of CVD with aging.

Cardiovascular disease in aging men with T2D and lowered testosterone levels

Lowered T-levels and T2D are each associated with CVD (18, 89, 90) however it is unclear whether lowered T-levels are causally related to CVD or merely a marker of illness e.g. T2D and/or obesity (18). In endothelial cells, the compensatory hyperinsulinemia in T2D may enhance pro-hypertensive and atherogenic actions of insulin (51). The increased risk for CVD in aging men with T2D with lowered T-levels might be attributed to a decreased muscle mass, an increased total and ectopic fat mass, insulin resistance, increased inflammation including a deranged adipokine profile (52) with reduced adiponectin levels (16, 91) and high leptin levels (17) (Fig. 2).

Figure 2 Associations between prevalent characteristics in aging men with T2D



Regarding CVD, thigh subcutaneous fat area (TFA) is more protective compared to subcutaneous abdominal adipose tissue (SAT) (92, 93) possibly due to a long-term entrapment of excess FFA (94, 95) and a more favorable adipokine secreting profile (96). In contrast, ectopic fat such as VAT and NAFLD are associated with an increased CVD risk (95).

The safety of long-term TRT has not been clarified regarding CVD (11-13, 89, 97, 98) and the committee established by the FDA recently concluded that the totality of the evidence regarding CVD suggests a weak signal of cardiovascular risk and recommended updating drug labels to reflect this information (8). The FDA also stressed that to date, no randomized, controlled trials (RCTs) have been appropriately designed to evaluate cardiovascular outcomes with testosterone use (8).

AIM OF THE STUDY

The aim of the study was to contribute to the clarification of the beneficial and potential harmful effects of TRT in aging men with T2D and lowered T-levels.

In manuscript I, we evaluate the effect of TRT on lean body mass, fat mass, insulin sensitivity, and oxidative metabolism in aging men with T2D and lowered T-levels. In manuscript II, we examine whether TRT reduces the volume of ectopic fat and improves the adipokine profile in aging men with T2D. In manuscript III, we evaluate whether TRT improves muscle mechanical and physical function in addition to increased lean body mass in aging men with T2D and lowered T-levels.

MATERIAL AND METHODS

All text in this section refers to the three manuscripts.

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.

Study population

Caucasian men, aged 50–70 years, BioT levels <7.3 nmol/l, and a diagnosis of T2D within three months to 10 years were eligible for inclusion. A total of 59 patients were screened, 43 patients were included, and 39 men completed the study. To find eligible patients, we used advertisement in newspapers, in magazines, at general practitioners, and through written invitations to patients with newly diagnosed T2D, who were referred to our department at Odense University Hospital.

The patients were all treated for T2D and metformin was the only allowed anti-diabetic drug. This treatment had to be stable for at least 3 months prior to inclusion and no change in anti-diabetic

medication dose was allowed during the study. The exclusion criteria were BMI ≥40 kg/m2, glycated hemoglobin (HbA1c) ≥9.0% (75 mmol/mol), hematocrit >50%, prostate specific antigen (PSA) >3 ng/dl, abnormal routine blood samples, nocturia >3 times, clinically significant disease of the heart, lung or kidneys, known malignant disease, severe hypertension, abuse of alcohol, medicine, or drugs within a year, significant electrocardiogram changes, primary or secondary hypogonadism, ongoing severe mental illness, wish of fatherhood within the trial period, severe claustrophobia, and treatment with vitamin K antagonist, morphine, 5α reductase inhibitors, oral glucocorticoid or antipsychotics. Diagnosis of hemochromatosis was excluded at the 24-week visit by measuring iron and transferrin in plasma.
The patients were allowed to continue on habitual physical activities throughout the study and were informed not to change their diet. Patients were instructed to avoid strenuous physical activity 48h before the clamp.

Symptoms and signs suggestive of hypogonadism
At the screening visit, patients were asked about symptoms and signs suggestive of hypogonadism in accordance to a clinical practice guideline (28).

Metformin and concomitant medication
The use of metformin was equally distributed in regard to low dosage and maximum dosage between the two groups. A total of 69.2% (27/39), 84.6% (33/39), 33.3% (13/39), 7.7% (3/39), and 7.7% (3/39) patients were on antihypertensive drugs, cholesterol-lowering drugs, anti-thrombotic drugs, inhalation steroids, and anti-depressants, respectively. The concomitant medication was equally distributed between the two groups. No cholesterol-lowering drugs were introduced during the study. All patients took their usual medication in the morning they attended the clinic. Any antithrombotic agents were stopped 3–7 days before the clamp.

Randomization and blinding
Eligible patients were randomized and numbers were assigned according to enrolment into the study. Randomization was performed by Hospital Pharmacy Fyn, Odense University Hospital, using the computer program www.randomization.com with four patients in each block. Trial investigators and patients were blinded to the intervention allocation.

Intervention, outcomes, and sample size
Patients were assigned to 5 g gel daily dispensed in visually identical tubes, containing placebo (n=21) or 50 mg testosterone, Testim® (TRT, n=22). T-levels were evaluated after three weeks of treatment and the dose was increased to 10 g gel daily if BioT level was <7.3 nmol/l. Compliance was monitored at weeks 3, 12, and 24 concerning gel application, timing, cutaneous area, and adverse skin reactions.

We examined the patients on two consecutive days before and after 24 weeks of TRT. On the first day, patients underwent physical testing, physical examination, scans (DXA, MRI, MRS, high-resolution peripheral quantitative computed tomography (HR-pQCT)), and they answered questionnaires. On the second day, fasting blood samples and euglycemic-hyperinsulinemic clamp were performed. T-levels were measured at the screening visit, week 3, and week 24 at time 0 of the clamp. Patients were told to stop gel therapy 24h before blood sampling to ensure measurement of a stable level of testosterone during TRT (41, 99). Our primary outcome was change in LBM between groups after TRT (20) with an assumption of a type 1 error (α)=0.05, type 2 error (β) =0.1, SD=1.3 kg, resulting in 15 patients in each group. We included 20 subjects in each group to allow for drop-outs. Secondary outcomes included changes TFM, whole-body insulin-sensitivity, oxidative metabolism, SAT, TFA, VAT, hepatic fat, HbA1c, fasting lipids, levels of adiponectin, leptin, leptin:adiponectin ratio, muscle mechanical function (power and strength), physical function (gait speed), lean leg mass, and leg fat mass between groups. Physical activity was assessed by accelerometry and considered as an explanatory outcome measure.

Dose titration and safety monitoring
The target level of BioT was >7.3 nmol/l (31). The patients were instructed to stop gel therapy 24h before the measurements of T-levels. After three weeks of treatment, all patients in the placebo group were increased to 10 g gel daily, while 16/21 patients were increased in the testosterone group. One patient in the testosterone group was decreased to 5 g gel once every other day after 12 weeks TRT due to elevated hematocrit level. This patient was excluded from the analyses. Seven patients in the testosterone group had paused TRT for 48h prior to the clamp day of the final visit. Digital rectal examination of the prostate along with measurements of PSA, hematocrit, and hemoglobin were evaluated after 3, 12, and 24 weeks of treatment. Dose titration and safety monitoring were externally handled to ensure continued blinding.

Dual-energy X-ray Absorptiometry
LBM, lean leg mass, TFM, and regional fat mass were measured by DXA scans using a Hologic Discovery device (Waltham, MA, US). The intra-assay percent root mean square CV (RMS-CV) was 1.4% for LBM and 1.8% for TFM.

Magnetic resonance imaging (MRI)
MRI was performed with a 3.0 Tesla High field MR Unit (Phillips Achieva, Phillips Healthcare, Best, Holland). Three abdominal slices (10 mm thick, 20 mm apart, lower slice at the dorsal, intervertebral space of L4/L5) were recorded using an axial, T1-weighted gradient-echo sequence. Computer software (100) was used to trace the different compartments of fat on the abdomen and for assessment of the areas of SAT and VAT. The TFA and thigh muscle area (TMA) were determined on three femoral slices (100). Computer software (100) was used to trace fat and muscle compartments on the thigh to assess TFA.

Magnetic resonance spectroscopy (MRS)
Single-voxel liver 1H MRS was performed to measure the hepatic fat fraction. MRS measurements were performed using a Philips Achieva 3.0-T MR scanner (Philips Healthcare, Best, Holland). 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 (30x30x30mm3) 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 (lipids/unsuppressed water) were calculated for each patient. Automated spectral results

were reviewed by an experienced MR spectroscopist who was blinded to the treatment allocation.

Euglycemic-hyperinsulinemic clamp
After a 10h overnight fast, a 2h basal tracer equilibration period was followed by a 4h period with insulin infusion (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) at a rate of 40 mU/m2/min resulting in physiological hyperinsulinemia at approximately 500–600 pmol/l in the insulin-stimulated period. A primed-constant [3-3H]-glucose infusion was used throughout the 6h study, and [3-3H]-glucose was added to the glucose infusates to maintain plasma specific activity constant at baseline levels during the 4h clamp period as described in detail previously (101). Plasma glucose was kept constant at approximately 5.5 mmol/l by varying the infusion of 20% glucose based on bedside plasma glucose measurements every 10-20 minutes. Plasma glucose and lactate were measured using an ABL800 FLEX analyzer (Radiometer, Copenhagen, Denmark). Serum insulin and C-Peptide were measured using COBAS immunoassay platforms (Roche Diagnostics, IN, USA). HOMA-IR was calculated as described (102). Plasma FFA levels were measured using a colorimetric assay kit (Wako Chemicals, Richmond, VA, USA) and a COBAS FARA 2 auto-analyzer (Roche Diagnostic, Rotkreuz, Switzerland).
The basal and insulin-stimulated steady-state periods were defined as the last 20 minutes of each period. Tritiated glucose-specific activity was determined on samples deproteinized with barium and zinc as described elsewhere (101). Steele's non-steady-state formulas were used to calculate rates of total glucose appearance and glucose disposal rate (Rd), assuming a glucose distribution volume of 200 ml/kg body weight and a pool fraction of 0.65 (101). Hepatic glucose production (HGP) was calculated as the difference between rate of total glucose appearance and the glucose infusion rate. Insulin-stimulated Rd was taken as an estimate of whole-body insulin sensitivity.

Indirect calorimetry
Indirect calorimetry was performed during the last 40 minutes of the basal and insulin-stimulated periods using a ventilated hood system (Parvo Medics TrueOne ® 2400 Metabolic Measurement System). The average gas exchanges recorded during the last 30 minutes of steady state in the basal and insulin-stimulated periods were used to calculate glucose oxidation rate (GOX), lipid oxidation rate (LOX), and the respiratory quotient (RQ) (103). NOGD was calculated as the difference between Rd and GOX.

Assays
The assays are described in the manuscripts.

Isokinetic Dynamometry (muscle mechanical function)
Knee-extensor muscle mechanical function was assessed by experienced sports physiologists using an isokinetic dynamometer (KinCom 500H, Chattecx, USA) (104). Following several warm-up trials, patients performed five maximal voluntary dynamic contractions at 180°/s (Dyn180) in a range of 90° to 20° (0°=full extension) and three maximal voluntary isometric contractions (MVC) at a 70° knee joint angle (104). Patients were instructed to contract the knee-extensors as fast and forcefully as possible and maintain maximal force exertion for 2–3 s during the MVCs. Rapid muscle force capacity was calculated as the contractile rate of force development (Δforce/Δtime) in the time interval between 0–100 ms relative to the onset of contraction (RFD100). Patients were given 1 min rest period between knee extension trials. The settings were recorded at baseline and used in subsequent tests. Onset of contraction was defined as the point where force production exceeded the baseline level force by 3% of the maximal force value. All trials with a visible initial countermovement were discarded from the analysis, and all approved trials were gravity corrected and normalized to moment arm. The trial with the highest force for each leg was selected from the dynamic and isometric knee-extensor muscle strength (from which RFD100 was calculated). MVC and RFD100 values from the right and left leg were averaged and presented as absolute values (Nm and Nm/s, respectively).

Nottingham Leg Rig (muscle mechanical function)
As an assessment of knee-extensor muscle mechanical function, unilateral leg extensor power was obtained for both legs separately using the Nottingham Leg Rig (Nottingham, UK) (105). Patients were seated in the power-rig chair in a 20° knee joint angle at full extension and pushed away the footplate connected to a flywheel as hard and fast as possible. A minimum of 5 trials were performed for each leg with visual feedback on a PC screen after each trial. The trial with the highest power was selected, averaged for the right and left leg, and presented as absolute values (W).

Gait speed (physical function)
Normal and maximal walking speed were assessed over a 10 meter course using a stop watch. The better of two trials was selected for normal walking speed and for maximal walking speed, respectively, and presented in absolute values (m/s).

Accelerometry (physical activity)
The accelerometer (ActiGraph GT3X+) was worn for seven consecutive days before treatment and during the last week of intervention. The patients were instructed to wear the device over the hip for all waking hours except when showering or swimming. The accelerometer was initialized before each measurement according to the manufacturer's specifications. Data were analyzed using the software program Propero (University of Southern Denmark). The epoch length was set to 30 s, and strings of 60 min or more of consecutive zero counts, were interpreted to represent non-wear time and were excluded from the analyses. Only data from patients accumulating at least 10 hours of activity per day for at least 4 days were analyzed. The average duration of physical activity and time spent at different physical activity intensities levels were assessed in minutes per day (min/d). The range (counts per minute, CPM) for activity intensities were 0-99 (sedentary), 100-2019 (light activities), 2020-5999 (moderate activities), and above 6000 (vigorous activities) (106).

STATISTICS
Statistical methods are described in each manuscript.

RESULTS
This section is based on the three manuscripts.

Manuscripts I-III
The testosterone and the placebo groups were comparable regarding all baseline measurements except for a lower basal GOX, higher basal NOGD, and higher FFA-levels in the testosterone group compared to placebo. Changes during TRT compared with placebo are summarized in Table 2.

Table 2 Changes during TRT compared with placebo

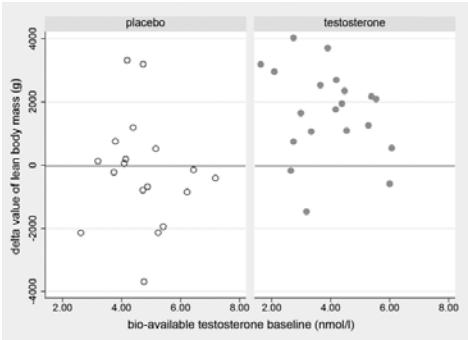
Body composition	→ Weight	→ BMI	→ WC
DXA/MRI/MRS	↑ LBM	↓ TFM	↓ TFA
	↓ SAT	→ VAT	→
	Hepatic fat		
Glycemic outcomes	→ Rd clamp	→ HOMA-IR	→ HbA1c
Oxidative metabolism	→ GOX	→ LOX	→ RQ
Lipid profile	→ T-cholesterol		
	→ LDL	↓ HDL	→ TG
Adiponectin and leptin	↓ Adiponectin	↓ Leptin	
	↓ Leptin:adiponectin ratio		
Physical performance	↑ Muscle strength		
	→ Physical function	→ Physical	
	activity		

Manuscripts I and III

Lean body mass and clinical characteristics

Total LBM and lean leg mass increased significantly during testosterone compared with placebo. The increase in LBM and lean leg mass were not associated with baseline T-levels (Fig. 3).

Figure 3 A modified Bland-Altman plot (difference plot) with BioT at baseline vs. changes in total lean body mass



Within the testosterone group, the increase in lean leg mass accounted for approximately 15% of the increase in LBM. Within the placebo group, lean leg mass decreased after 24 weeks and the decline in lean leg mass accounted for approximately 99% of the reduction in LBM.

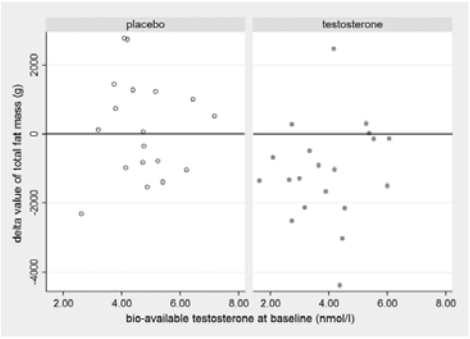
Body weight, BMI, and WC were unchanged during TRT.

Manuscripts I and II

Total and regional body fat distribution

TFM, fat trunk mass, leg fat mass, and arm fat mass decreased significantly during testosterone compared with placebo. The decrease in TFM was not associated with BioT-levels at baseline (Fig. 4). SAT and TFA decreased significantly during TRT compared with placebo. There was no change in hepatic fat volume and VAT.

Figure 4 A modified Bland–Altman plot (difference plot) with BioT at baseline vs. changes in total fat mass



Manuscript I

Insulin-sensitivity, glycemic control, and oxidative metabolism

HOMA-IR and HbA1c were unchanged after TRT. In the basal state, Rd, HGP, GOX, LOX, NOGD, RQ, serum-insulin, serum-C-peptide-levels, P-glucose, and FFA-levels were unaltered during TRT compared with placebo.

Insulin-stimulated Rd, HGP, GOX, LOX, NOGD, RQ, serum-C-peptide, P-glucose, FFAs, and insulin-clearance were unchanged during TRT compared with placebo. Clamp serum-insulin-levels increased significantly during placebo compared with testosterone. This caused an increase in Rd corrected for clamp insulin-levels during TRT compared with placebo.

Insulin-stimulated Rd was not associated with baseline BioT-levels, baseline HOMA-IR, or baseline HbA1c in either group.

Manuscripts I-III

Paraclinical characteristics

The paraclinical characteristics of the 39 patients are listed according to treatment group in all tables. Hemoglobin and hematocrit increased during testosterone compared with placebo.

SHBG, adiponectin, leptin, leptin:adiponectin ratio, HDL-cholesterol, and gamma-glutamyltransferase (GGT) levels decreased.

Levels of total-cholesterol, LDL-cholesterol, triglycerides, alanin-transaminase (ALAT), and PSA were unchanged.

Manuscript II

Correlations

At baseline in all patients

Adiponectin was positively associated with age and HDL-cholesterol, while adiponectin was negatively associated with TG, HOMA-IR, and leptin:adiponectin ratio.

Leptin was positively associated with BMI, WC, TFM, VAT, hepatic fat volume, HOMA-IR, and leptin:adiponectin ratio, whereas leptin was negatively associated with T-levels (TT, BT, FT, DHT), SHBG, and insulin-stimulated Rd.

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

Hepatic fat volume was positively associated with BMI, WC, TFM, ALAT, leptin, leptin:adiponec-tin ratio, and HOMA-IR, whereas hepatic fat volume was negatively associated with insulin-stimulated Rd.

At 24 weeks of treatment in the testosterone group

Δ adiponectin levels were positively associated with Δ HDL-cholesterol and Δ leptin, whereas Δ adiponectin levels were negatively associated with Δ SHBG. Δ leptin levels showed a trend towards negative correlation with Δ insulin-stimulated Rd. Δ

leptin:adiponectin ratio was positively associated with Δ leptin and negatively associated with Δ estradiol.
No other significant correlations were observed between Δ adiponectin levels, Δ leptin levels, Δ leptin:adiponectin ratio and changes in clinical/biochemical parameters during TRT.
Δ hepatic fat volume was not associated with any change in the above-mentioned clinical/biochemical parameters.

Manuscript III

Muscle mechanical function, physical function and physical activity

Knee extension MVC, RFD100, and Dyn180 increased significantly during TRT compared with placebo. Within the testosterone group, MVC, RFD100 and Dyn180 remained unaffected after treatment. Within the placebo group, RFD100 and Dyn180 decreased significantly after 24 weeks treatment. No changes were observed within or between the two groups after treatment in leg muscle quality evaluated by MVC normalized to lean leg mass, leg power, normal walking speed, maximum walking speed, physical activity, or time spent at different physical activity intensity levels. Leg muscle quality evaluated as MVC normalized to lean leg mass was unchanged during TRT compared with placebo.

GENERAL DISCUSSION

Manuscripts I and III

Muscle mass during TRT

TRT had substantial beneficial effects on total LBM and lean leg mass measured by DXA scan as reported in former studies in patients with (27) and without T2D (19, 20). The lack of association between baseline T-levels and the increase in LBM is in accordance with no testosterone threshold for this biological response (27). The anabolic effects of TRT on muscle mass are achieved through an action on several cellular targets such as an increasing number of satellite cells and through effects on protein metabolism (107). This is the first study to report a counteractive effect of TRT on lean leg mass compared with placebo in aging men with T2D and lowered BioT levels. Comparable increases in lower (108, 109) and upper (110) extremity lean mass were previously demonstrated in RCTs including aging men without T2D (108-110). While the increase in lean leg mass accounted for approximately 15% of the increase in total LBM in the testosterone group, the decline in lean leg mass accounted for approximately 99% of the decrease in total LBM in the placebo group. Hence, without interventions such as TRT, the lower extremities appear especially susceptible toward muscle atrophy in aging men with T2D and lowered BioT levels. Strikingly, the observed decrease in lean leg mass in the placebo group occurred after a relatively short period of time (24 weeks). Yet the finding is consistent with a longitudinal study (3 years) showing an accelerated loss of muscle mass in aging patients with T2D compared to aging persons without T2D (111). If men with T2D have an accelerated loss of muscle mass compared with control persons, the patients might have a higher risk of developing sarcopenia (112, 113).

Manuscripts I and II

Fat mass during TRT

TRT markedly improved TFM evaluated by DXA scan which was in line with previous RCTs in aging men with T2D (27) and without T2D (19, 114-117). In aging men with T2D, the majority of previous RCTs have evaluated the effect of TRT on body fat by less accurate markers thus providing conflicting results, e.g. body fat was unchanged measured by bioelectrical impedance (22, 25), whereas BMI was unchanged in all (22, 23, 25, 26) except one former study (24) (Table 1).
We evaluated regional abdominal adipose tissue by MRI and found a considerable reduction in SAT, whereas VAT was unaltered during TRT. Reports on the effect of TRT on regional abdominal fat mass in aging men without T2D have been inconsistent with different ways of assessment of regional abdominal adipose tissue (CT, MRI, or ultrasound), differing patient cohorts included, and numerous ways of TRT doses and administration (oral, patch, gel, or injections) (92). Studies using a sufficient testosterone dose in aging men without T2D (92) have similarly reported a significant decrease in SAT and no change in VAT during TRT (117, 118), whereas an old study by Marin et al (119) in 31 middle-aged obese men with higher baseline T-levels reported unchanged SAT and a decrease in VAT assessed by CT despite no change in TFM and LBM (119). Therefore, the decrease in VAT during TRT in the study by Marin et al (119) might be attributed to other changes during the experiment. In aging men with T2D, only one previous study has correspondingly evaluated abdominal fat mass during TRT by MRI with an agreeing report of reduced SAT and unchanged VAT (27). The other RCTs have used WC as an imprecise surrogate marker for VAT and these studies reported WC as decreased (22, 24, 26) or unchanged (23, 25) during TRT.
To the best of our knowledge, we are the first to report TFA evaluated by MRI during TRT in aging men with T2D and we found a substantial reduction in TFA. In aging men without T2D, two former studies correspondingly reported a reduction in TFA during TRT (92, 116).
The mechanisms behind a tissue specific TRT-induced reduction in fat mass could be caused by an inverse correlation between the number of CAG repeats (CAGn) within the CAG repeat polymorphism of the androgen receptor and the activity of the receptor (92, 120, 121). Thus, men with lower number of CAG repeats of the androgen receptor are more sensitive to androgens (121-123). In healthy young men, TFM increased whereas LBM decreased with increasing CAGn, suggesting a direct effect of longer CAG repeats on the testosterone-induced increase in LBM and decrease in TFM (124). Nielsen et al. (124) also reported a significant positive association between increasing CAG repeats and subcutaneous fat on the abdomen and thigh; however, no significant association with VAT was seen (124). Theoretically, TRT might not have an effect on VAT due to a low concentration of androgen receptors in contrast to a high concentration of androgen receptors in the physiological subcutaneous fat depots (TFA and SAT).
To date, we are the first to assess the effect of TRT on amount of hepatic fat with the "gold-standard" imaging method MRS (125) in a homogenous cohort of aging men with T2D. We hypothesized that hepatic fat volume would decrease during TRT as lipolysis is usually increased during TRT (27), however, we observed no change in hepatic fat volume measured by MRS which might also be explained by a low concentration of androgen receptors in the ectopic hepatic fat. The included patients were markedly insulin resistant as evaluated by their insulin-stimulated Rd as well as HOMA-IR (126) which could reflect the presence of NAFLD at baseline (95). Our results were not compromised by different treatment regimens regarding anti-diabetic or cholesterol-lowering drugs as the use of only metformin and statins were equally distributed between the two groups with no change in the treatment regimens or doses allowed throughout the entire study. The impact of the decreased GGT levels during TRT is probably limited as there was no other beneficial changes in metabolism (insulin-stimulated Rd, hepatic fat by MRS, lipids) and the liver function parameters were actually within the normal range in all patients

except one. The unchanged hepatic fat volume during TRT could be explained by the portal/visceral hypothesis stating that increase in VAT is associated with rise in portal vein plasma FFAs and thus NAFLD (52). Accordingly, overnight fasting FFA levels were unchanged during TRT. Four previous studies have evaluated hepatic fat volume during TRT in men without T2D showing inconsistent results with both unchanged (115, 127) and improved hepatic fat volume (128, 129). However, these studies used different assessment methods for hepatic fat volume (115, 127-129) and only Sattler et al (115) used the "gold-standard" method MRS (115). Although Sattler et al (115) also reported unchanged hepatic fat volume during TRT, the study has some limitations as it was a single-arm open-labelled small (n=20) study conducted in men without T2D, and changes of concomitant medication during the trial were not reported (115). Huang et al (127) also reported unchanged hepatic fat volume during TRT evaluated by the less accurate MRI in a subgroup of 73 out of originally 209 men (127). Of these 73 men, 17 men actually had a diagnosis of T2D however there was no description of the antidiabetic treatment regimen (127). Hence, the results of TRT on hepatic fat volume might have been affected by different anti-diabetic treatment regimens and by baseline differences regarding presence of hyperlipidemia and the use of statins between the groups (127). In the RCT by Hoyos et al (129), hepatic fat volume was improved during TRT assessed by the less precise CT however the included patients were considerably different compared to our patients as they were not diagnosed with T2D, were younger, more obese, received a weight loss program in addition to the TRT, and the permission of change in concomitant medication was not reported (129). In addition, we could speculate that the reduction of hepatic fat volume during TRT was confounded by baseline differences between the two groups as the testosterone group had lower WC compared with the placebo group (129). In the open-label, non-controlled study by Haider et al (128), hepatic fat volume was improved; however, the volume was assessed by liver function parameters (128) which are of limited use in patients with NAFLD as they often have normal transaminases (55) and there was no report of permission to change concomitant medication either (128).

Insulin resistance

We are the first to evaluate insulin sensitivity by the gold-standard euglycemic-hyperinsulinemic clamp during TRT in a RCT in aging men with T2D. Despite a low insulin-stimulated Rd at baseline and the beneficial effect of TRT on body composition, i.e. LBM and TFM, insulin-stimulated Rd and HOMA-IR were unchanged during TRT in our study. Moreover, high baseline HOMA-IR was not associated with change in insulin-stimulated Rd after treatment in our study. We could speculate that despite increased muscle mass the muscle cells were still insulin resistant with impaired influx of glucose and glycogen synthesis to such a degree that the amount of increased LBM could not improve insulin resistance. Any other direct beneficial role of TRT on intracellular molecular mechanisms involved in insulin-signaling, and hence the regulations of insulin sensitivity independent of changes in body composition have not been assessed in this thesis except for levels of adiponectin and leptin.

We could not demonstrate any changes in VAT or FFAs during TRT. Thus, the portal/visceral hypothesis might partly explain the link between adiposity and insulin resistance (52). In a previous study from our group, we observed a small but significant decrease in the ability of insulin to suppress FFA levels during the clamp after three months TRT, but not after six months TRT, suggesting at least a transient negative effect on the inhibitory action of insulin on lipolysis in aging men without T2D (19). The unchanged insulin sensitivity during TRT is also in line with the ectopic fat storage syndrome linking adiposity with insulin resistance because we observed no change in VAT or hepatic fat during TRT. Based on the endocrine hypothesis, the reduction of SAT and TFA during TRT might partly explain the inability of TRT to improve insulin sensitivity because of the reduced levels of adiponectin during TRT. However, the decreasing leptin levels during TRT may have had a positive effect on insulin resistance as the changes in leptin levels during TRT showed a trend towards negative correlation with changes in insulin-stimulated Rd. A dysregulated secretion of leptin in T2D and/or obesity might contribute to the insulin resistance because leptin suppresses insulin secretion in a negative feedback loop where insulin stimulates the release of leptin (130). However, this feedback loop might be comprised by the insulin and leptin resistance often seen in T2D.

In previous RCTs in patients without T2D, insulin sensitivity assessed by euglycemic-hyperinsulinemic clamp was unchanged (19) or improved (115, 131, 132) during TRT. In two of the studies, the improved insulin sensitivity was limited to men with reductions in trunk and extremity fat greater than median declines for the entire group (62–78 years-old) (115) or during combined TRT and dutasteride treatment (24–51 years-old) (131). In previous RCTs in aging men with T2D, the effect of TRT on insulin sensitivity assessed by HOMA-IR has been conflicting, showing unchanged HOMA-IR (23, 26, 27) as in our study or decreased HOMA-IR (22, 24, 25). The extern validity of the three studies reporting decreased HOMA-IR might have been weakened by different anti-diabetic treatment regimens applied at baseline (22, 24, 25), improvement in insulin sensitivity only seen in the intention-to-treat population (25, 133), the use of statistically insignificant explanatory variables for the calculation of HOMA-IR (24, 25), and a cross-over design undermining the blinding if withdrawal of TRT caused symptoms of hypogonadism (22).

The insulin levels during clamp increased in the placebo group causing a significant difference between groups during TRT. This increase in insulin levels cannot be explained by differences in insulin-infusion rates or glucose levels during the euglycemic-hyperinsulinemic clamp. Consistently, serum-C-peptide levels, which are a measure of endogenous insulin-secretion, were similar among groups during the clamp. The only possible explanation for increased insulin levels during TRT was a tendency for reduced insulin clearance in the placebo group after treatment. We could speculate that insulin clearance is reduced as a result of time (24 weeks) and hence the natural course of the T2D in men with lowered T-levels. This may suggest that treatment with testosterone protects against this decline in insulin clearance. However, we have no solid data to provide evidence for this potential mechanism. In the above-mentioned randomized trials, only measurements of fasting serum-insulin were made, and the results were in line with our results of unchanged basal insulin levels (22, 24, 25, 27).

Manuscript I

Glycemic control

The average duration of T2D was relatively short (3¬¬–4 years). Moreover, the included patients with T2D were relatively well-controlled on the antidiabetic treatment with metformin alone, and they had fasting insulin levels demonstrating 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 antidiabetic medications during the trial that primarily enhance or substitute endogenous insulin secretion rather than improve insulin resistance. 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 thus a need for further antidiabetic drugs. Insulin sensitivity was the main focus of the trial in regards to the effect of TRT on T2D rather than the glycemic control. Although our patients had considerable insulin resistance with HOMA-IR >2.5, which is assumed necessary to detect a change in insulin sensitivity during TRT (126), there was no association between degree of insulin resistance and change in insulin sensitivity during TRT. Contrary to the study by Jones et al (25) we did not allow any change in anti-diabetic treatment regimen. Hence, severely dysregulated patients were excluded at baseline as they could not maintain a stable anti-diabetic therapy throughout the study and any change would undermine the results in the glycemic outcomes.

Metabolic inflexibility

Studies have reported an enhancement of basal LOX after TRT in patients without T2D (19, 62). Based on these findings, we also expected basal LOX to increase and RQ to decrease after TRT in patients with T2D. Nevertheless, we could not demonstrate any changes in oxidative metabolism or RQ during TRT in the present study. In patients with T2D, insulin-stimulation of glucose-oxidation and suppression of lipid-oxidation are impaired compared with matched controls (51, 61). Conversely, muscle glucose-oxidation is increased and reliance on lipid-oxidation is decreased in T2D during fasting conditions compared with healthy controls (51, 61). We cannot exclude the possibility that a similar "metabolic inflexibility" in response to TRT is responsible for the lack of changes in substrate partitioning in our study. Another explanation of unchanged substrate-oxidation after TRT could be a larger heterogeneity within study populations with T2D and possibly only a proportion of men with lowered T-levels show this response to TRT in regards to substrate-oxidation. Thus, it may be difficult to detect an effect of TRT on whole-body substrate metabolism in T2D.

Testosterone and SHBG levels

The patients were told to stop gel therapy 24h before blood sampling at weeks 3 and 24 in order to measure a stable level of testosterone. The 2h post gel-application serum T-levels in older men (≥65 years) is a poor indicator of average serum T-levels due to large inter- and intra-individual variability (99). Unfortunately, seven patients in the testosterone group had paused TRT for 48h prior to the blood sampling at week 24 and they had BioT levels <7.3 nmol/l. All these 7 patients who had stopped gel therapy for 48h were in a similar hormonal milieu with a considerable effect on body composition and there was no difference in response of delta insulin-stimulated Rd compared to the others in the testosterone group. Testosterone gel-application can efficiently elevate T-levels in hypogonadal men into the mid- to upper normal range within the first day of application, achieve steady state within a few days, and maintain serum T-levels with once daily repeated applications according to Swerdloff et al (41) and Wang et al (42). It is difficult to assess T-levels during TRT because of the negative feed-back mechanism where TRT inhibits the pituitary secretion of LH causing decreasing levels of endogenous testosterone while serum T-levels rise from exogenous administration (41). After withdrawal of gel application, serum T-levels decrease about 40–50% by 48h and to baseline hypogonadal levels by 96h (42). Overall adiposity and abdominal fat accumulation have been associated with decreased SHBG concentrations (134). Surprisingly, SHBG levels decreased significantly in the testosterone group compared to placebo despite the improved body composition in our study. A similar decrease in SHBG was seen in two other RCTs in aging men with T2D (27, 135). Obese subject are often in a hyperinsulinemic state (51) and fasting insulin levels are shown inversely correlated with SHBG levels possibly due to inhibition of the hepatic production of SHBG (136). However, insulin levels during clamp and in the fasting state were unchanged in the testosterone group during TRT, whereas the insulin levels increased in the placebo group causing a significant change in insulin levels after TRT. Thus, other (unknown) mechanisms could be responsible for the reduced SHBG levels.

Manuscript II

Adiponectin and leptin levels during TRT

There was a potentially harmful reduction in adiponectin levels during TRT even though the placebo and TRT groups had similar baseline adiponectin levels comparable to adiponectin levels of the lowest quartile in a study in 741 men with T2D (137). In contrast to our baseline correlations, 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 (96). In the present study, we found that the changes in adiponectin levels during TRT were not associated with changes in total or regional fat compartments. These data could suggest that TRT reduced adiponectin levels through either a direct inhibition of the production or secretion and/or an increased breakdown of adiponectin (80). In aging men with T2D, previous studies have reported that adiponectin levels were decreased (21), unchanged (27), or increased (138) during TRT. In an un-blinded study including a diet and exercise program, both groups actually had an increase in adiponectin levels during TRT (138). However, the higher increase in adiponectin levels in the testosterone group might have been attributed to a more dedicated TRT group regarding the diet and exercise program (138). In the RCT by Gianatti et al (27), adiponectin levels were unchanged during TRT however the patients had higher baseline levels of adiponectin and were more obese compared to the patients in our study (27). Hence, a hypothetically non-linear relation between SAT/TFA and adiponectin secretion may explain the unchanged levels of adiponectin during TRT as Gianatti et al (27) reported effects of TRT on SAT and VAT in accordance with our results, whereas TFA was not reported (27).

We confirmed that overall obesity (TFM, BMI) was associated with higher leptin levels at baseline (73) and our data suggest that leptin secretion is more attributed to VAT rather than the subcutaneous fat compartments (SAT, TFA) at baseline. Previous studies report that leptin levels are more strongly associated with SAT than VAT (93, 139). However, in the study by Neeland et al (93) leptin levels were actually associated with VAT in obese men but not in women (93). In further support, biopsies collected from SAT and VAT in 28 men with and without T2D undergoing abdominal aortic surgery, showed a significant higher secretion of leptin from the mesenteric VAT compared to SAT (140).

In accordance with our findings, other RCTs have reported decreased leptin levels during TRT in men with T2D (21, 24). The significantly reduced leptin levels during TRT were not associated with changes in any of the regional fat compartments (TFA, SAT, VAT, hepatic fat). Hence, the reduced leptin levels during TRT

could be due to a direct suppressive effect on the production, secretion, or breakdown (21) as with adiponectin levels. No other study has evaluated leptin levels in relation to regional fat compartments assessed by MRI, CT, and/or MRS in aging men with T2D.

Manuscript III

Muscle mechanical function

TRT was shown to preserve knee-extensor muscle mechanical function (isometric and dynamic strength (MVC and Dyn180), and explosive force (RFD100)). These measures have previously been shown to be functionally important, e.g. by discriminating older fallers from non-fallers (141, 142). The placebo group experienced potentially harmful decreases in muscle mechanical function following the 24-week intervention period, except for unchanged leg power. The evaluation method applied for leg power, Nottingham Leg Rig, is not as sensitive as the KinCom method which may explain why we were not able to detect potential small changes in leg power during TRT. Although difficult to determine, it appears that there is an age-threshold for the initiation of a rapid decline in muscle mechanical function occurring around 60–70 years of age (143, 144). It is plausible that such a decline starts earlier in men with T2D and lowered T-levels (111) and are linked to other mechanisms such as peripheral neuropathy (145, 146) and possibly increased skeletal intramuscular fat (147). Although physical activity is necessary to preserve good physical function (148), the observed changes in muscle mechanical function in the testosterone group were not due to changes in physical activity levels (assessed by accelerometry) as these remained unaffected in both groups after treatment. In support of unchanged physical activity during TRT, a previous study in aging men without T2D reported unaltered daily physical activity during 12-months TRT (110). The observed preservation of muscle mechanical function in our study was probably caused by the increase in muscle mass during TRT. In accordance, a large cross-sectional cohort study reported that lower strength with older age was predominantly due to a lower muscle mass (149). However, the same study showed that factors such as prevention of fat mass gain and intramuscular fat infiltration are also important features in maintaining muscle mechanical function and muscle quality in aging persons (147, 149). Although leg fat mass decreased, leg muscle quality remained unchanged in our study. In contrast to our finding of preserved muscle mechanical function, there was no change in lower leg muscle mechanical function during TRT in two previous RCTs in healthy, slightly older men (108, 110). It is possible that the observed preservation of muscle mechanical function during TRT in our study were simply due to the patients being younger than those in the two other studies (108, 110). Nevertheless, T2D appears to have a detrimental effect on muscle mechanical function, adding to the age-related changes. Taken together, TRT may be more efficacious in men with T2D regarding leg muscle mechanical function compared to men without T2D.

Physical function

The remarkable preservation observed in muscle mechanical function after TRT in our study did not translate into improvements in physical function evaluated by gait speed. However, the patients included in our study were not yet limited in their mobility as reflected by their high gait speed at baseline. There is a non-linear relationship between leg strength and gait speed which explains how small changes in physiological capacity may have substantial effects on performance in frail older persons, whereas large changes in capacity have small or no effect in healthy older persons (150). Thus, a potential improvement in physical function during TRT could have been undetectable by the measurement methods applied in our study because of a well preserved physiological capacity in the patients in the placebo group. Our results are in line with previous studies in aging men without T2D reporting that physical function was unchanged after TRT (109, 110).

Manuscripts I and II

Cardiovascular disease

There were no cardiovascular adverse events in our study. The study by Basaria et al (151) has especially questioned whether TRT is safe in aging men regarding CVD as the trial was stopped before completion of enrollment because of an incidence of adverse cardiovascular events that was higher in the testosterone group compared to the placebo group (151). Our study differs in some aspects from the study by Basaria et al (151) as our patients were younger (mean age 60 vs. 74 years), more robust (generally without limitations in mobility), the initial dose of testosterone gel was smaller (50 mg vs. 100 mg testosterone), and our patients could maximally receive 100 mg testosterone vs. 150 mg (151). Our dose administration was consistent with the current practice guidelines which recommends serum testosterone target concentrations for older men within the mid-reference range of young adult men (28) in the absence of definitive evidence of cardiovascular safety (8).

Regarding the risk of CVD during TRT, it is necessary to evaluate known cardiovascular risk factors. An adverse lipid profile is a known modifiable risk factor for CVD (152). The significant reduction in HDL-cholesterol during TRT might increase the risk for CVD because an average decrease in HDL-cholesterol of 0.11 mmol/l corresponds to a considerable portion of the patients having HDL-cholesterol <1.03 mmol/l which is a known risk factor for developing CVD (153). The reduced HDL-cholesterol levels during TRT are consistent with previous studies in men with T2D (25, 27) and without T2D (19). Moreover, the effect of TRT on levels of HDL¬-cholesterol was significant despite no observed changes in TG or LDL-cholesterol and despite 85% of our patients was actually on cholesterol-lowering drugs.

Loss of gluteofemoral fat is associated with an increased CVD risk as observed in Cushing's syndrome and lipodystrophy (94). This protection by gluteofemoral fat could be mediated through higher adiponectin levels as TFA was positively associated with serum adiponectin in a population-based study in young (20–29 years) healthy men (96). Our data support the association between high adiponectin levels and a healthier cardio-metabolic profile (154) as adiponectin levels were positively related to levels of HDL-cholesterol and negatively related to levels of TG and HOMA-IR at baseline. In patients with T2D, Swellam et al (91) showed that adiponectin levels were lower in patients with complications compared to patients without complications, and Swellam et al (91) suggested that reduced adiponectin levels might not only play an important role in the pathophysiology of T2D but also in complications to T2D (91). Theoretically, adiponectin may reduce hepatic glucose production, increase FFA oxidation, and augment glucose uptake in skeletal muscles which may improve insulin sensitivity, inflammation, and atherosclerosis (155). In accordance with the decreased levels of adiponectin, we have showed that FFA levels and insulin sensitivity were unchanged during TRT. Surprisingly, there was no correlation between adiponectin levels and insulin sensitivity at baseline however this lack of association might be due to the low levels of

adiponectin within a narrow range. Thus, the decreased adiponectin levels during TRT in our study might suggest a worsened cardio-metabolic profile although the actual effect is unknown considering that the levels of adiponectin were low at baseline. On the other hand, the reduced leptin levels and leptin:adiponectin ratio during TRT could reflect an amelioration in the hyperleptinaemia which may be beneficial regarding the CVD risk (156). At baseline, leptin:adiponectin ratio was related to a more adverse cardio-metabolic profile regarding increased ectopic fat, a poorer lipid profile, and higher insulin resistance in our study. Moreover, leptin:adiponectin ratio has been shown to correlate positively with arterial stiffness in patients with T2D (84). However, our study was not powered to differentiate whether the reduced leptin:adiponectin ratio reflects a genuine reduced risk of CVD where the decreased leptins levels outweigh the potential harmful decline in adiponectin levels.

Men with lower number of CAG repeats of the androgen receptor are more sensitive to androgens (121-123). The contention that androgens protect against CVD might be challenged by the finding of a lower number of CAG repeats in otherwise healthy aging men with a less favorable cardio-metabolic profile (123). Additionally, the association between acquired hypogonadism and risk of CVD is still unresolved in men with prostate cancer treated with androgen deprivation (157).

Manuscripts I-III

The suggested beneficial and harmful effects detected during TRT are summarized in Table 3.

Table 3 Effects of TRT

	Beneficial	Harmful	None	Unclear
Body composition	↑ LBM ↓ TFM	↓ TFA	→ weight → BMI → WC → VAT → hepatic fat	↓ SAT
Insulin resistance			→ Rd clamp → HOMA-IR	→ HbA1c
Lipid profile		↓ HDL	→ total-cholesterol → LDL → triglycerides	
Adipokines	↓ leptin ↓ leptin:adiponectin ratio	↓ adiponectin		
Physical performance	↑ muscle strength		→ physical function → physical activity	

METHODOLOGICAL CONSIDERATIONS

I consider the inclusion of patients based on biochemical hypogonadism as an advantage due to the lack of coherence between measurements of biochemical hypogonadism and symptoms consistent with hypogonadism (9, 10). We used the gold-standard method for testosterone evaluation, liquid chromatography tandem mass spectrometry. Other strengths of the study were the performance of per-protocol-analyses, a low drop-out rate, and no drop-outs were due to lack or adverse effects of the gel.

Body composition was assessed by two different and accurate imaging techniques (whole-body DXA and MRI scans) instead of the imprecise methods, i.e. BMI, bioelectrical impedance, or WC as a surrogate marker for VAT.

Hepatic MRS is the most accurate imaging method available in evaluating hepatic fat (125). The primary outcome of our study was not change in hepatic fat, and thus the study might have been under-powered to detect such a change (127). We were not able to establish a quantitative measure for amount of hepatic fat, only a change. Thus, we cannot determine whether hepatic fat was actually increased at baseline in our T2D patients. Our study was limited by the dimensions of the MR scanner, failure due to technical difficulties, and claustrophobia resulting in the exclusion of several patients from MRI and/or MRS.

Insulin sensitivity was assessed by the gold-standard method, euglycemic-hyperinsulinemic clamp in a population of men with recognized T2D and a considerable insulin resistance (HOMA-IR>2.5) (126). The primary outcome of the study was a change in total LBM and our sample size was based on this known effect of TRT on LBM. No previous study has evaluated insulin sensitivity by the clamp method in aging men with T2D and lowered testosterone levels. Therefore, we were not able to calculate a sample size based on the influence of TRT on insulin sensitivity evaluated by clamp. We hypothesized that TRT could improve insulin sensitivity due to the beneficial effects on body composition. I do not believe that an increased number of patients in our study would have changed the results regarding insulin sensitivity. The modified Bland-Altman plot (manuscript I) illustrates that there was no sign of improvement in whole-body insulin sensitivity during TRT. However, I do acknowledge that our sample size of 39 patients might limit the interpretation of our null result regarding insulin sensitivity during TRT, and the likelihood of a type 2 error (a failure to detect an effect that is present) would have been reduced by a better sample size.

In contrast to the prior RCTs in men with T2D (Table 1), we only allowed metformin as the anti-diabetic treatment and no change in metformin was permitted throughout the study as any change would undermine the results in the glycemic outcomes. The patients were well-regulated in regard to glycemic control, because severely dysregulated patients at baseline could not maintain a stable anti-diabetic therapy throughout the study.

Due to misunderstandings, the treatment with gel was not optimal on the last day of the trial in seven patients of the testosterone group, because they had stopped testosterone gel for 48h prior to the blood sampling at weeks 24. These seven patients had BioT-levels <7.3 nmol/l. In theory, the clamp data might have been impacted by a shift in T-levels over the preceding 24h. However, the serum-T-levels are relatively stable until the levels gradually decline over 48–96h since last gel application (42).

GENERAL CONCLUSION

The aim of the study was to contribute to the clarification of the beneficial and potential harmful effects of testosterone therapy in aging men with T2D.

Our study is especially necessary and justified in aging men with T2D in order to establish whether the diagnosis of T2D in this group of men in itself is an indication for testosterone therapy.

Our results did not support evidence of beneficial effects of testosterone therapy on insulin resistance or on glycemic control in aging men with T2D and we cannot recommend TRT as a novel or additional treatment for T2D.

Testosterone therapy has considerable advantageous effects on muscle mass and strength in the applied physiological doses. Without TRT, leg lean mass and knee extensor muscle mechanical function deteriorated significantly within 24 weeks and the observed deteriorations were counteracted by TRT in aging men with T2D and lowered BioT levels. Although physical function was unchanged, TRT may potentially diminish the risk of developing sarcopenia resulting in a longer independent life and shorten the length of rehabilitation periods.

Regarding CVD, the substantially reduced subcutaneous fat (TFA and SAT) and HDL-cholesterol levels along with unchanged ectopic fat (VAT and hepatic) during TRT might suggest an increased CVD risk. However, TRT has an ambiguous impact on the adipokine profile with a potential harmful decrease in levels of adiponectin, whereas the decrease in leptin levels and leptin:adiponectin ratio may reflect an amelioration of the CVD risk linked to hyperleptinaemia in aging men with T2D.
It is still unclear whether the positive effects of TRT on muscle mass and muscle mechanical function outweigh potential negative effects especially regarding the risk for CVD.
In conclusion, testosterone replacement therapy is indicated in men with clinically symptomatic hypogonadism regardless of status for T2D.

PERSPECTIVES

Several more aspects can be evaluated in this particular study in order to help establish whether TRT is beneficial or not. The examination of ectopic fat in the muscles by MRI could help clarify if the unchanged insulin resistance could be due to unaltered fat accumulation in the muscles during TRT. Intra-myocellular total volume fraction of lipid assessed by transmission electron microscope was unchanged during TRT in aging without T2D (personal communication). In order to determine whether TRT even has an effect on ectopic fat, it could be useful to assess androgen receptor concentration in the ectopic muscle fat from our muscle biopsies if possible. Moreover, we are planning to measure other markers for risk of CVD e.g. klotho and calprotectin.
From a broader perspective, it is necessary to clarify whether TRT is safe regarding CVD particularly in men with T2D who have an increased risk for CVD to begin with regardless of T-levels. As stated by the FDA, only a controlled clinical trial will be able to definitively determine the effects of testosterone therapy on cardiovascular outcomes (8). Several concerns can be raised regarding the funding of such a study. Xu et al (11) showed in a meta-analysis that the effects of testosterone on cardiovascular-related events varied with source of funding; however, overall, TRT increased the risk of cardiovascular-related events (11). Based on the same studies and results, another recent paper by Tanna et al (12) concluded that testosterone therapy can be safely considered in men with appropriately diagnosed clinical androgen deficiency and increased cardiovascular risk after a thorough discussion of potential risks with the patient (12). However, it is difficult to imagine how a well-designed, large-scaled RCT addressing the safety of TRT in regard of CVD could ever take place unless several centers work together. Such a multi-center study should have reliable measurements of T-levels obtained in ideally the same laboratory or at least in laboratories using similar techniques and kits. Such a study design would be very time-consuming and expensive. Thus, it would be difficult to fund without any support from the pharmaceutical industry or a government subsidy.

SUMMARY

The prevalence of chronic diseases including obesity and type 2 diabetes mellitus (T2D) are increasing. The usage of testosterone replacement therapy (TRT) has escalated in the Western countries during the past decades especially in aging men without clear organic indication for TRT although the safety of long-term TRT has not been clarified regarding the risk of cardiovascular disease (CVD). Aging men with T2D have an increased risk of CVD and these patients are often characterized by lowered T-levels, ectopic fat depots, a deranged adipokine profile with e.g. low adiponectin levels and hyperleptinaemia. However, the causal relations are unclear, and lowered T-levels could simply be a marker of illness, i.e. T2D and obesity.
The aim of this randomized, double-blind, placebo-controlled study was to contribute to the clarification of the beneficial and potential harmful effects of testosterone therapy in aging men with T2D.
Our results did not support evidence to beneficial effects of testosterone therapy on insulin resistance, glycemic control, or on substrate-oxidation in aging men with T2D and we cannot recommend TRT as a novel treatment for T2D.
Regarding risk of CVD, the substantially reduction in subcutaneous fat (thigh and abdomen) and HDL-cholesterol levels along with unchanged ectopic fat (visceral and hepatic) during TRT might suggest an increased CVD risk. However, TRT has an ambiguous impact on the adipokine profile with a potential harmful decrease in levels of adiponectin, whereas the decrease in leptin levels and leptin:adiponectin ratio could reflect an amelioration of the CVD risk linked to hyperleptinaemia in aging men with T2D.
We found that TRT for 24 weeks in aging men with T2D and lowered bio-available T-levels improved body composition with an increase in LBM and a reduction in regional and TFM. In addition to increased lean leg mass, TRT preserved knee-extensor muscle mechanical function. Although physical function was unchanged, TRT may potentially diminish the risk of developing sarcopenia resulting in a longer independent life and shorten the length of rehabilitation periods. It is still unclear whether the positive effects of TRT on muscle mass and muscle mechanical function outweigh potential negative effects especially regarding the risk for CVD.
In conclusion, testosterone replacement therapy is indicated in men with clinically symptomatic hypogonadism regardless of status for T2D.

REFERENCES

1. Magnussen L. Insulin sensitivity assessed by clamp method during testosterone in type 2 diabetes, a randomized controlled trial. 2016.
2. Magnussen L. Changes in ectopic fat, adiponectin, and leptin levels during testosterone therapy in type 2 diabetes. A randomized, double-blinded, placebo-controlled trial. 2016.
3. Magnussen L. Testosterone therapy increases lean leg mass and preserves muscle mechanical function in aging men with type 2 diabetes, a randomized, double-blinded, placebo-controlled trial. 2016.
4. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet. 2006;367(9524):1747-57.
5. Adeyi OS, Owen; Robles, Sylvia. Public Policy and the Challenge of Chronic Noncommunicable Diseases. 2007.
6. Crimmins EM, Alley D, Reynolds SL, Johnston M, Karlamangla A, Seeman T. Changes in biological markers of health: older Americans in the 1990s. The journals of gerontology Series A, Biological sciences and medical sciences. 2005;60(11):1409-13.
7. Grossmann M. Low testosterone in men with type 2 diabetes: significance and treatment. The Journal of clinical endocrinology and metabolism. 2011;96(8):2341-53.
8. Nguyen CP, Hirsch MS, Moeny D, Kaul S, Mohamoud M, Joffe HV. Testosterone and "Age-Related Hypogonadism"--FDA Concerns. The New England journal of medicine. 2015;373(8):689-91.

9. Araujo AB, Esche GR, Kupelian V, O'Donnell AB, Travison TG, Williams RE, et al. Prevalence of symptomatic androgen deficiency in men. The Journal of clinical endocrinology and metabolism. 2007;92(11):4241-7.
10. Wu FC, Tajar A, Beynon JM, Pye SR, Silman AJ, Finn JD, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. The New England journal of medicine. 2010;363(2):123-35.
11. 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.
12. Tanna MS, Schwartzbard A, Berger JS, Alukal J, Weintraub H. The role of testosterone therapy in cardiovascular mortality: culprit or innocent bystander? Curr Atheroscler Rep. 2015;17(3):490.
13. Hwang K, Miner M. Controversies in testosterone replacement therapy: testosterone and cardiovascular disease. Asian journal of andrology. 2015;17(2):187-91.
14. Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, Macisaac RJ, Clarke S, et al. Low testosterone levels are common and associated with insulin resistance in men with diabetes. The Journal of clinical endocrinology and metabolism. 2008;93(5):1834-40.
15. Kotronen A, Juurinen L, Hakkarainen A, Westerbacka J, Corner A, Bergholm R, et al. 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(1):165-9.
16. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. The Journal of clinical endocrinology and metabolism. 2001;86(5):1930-5.
17. Fischer S, Hanefeld M, Haffner SM, Fusch C, Schwanebeck U, Kohler C, et al. Insulin-resistant patients with type 2 diabetes mellitus have higher serum leptin levels independently of body fat mass. Acta Diabetol. 2002;39(3):105-10.
18. 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. The Journal of clinical endocrinology and metabolism. 2011;96(10):3007-19.
19. Frederiksen L, Hojlund K, Hougaard DM, Brixen K, Andersen M. Testosterone therapy increased muscle mass and lipid oxidation in aging men. Age (Dordrecht, Netherlands). 2012;34(1):145-56.
20. Isidori AM, Giannetta E, Greco EA, Gianfrilli D, Bonifacio V, Isidori A, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. Clinical endocrinology. 2005;63(3):280-93.
21. 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 / European Federation of Endocrine Societies. 2007;156(5):595-602.
22. Kapoor D, Goodwin E, Channer KS, Jones TH. Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. European journal of endocrinology / European Federation of Endocrine Societies. 2006;154(6):899-906.
23. Gopal RA, Bothra N, Acharya SV, Ganesh HK, Bandgar TR, Menon PS, et al. Treatment of hypogonadism with testosterone in patients with type 2 diabetes mellitus. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2010;16(4):570-6.
24. 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(5):602-12.
25. Jones TH, Arver S, Behre HM, Buvat J, Meuleman E, Moncada I, et al. Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). Diabetes care. 2011;34(4):828-37.
26. Hackett G, Cole N, Bhartia M, Kennedy D, Raju J, Wilkinson P. Testosterone replacement therapy improves metabolic parameters in hypogonadal men with type 2 diabetes but not in men with coexisting depression: the BLAST study. The journal of sexual medicine. 2014;11(3):840-56.
27. Gianatti EJ, Dupuis P, Hoermann R, Strauss BJ, Wentworth JM, Zajac JD, et al. Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: a randomized controlled trial. Diabetes care. 2014;37(8):2098-107.
28. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, et al. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. The Journal of clinical endocrinology and metabolism. 2010;95(6):2536-59.
29. Surampudi PN, Wang C, Swerdloff R. Hypogonadism in the aging male diagnosis, potential benefits, and risks of testosterone replacement therapy. International journal of endocrinology. 2012;2012:625434.
30. Wang C, Nieschlag E, Swerdloff R, Behre HM, Hellstrom WJ, Gooren LJ, et al. Investigation, treatment and monitoring of late-onset hypogonadism in males: ISA, ISSAM, EAU, EAA and ASA recommendations. European journal of endocrinology / European Federation of Endocrine Societies. 2008;159(5):507-14.
31. Nielsen TL, Hagen C, Wraae K, Brixen K, Petersen PH, Haug E, et al. Visceral and subcutaneous adipose tissue assessed by magnetic resonance imaging in relation to circulating androgens, sex hormone-binding globulin, and luteinizing hormone in young men. The Journal of clinical endocrinology and metabolism. 2007;92(7):2696-705.
32. Nielsen TL, Wraae K, Brixen K, Hermann AP, Andersen M, Hagen C. Prevalence of overweight, obesity and physical inactivity in 20- to 29-year-old, Danish men. Relation to sociodemography, physical dysfunction and low socioeconomic status: the Odense Androgen Study. Int J Obes (Lond). 2006;30(5):805-15.
33. Dhindsa S, Miller MG, McWhirter CL, Mager DE, Ghanim H, Chaudhuri A, et al. Testosterone concentrations in diabetic and nondiabetic obese men. Diabetes care. 2010;33(6):1186-92.

34. Dhindsa S, Prabhakar S, Sethi M, Bandyopadhyay A, Chaudhuri A, Dandona P. Frequent occurrence of hypogonadotropic hypogonadism in type 2 diabetes. The Journal of clinical endocrinology and metabolism. 2004;89(11):5462-8.
35. Frost M, Wraae K, Nielsen TL, Hougaard DM, Brixen K, Hagen C, et al. Similar reference intervals for total testosterone in healthy young and elderly men: results from the Odense Androgen Study. Clinical endocrinology. 2013;78(5):743-51.
36. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. The Journal of clinical endocrinology and metabolism. 2001;86(2):724-31.
37. Vermeulen A, Kaufman JM. Diagnosis of hypogonadism in the aging male. The aging male : the official journal of the International Society for the Study of the Aging Male. 2002;5(3):170-6.
38. Giton F, Urien S, Born C, Tichet J, Guechot J, Callebert J, et al. Determination of bioavailable testosterone [non sex hormone binding globulin (SHBG)-bound testosterone] in a population of healthy French men: influence of androstenediol on testosterone binding to SHBG. Clin Chem. 2007;53(12):2160-8.
39. Hammond GL, Wu TS, Simard M. Evolving utility of sex hormone-binding globulin measurements in clinical medicine. Current opinion in endocrinology, diabetes, and obesity. 2012;19(3):183-9.
40. Deutschbein T, Mann K, Petersenn S. Total Testosterone and Calculated Estimates for Free and Bioavailable Testosterone: Influence of Age and Body Mass Index and Establishment of Sex-Specific Reference Ranges. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2015.
41. Swerdloff RS, Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, et al. Long-term pharmacokinetics of transdermal testosterone gel in hypogonadal men. The Journal of clinical endocrinology and metabolism. 2000;85(12):4500-10.
42. Wang C, Berman N, Longstreth JA, Chuapoco B, Hull L, Steiner B, et al. Pharmacokinetics of transdermal testosterone gel in hypogonadal men: application of gel at one site versus four sites: a General Clinical Research Center Study. The Journal of clinical endocrinology and metabolism. 2000;85(3):964-9.
43. 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(11):5081-8.
44. Faulkner JA, Larkin LM, Claflin DR, Brooks SV. Age-related changes in the structure and function of skeletal muscles. Clinical and experimental pharmacology & physiology. 2007;34(11):1091-6.
45. Srinivas-Shankar U, Roberts SA, Connolly MJ, O'Connell MD, Adams JE, Oldham JA, et al. Effects of testosterone on muscle strength, physical function, body composition, and quality of life in intermediate-frail and frail elderly men: a randomized, double-blind, placebo-controlled study. The Journal of clinical endocrinology and metabolism. 2010;95(2):639-50.
46. Bhasin S, Taylor WE, Singh R, Artaza J, Sinha-Hikim I, Jasuja R, et al. The mechanisms of androgen effects on body composition: mesenchymal pluripotent cell as the target of androgen action. The journals of gerontology Series A, Biological sciences and medical sciences. 2003;58(12):M1103-10.
47. Katznelson L, Rosenthal DI, Rosol MS, Anderson EJ, Hayden DL, Schoenfeld DA, et al. Using quantitative CT to assess adipose distribution in adult men with acquired hypogonadism. AJR Am J Roentgenol. 1998;170(2):423-7.
48. Unger RH. The physiology of cellular liporegulation. Annu Rev Physiol. 2003;65:333-47.
49. Carter S, Caron A, Richard D, Picard F. Role of leptin resistance in the development of obesity in older patients. Clin Interv Aging. 2013;8:829-44.
50. Xu XF, De Pergola G, Bjorntorp P. Testosterone increases lipolysis and the number of beta-adrenoceptors in male rat adipocytes. Endocrinology. 1991;128(1):379-82.
51. Hojlund K. Metabolism and insulin signaling in common metabolic disorders and inherited insulin resistance. Danish medical journal. 2014;61(7):B4890.
52. 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 : journal of the International Association for the Study of Obesity. 2004;28 Suppl 4:S12-21.
53. Bjorntorp P. "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis. 1990;10(4):493-6.
54. Marin P, Oden B, Bjorntorp P. Assimilation and mobilization of triglycerides in subcutaneous abdominal and femoral adipose tissue in vivo in men: effects of androgens. The Journal of clinical endocrinology and metabolism. 1995;80(1):239-43.
55. Yki-Jarvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. The lancet Diabetes & endocrinology. 2014;2(11):901-10.
56. Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. The Journal of clinical endocrinology and metabolism. 2004;89(7):3313-8.
57. Muthusamy T, Murugesan P, Balasubramanian K. Sex steroids deficiency impairs glucose transporter 4 expression and its translocation through defective Akt phosphorylation in target tissues of adult male rat. Metabolism: clinical and experimental. 2009;58(11):1581-92.
58. Ramamani A, Aruldhas MM, Govindarajulu P. Differential response of rat skeletal muscle glycogen metabolism to testosterone and estradiol. Canadian journal of physiology and pharmacology. 1999;77(4):300-4.
59. Sato K, Iemitsu M, Aizawa K, Ajisaka R. Testosterone and DHEA activate the glucose metabolism-related signaling pathway in skeletal muscle. American journal of physiology Endocrinology and metabolism. 2008;294(5):E961-8.
60. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. The New England journal of medicine. 1990;322(4):223-8.

61. Kelley DE. Skeletal muscle fat oxidation: timing and flexibility are everything. The Journal of clinical investigation. 2005;115(7):1699-702.
62. 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(5):715-21.
63. Diehl AM, Li ZP, Lin HZ, Yang SQ. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. Gut. 2005;54(2):303-6.
64. Stojsavljevic S, Gomercic Palcic M, Virovic Jukic L, Smircic Duvnjak L, Duvnjak M. Adipokines and proinflammatory cytokines, the key mediators in the pathogenesis of nonalcoholic fatty liver disease. World J Gastroenterol. 2014;20(48):18070-91.
65. Viengchareun S, Zennaro MC, Pascual-Le Tallec L, Lombes M. Brown adipocytes are novel sites of expression and regulation of adiponectin and resistin. FEBS Lett. 2002;532(3):345-50.
66. Ziemke F, Mantzoros CS. Adiponectin in insulin resistance: lessons from translational research. The American journal of clinical nutrition. 2010;91(1):258S-61S.
67. Kadowaki T, Hara K, Yamauchi T, Terauchi Y, Tobe K, Nagai R. Molecular mechanism of insulin resistance and obesity. Experimental biology and medicine (Maywood, NJ). 2003;228(10):1111-7.
68. Xu A, Chan KW, Hoo RL, Wang Y, Tan KC, Zhang J, et al. Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. The Journal of biological chemistry. 2005;280(18):18073-80.
69. Gavrila A, Chan JL, Yiannakouris N, Kontogianni M, Miller LC, Orlova C, et al. Serum adiponectin levels are inversely associated with overall and central fat distribution but are not directly regulated by acute fasting or leptin administration in humans: cross-sectional and interventional studies. The Journal of clinical endocrinology and metabolism. 2003;88(10):4823-31.
70. Hulthe J, Hulten LM, Fagerberg B. Low adipocyte-derived plasma protein adiponectin concentrations are associated with the metabolic syndrome and small dense low-density lipoprotein particles: atherosclerosis and insulin resistance study. Metabolism: clinical and experimental. 2003;52(12):1612-4.
71. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, et al. Plasma adiponectin levels in overweight and obese Asians. Obesity research. 2002;10(11):1104-10.
72. Scheen AJ, Luyckx FH. Obesity and liver disease. Best practice & research Clinical endocrinology & metabolism. 2002;16(4):703-16.
73. Stanley S, Wynne K, McGowan B, Bloom S. Hormonal regulation of food intake. Physiol Rev. 2005;85(4):1131-58.
74. Lonnqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nat Med. 1995;1(9):950-3.
75. Rosenbaum M, Leibel RL. 20 years of leptin: role of leptin in energy homeostasis in humans. The Journal of endocrinology. 2014;223(1):T83-96.
76. Fasshauer M, Bluher M. Adipokines in health and disease. Trends Pharmacol Sci. 2015;36(7):461-70.
77. Bluher M, Mantzoros CS. From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. Metabolism: clinical and experimental. 2015;64(1):131-45.
78. Marette A. Pathogenic role of inflammatory cytokines in obesity: from insulin resistance to diabetes mellitus. Nestle Nutr Workshop Ser Clin Perform Programme. 2004;9:141-50; discussion 51-3.
79. Wannamethee SG. Plasma leptin: Associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. Atherosclerosis. 2007(191):418–26.
80. De Maddalena C, Vodo S, Petroni A, Aloisi AM. Impact of testosterone on body fat composition. J Cell Physiol. 2012;227(12):3744-8.
81. Kosaki A, Yamada K, Kuzuya H. Reduced expression of the leptin gene (ob) by catecholamine through a G(S) protein-coupled pathway in 3T3-L1 adipocytes. Diabetes. 1996;45(12):1744-9.
82. Wabitsch M, Blum WF, Muche R, Braun M, Hube F, Rascher W, et al. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. The Journal of clinical investigation. 1997;100(4):808-13.
83. 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. Diabetol Metab Syndr. 2014;6(1):99.
84. Satoh N, Naruse M, Usui T, Tagami T, Suganami T, Yamada K, et al. Leptin-to-adiponectin ratio as a potential atherogenic index in obese type 2 diabetic patients. Diabetes care. 2004;27(10):2488-90.
85. Peterson MD, Rhea MR, Sen A, Gordon PM. Resistance exercise for muscular strength in older adults: a meta-analysis. Ageing Res Rev. 2010;9(3):226-37.
86. Frost M, Nielsen TL, Brixen K, Andersen M. Peak muscle mass in young men and sarcopenia in the ageing male. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2014.
87. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age and ageing. 2010;39(4):412-23.
88. Dulloo AG, Montani JP. Body composition, inflammation and thermogenesis in pathways to obesity and the metabolic syndrome: an overview. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2012;13 Suppl 2:1-5.
89. Afiadata A, Ellsworth P. Testosterone replacement therapy: who to evaluate, what to use, how to follow, and who is at risk? Hosp Pract (1995). 2014;42(5):69-82.
90. Laakso M. Cardiovascular disease in type 2 diabetes from population to man to mechanisms: the Kelly West Award Lecture 2008. Diabetes care. 2010;33(2):442-9.
91. Swellam M, Sayed M, Abdel-Fatah Ali A. Clinical implications of adiponectin and inflammatory biomarkers in type 2 diabetes mellitus. Dis Markers. 2009;27(6):269-78.
92. Frederiksen L, Hojlund K, Hougaard DM, Mosbech TH, Larsen R, Flyvbjerg A, et al. Testosterone therapy decreases subcutaneous fat and adiponectin in aging men.

European journal of endocrinology / European Federation of Endocrine Societies. 2012;166(3):469-76.
93. Neeland IJ, Ayers CR, Rohatgi AK, Turer AT, Berry JD, Das SR, et al. Associations of visceral and abdominal subcutaneous adipose tissue with markers of cardiac and metabolic risk in obese adults. Obesity (Silver Spring, Md). 2013;21(9):E439-47.
94. Manolopoulos KN, Karpe F, Frayn KN. Gluteofemoral body fat as a determinant of metabolic health. Int J Obes (Lond). 2010;34(6):949-59.
95. Neeland IJ, Turer AT, Ayers CR, Berry JD, Rohatgi A, Das SR, et al. Body fat distribution and incident cardiovascular disease in obese adults. Journal of the American College of Cardiology. 2015;65(19):2150-1.
96. Frederiksen L, Nielsen TL, Wraae K, Hagen C, Frystyk J, Flyvbjerg A, et al. Subcutaneous rather than visceral adipose tissue is associated with adiponectin levels and insulin resistance in young men. The Journal of clinical endocrinology and metabolism. 2009;94(10):4010-5.
97. Fernandez-Balsells MM, Murad MH, Lane M, Lampropulos JF, Albuquerque F, Mullan RJ, et al. Clinical review 1: Adverse effects of testosterone therapy in adult men: a systematic review and meta-analysis. The Journal of clinical endocrinology and metabolism. 2010;95(6):2560-75.
98. Muraleedharan V, Marsh H, Kapoor D, Channer KS, Jones TH. Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. European journal of endocrinology / European Federation of Endocrine Societies. 2013;169(6):725-33.
99. Swerdloff RS, Pak Y, Wang C, Liu PY, Bhasin S, Gill TM, et al. Serum Testosterone (T) Level Variability in T Gel-Treated Older Hypogonadal Men: Treatment Monitoring Implications. The Journal of clinical endocrinology and metabolism. 2015:JC20151542.
100. Mosbech TM LRVA. Automatic segmentation of
    1. abdominal adipose tissue in MRI. Lecture Notes in Computer
    2. Science, Proceedings of the 17th Scandinavian Conference on Image
    3. Analysis 1-1-2011. 2011.
101. Hother-Nielsen O, Henriksen JE, Holst JJ, Beck-Nielsen H. Effects of insulin on glucose turnover rates in vivo: isotope dilution versus constant specific activity technique. Metabolism: clinical and experimental. 1996;45(1):82-91.
102. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes care. 2004;27(6):1487-95.
103. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol Respir Environ Exerc Physiol. 1983;55(2):628-34.
104. Hvid LG, Suetta C, Nielsen JH, Jensen MM, Frandsen U, Ortenblad N, et al. Aging impairs the recovery in mechanical muscle function following 4 days of disuse. Exp Gerontol. 2014;52:1-8.
105. Caserotti P, Aagaard P, Larsen JB, Puggaard L. Explosive heavy-resistance training in old and very old adults: changes in rapid muscle force, strength and power. Scandinavian journal of medicine & science in sports. 2008;18(6):773-82.
106. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. Medicine and science in sports and exercise. 2008;40(1):181-8.
107. O'Connell MD, Wu FC. Androgen effects on skeletal muscle: implications for the development and management of frailty. Asian journal of andrology. 2014;16(2):203-12.
108. Kvorning T, Christensen LL, Madsen K, Nielsen JL, Gejl KD, Brixen K, et al. Mechanical muscle function and lean body mass during supervised strength training and testosterone therapy in aging men with low-normal testosterone levels. Journal of the American Geriatrics Society. 2013;61(6):957-62.
109. Storer TW, Woodhouse L, Magliano L, Singh AB, Dzekov C, Dzekov J, et al. Changes in muscle mass, muscle strength, and power but not physical function are related to testosterone dose in healthy older men. Journal of the American Geriatrics Society. 2008;56(11):1991-9.
110. Hildreth KL, Barry DW, Moreau KL, Vande Griend J, Meacham RB, Nakamura T, et al. Effects of testosterone and progressive resistance exercise in healthy, highly functioning older men with low-normal testosterone levels. The Journal of clinical endocrinology and metabolism. 2013;98(5):1891-900.
111. Park SW, Goodpaster BH, Strotmeyer ES, de Rekeneire N, Harris TB, Schwartz AV, et al. Decreased muscle strength and quality in older adults with type 2 diabetes: the health, aging, and body composition study. Diabetes. 2006;55(6):1813-8.
112. Kalyani RR, Tra Y, Yeh HC, Egan JM, Ferrucci L, Brancati FL. Quadriceps strength, quadriceps power, and gait speed in older U.S. adults with diabetes mellitus: results from the National Health and Nutrition Examination Survey, 1999-2002. Journal of the American Geriatrics Society. 2013;61(5):769-75.
113. Volpato S, Bianchi L, Lauretani F, Lauretani F, Bandinelli S, Guralnik JM, et al. Role of muscle mass and muscle quality in the association between diabetes and gait speed. Diabetes care. 2012;35(8):1672-9.
114. Page ST, Amory JK, Bowman FD, Anawalt BD, Matsumoto AM, Bremner WJ, et al. Exogenous testosterone (T) alone or with finasteride increases physical performance, grip strength, and lean body mass in older men with low serum T. The Journal of clinical endocrinology and metabolism. 2005;90(3):1502-10.
115. Sattler F, He J, Chukwuneke J, Kim H, Stewart Y, Colletti P, et al. Testosterone Supplementation Improves Carbohydrate and Lipid Metabolism in Some Older Men with Abdominal Obesity. Journal of gerontology & geriatric research. 2014;3(3):1000159.
116. Schroeder ET, Zheng L, Ong MD, Martinez C, Flores C, Stewart Y, et al. Effects of androgen therapy on adipose tissue and metabolism in older men. The Journal of clinical endocrinology and metabolism. 2004;89(10):4863-72.
117. 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(4):378-87.
118. Munzer T, Harman SM, Hees P, Shapiro E, Christmas C, Bellantoni MF, et al. Effects of GH and/or sex steroid administration on abdominal subcutaneous and visceral fat in healthy aged women and men. The Journal of clinical endocrinology and metabolism. 2001;86(8):3604-10.
119. Marin P, Holmang S, Gustafsson C, Jonsson L, Kvist H, Elander A, et al. Androgen treatment of abdominally obese men. Obesity research. 1993;1(4):245-51.

120. Mhatre AN, Trifiro MA, Kaufman M, Kazemi-Esfarjani P, Figlewicz D, Rouleau G, et al. Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. Nat Genet. 1993;5(2):184-8.
121. Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. The Journal of clinical endocrinology and metabolism. 1997;82(11):3777-82.
122. Zitzmann M, Gromoll J, von Eckardstein A, Nieschlag E. The CAG repeat polymorphism in the androgen receptor gene modulates body fat mass and serum concentrations of leptin and insulin in men. Diabetologia. 2003;46(1):31-9.
123. Skjaerpe PA, Giwercman YL, Giwercman A, Svartberg J. Androgen receptor gene polymorphism and the metabolic syndrome in 60-80 years old Norwegian men. International journal of andrology. 2010;33(3):500-6.
124. Nielsen TL, Hagen C, Wraae K, Bathum L, Larsen R, Brixen K, et al. The impact of the CAG repeat polymorphism of the androgen receptor gene on muscle and adipose tissues in 20-29-year-old Danish men: Odense Androgen Study. European journal of endocrinology / European Federation of Endocrine Societies. 2010;162(4):795-804.
125. Koplay M, Sivri M, Erdogan H, Nayman A. Importance of imaging and recent developments in diagnosis of nonalcoholic fatty liver disease. World J Hepatol. 2015;7(5):769-76.
126. Jones TH. Comment on Gianatti et al. Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: a randomized controlled trial. Diabetes Care 2014;37:2098-2107. Diabetes care. 2014;37(12):e267-8.
127. Huang G, Bhasin S, Tang ER, Aakil A, Anderson SW, Jara H, et al. Effect of testosterone administration on liver fat in older men with mobility limitation: results from a randomized controlled trial. The journals of gerontology Series A, Biological sciences and medical sciences. 2013;68(8):954-9.
128. 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. Exp Clin Endocrinol Diabetes. 2010;118(3):167-71.
129. 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 / European Federation of Endocrine Societies. 2012;167(4):531-41.
130. Knights AJ, Funnell AP, Pearson RC, Crossley M, Bell-Anderson KS. Adipokines and insulin action: A sensitive issue. Adipocyte. 2014;3(2):88-96.
131. Juang PS, Peng S, Allehmazedeh K, Shah A, Coviello AD, Herbst KL. Testosterone with dutasteride, but not anastrazole, improves insulin sensitivity in young obese men: a randomized controlled trial. The journal of sexual medicine. 2014;11(2):563-73.
132. Marin P, Holmang S, Jonsson L, Sjostrom L, Kvist H, Holm G, et al. The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 1992;16(12):991-7.
133. Sonmez A, Taslipinar A, Tapan S, Serdar MA. Comment on: Jones et al. Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 Study). Diabetes Care 2011;34:828-837. Diabetes care. 2011;34(11):e172; author's reply e3.
134. Couillard C, Gagnon J, Bergeron J, Leon AS, Rao DC, Skinner JS, et al. Contribution of body fatness and adipose tissue distribution to the age variation in plasma steroid hormone concentrations in men: the HERITAGE Family Study. The Journal of clinical endocrinology and metabolism. 2000;85(3):1026-31.
135. Hackett G, Cole N, Bhartia M, Kennedy D, Raju J, Wilkinson P, et al. The response to testosterone undecanoate in men with type 2 diabetes is dependent on achieving threshold serum levels (the BLAST study). International journal of clinical practice. 2014;68(2):203-15.
136. Daka B, Rosen T, Jansson PA, Rastam L, Larsson CA, Lindblad U. Inverse association between serum insulin and sex hormone-binding globulin in a population survey in Sweden. Endocrine connections. 2013;2(1):18-22.
137. 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(7):1680-7.
138. 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(6):726-33.
139. Wium C, Eggesbo HB, Ueland T, Michelsen AE, Torjesen PA, Aukrust P, et al. 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(8):700-7.
140. Kranendonk ME, van Herwaarden JA, Stupkova T, de Jager W, Vink A, Moll FL, et al. Inflammatory characteristics of distinct abdominal adipose tissue depots relate differently to metabolic risk factors for cardiovascular disease: distinct fat depots and vascular risk factors. Atherosclerosis. 2015;239(2):419-27.
141. Pijnappels M, van der Burg PJ, Reeves ND, van Dieen JH. Identification of elderly fallers by muscle strength measures. European journal of applied physiology. 2008;102(5):585-92.
142. Wyszomierski SA, Chambers AJ, Cham R. Knee strength capabilities and slip severity. Journal of applied biomechanics. 2009;25(2):140-8.
143. Lauretani F, Russo CR, Bandinelli S, Bartali B, Cavazzini C, Di Iorio A, et al. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. Journal of applied physiology (Bethesda, Md : 1985). 2003;95(5):1851-60.
144. Lindle RS, Metter EJ, Lynch NA, Fleg JL, Fozard JL, Tobin J, et al. Age and gender comparisons of muscle strength in 654 women and men aged 20-93 yr. Journal of applied physiology (Bethesda, Md : 1985). 1997;83(5):1581-7.

145. Casellini CM, Vinik AI. Clinical manifestations and current treatment options for diabetic neuropathies. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2007;13(5):550-66.
146. Morley JE. Sarcopenia: diagnosis and treatment. The journal of nutrition, health & aging. 2008;12(7):452-6.
147. Delmonico MJ, Harris TB, Visser M, Park SW, Conroy MB, Velasquez-Mieyer P, et al. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. The American journal of clinical nutrition. 2009;90(6):1579-85.
148. Morey MC, Sloane R, Pieper CF, Peterson MJ, Pearson MP, Ekelund CC, et al. Effect of physical activity guidelines on physical function in older adults. Journal of the American Geriatrics Society. 2008;56(10):1873-8.
149. Newman AB, Haggerty CL, Goodpaster B, Harris T, Kritchevsky S, Nevitt M, et al. Strength and muscle quality in a well-functioning cohort of older adults: the Health, Aging and Body Composition Study. Journal of the American Geriatrics Society. 2003;51(3):323-30.
150. Buchner DM, Cress ME, Esselman PC, Margherita AJ, de Lateur BJ, Campbell AJ, et al. Factors associated with changes in gait speed in older adults. The journals of gerontology Series A, Biological sciences and medical sciences. 1996;51(6):M297-302.
151. Basaria S, Coviello AD, Travison TG, Storer TW, Farwell WR, Jette AM, et al. Adverse events associated with testosterone administration. The New England journal of medicine. 2010;363(2):109-22.
152. Sam S, Haffner S, Davidson MH, D'Agostino RB, Sr., Feinstein S, Kondos G, et al. Relationship of abdominal visceral and subcutaneous adipose tissue with lipoprotein particle number and size in type 2 diabetes. Diabetes. 2008;57(8):2022-7.
153. Wilson PW, Garrison RJ, Castelli WP, Feinleib M, McNamara PM, Kannel WB. Prevalence of coronary heart disease in the Framingham Offspring Study: role of lipoprotein cholesterols. Am J Cardiol. 1980;46(4):649-54.
154. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol. 2000;20(6):1595-9.
155. Goldstein BJ, Scalia R. Adiponectin: A novel adipokine linking adipocytes and vascular function. The Journal of clinical endocrinology and metabolism. 2004;89(6):2563-8.
156. Hou N, Luo JD. Leptin and cardiovascular diseases. Clinical and experimental pharmacology & physiology. 2011;38(12):905-13.
157. Azvolinsky A. Androgen Deprivation Therapy-Linked Cardiovascular Disease Risk: Still Unresolved. J Natl Cancer Inst. 2015;107(9).