

Androgens and the Regulation of Adiposity and Body Fat Distribution in Humans

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ABSTRACT

The sexual dimorphism in human body fat distribution suggests a causal role for sex hormones. This is of particular importance when considering the role of excess visceral adipose tissue accumulation as a critical determinant of obesity-related cardiometabolic alterations. Scientific literature on the modulation of body fat distribution by androgens in humans is abundant, remarkably inconsistent and difficult to summarize. We reviewed relevant literature on this topic, with a particular emphasis on androgen replacement, androgen effects on selected parameters of adipose tissue function and adipose tissue steroid-converting enzymes. In men, low androgenic status mostly reflected by reduced total testosterone is a frequent feature of visceral obesity and the metabolic syndrome. Regarding testosterone therapy, however, studies must be appreciated in the context of current controversies on their cardiovascular effects. Analyses of available studies suggest that decreases in waist circumference in response to testosterone are more likely observed in men with low levels of testosterone and high BMI at study onset. In women with androgen excess, higher testosterone and free testosterone levels are fairly consistent predictors of increased abdominal and/or visceral adipose tissue accumulation, which is not the case in nonhyperandrogenic women. Regarding mechanisms, androgens decrease adipogenesis and markers of lipid storage *in vitro* in men and women. Evidence also suggest that local steroid transformations by adipose tissue steroid-converting enzymes expressed in a depot-specific fashion may play a role in androgen-mediated modulation of body fat distribution. Accumulating evidence shows that androgens are critical modulators of body fat distribution in both men and women. © 2018 American Physiological Society. *Compr Physiol* 8:1253-1290, 2018.

Didactic Synopsis

Major teaching points

1. Reduced total testosterone is observed frequently in men with abdominal and/or visceral obesity and the metabolic syndrome.
2. Reports on testosterone replacement therapy in men show that:
 - a. Observational studies have reported decreases in waist circumference in response to testosterone more frequently than randomized controlled trials.
 - b. This may be explained in part by the lower average waist circumference or BMI values and higher testosterone levels at baseline in randomized controlled trials compared to observational studies.
 - c. Independent of study design, decreases in waist circumference in response to testosterone are observed more frequently in men with low levels of testosterone and high BMI at study onset.
3. In women with androgen excess, higher testosterone and free testosterone levels are frequent correlates of increased abdominal and/or visceral fat accumulation; this may not be the case in nonhyperandrogenic women.
4. Steroid-converting enzymes expressed in adipose tissues may be involved in androgen-mediated modulation of body fat distribution.

Introduction

Obesity is an important public health concern because of its association with serious disorders such as type 2 diabetes

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(T2D) and cardiovascular disease (279,308). It is a heterogeneous condition with variable cardiometabolic risk owing to a complex hormonal and metabolic interplay involving many organs and tissues in the processes leading to atherogenesis, dyslipidemia, and insulin resistance. Accumulating evidence suggests that regional body fat distribution is a critical factor in the relationship between obesity and cardiometabolic disease, where significant sexual dimorphisms are observed (292). Generally, women have higher body fat percentages than men, who in turn have higher lean body mass (LBM) including muscle and bone (164, 239, 285, 300). Moreover, men present an android fat distribution pattern and preferentially accumulate fat in the abdomen, while women present a gynoid fat distribution pattern and more likely accumulate lipids in the gluteal and femoral regions of the body (267). Compared to women, men also have more visceral or intra-abdominal fat, that is, adipose tissue located inside the abdominal cavity, regardless of total adiposity (267). In spite of this dimorphism, wide interindividual variation in the amount of visceral adipose tissue (VAT) is observed in both sexes (262). For example, visceral fat varies by approximately 10-fold in samples of lean to moderately obese Caucasian men and women (262). The interindividual variation in visceral fat accumulation for a similar body fat mass (BFM) is illustrated in Figure 1.

Excess accumulation of abdominal fat as assessed by the waist circumference (WC) is a highly prevalent feature among the clustering risk factors found in the metabolic syndrome (MetS) (75, 235). The latter affected approximately 23% of US adults in 2010, and 56% of individuals had a high WC (21). In Canada, MetS was found in 18% of individuals in 2009 (235). The prevalence of cardiometabolic alterations increased with age, and high WC was the most prevalent diagnostic criterion of MetS (21, 235). These conditions not only contribute to the risk of T2D and cardiovascular disease, but also increase the risk, or alter the outcome of some cancers including breast cancer and endometrial cancer (89,267). Indications that android obesity and MetS affect close to one fifth of our population and up to half of older age individuals highlight the urgency of addressing these conditions as a priority. Interestingly, the amount of visceral fat has been shown repeatedly to be among the most critical determinants of the presence of the clustering metabolic syndrome features in men as well as women (75, 246).

The difference in regional body fat deposition in males versus females and the changes seen after menopause or other altered states of androgen deficiency or excess suggest a causal role for sex steroid hormones in human body fat distribution patterning (26, 267, 273). However, the specific role

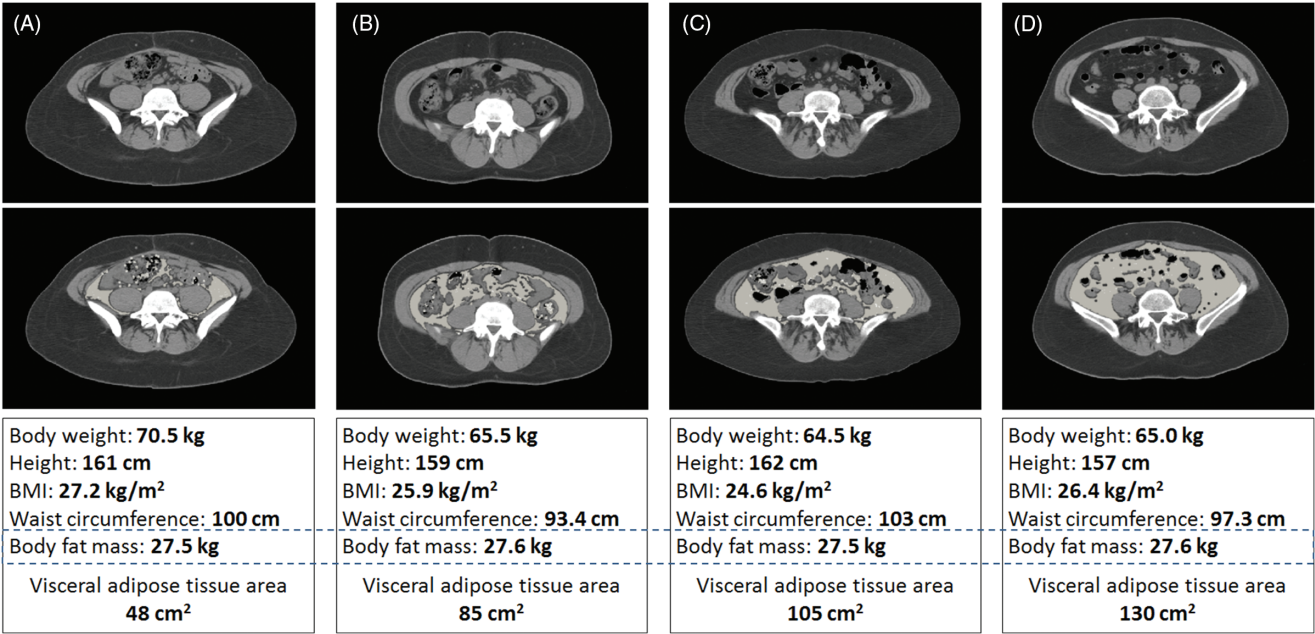


Figure 1 Illustration of the interindividual variability in visceral fat accumulation for a given total body fat mass in women. Computed tomography axial images were obtained at the L4-L5 vertebrae level in four women examined in the supine position. The visceral cavity was delineated and adipose tissue was highlighted and quantified as described in (73). Visceral adipose tissue (VAT) is shown in grey on the bottom scan of each panel. Total body fat mass was measured by dual-energy x-ray absorptiometry. For consistency, waist circumference (WC) values were obtained by measuring the perimeter of each scan by image analysis. Other anthropometric measurements were obtained in a standardized manner. The image in Panel A shows a cross-section of the abdomen of a woman with low VAT accumulation and a propensity for subcutaneous adipose tissue (SAT) storage. She is characterized by the highest BMI value and also has the highest SAT area (367 cm²). Images in Panels B and C show abdominal cross-sections from women with intermediary amounts of VAT. Their SAT areas are 260 and 327 cm², respectively. The image in Panel D shows a cross-section of the abdomen of a woman with a high propensity for VAT storage. SAT area is 281 cm². These substantial differences in VAT accumulation are noted in four women with similar heights (± 5 cm) and rigorously similar body fat mass values (± 100 g, 0.4% difference).

of each hormone remains quite elusive. In fact, through many decades of research on this topic, available literature, though widely abundant, remains remarkably inconsistent and difficult to summarize. This may be due to methodological caveats in some of the studies, including particularities of the study designs and populations examined of course, but most importantly, many reports have been plagued by difficulties in the accurate measurement of steroid levels. At the same time, the effects of sex steroids on each adipose tissue compartment are difficult to decipher at the cellular level. Access to abdominal adipose tissue samples and the complex dynamic interactions among all adipose tissue cell types clearly represent great challenges. As a particularly striking example, adipose tissues express a large number of steroid-converting enzymes that may either decrease or increase steroid action at the local level (273). The present overview article summarizes relevant literature on the role of androgens as modulators of body fat distribution patterns in men and women, with a particular emphasis on androgen replacement, androgen effects on selected parameters of adipose tissue function and adipose tissue steroid-converting enzymes.

Methodological Issues

Measurement of body composition, body fat distribution, and androgen levels

As illustrated above, assessing body shape and more specifically body fat distribution patterns has become a key issue in the evaluation of the metabolic risk associated with obesity (267). The reports reviewed in this manuscript are based on a large number of measurements or indices reflecting either body composition or body fat distribution. Many relied on anthropometric measurements whereas others used more detailed assessments of body composition and distribution such as imaging techniques. Anthropometric measurements of overall adiposity or body composition include body weight and the body mass index (BMI), the latter corresponding to body weight in kilograms divided by squared height in meters (156). More detailed measurements of body composition were performed using dual-energy x-ray absorptiometry (DXA) or occasionally hydrostatic weighing, which generate values for BFM, body fat percentage (%BF), and LBM or fat-free mass (FFM). Anthropometric indices of body fat distribution include WC and the waist-to-hip ratio (WHR). The former has been described as a superior proxy of VAT accumulation compared to the latter (211, 267) despite limitations that are clearly apparent in Figure 1.

Imaging techniques such as computed tomography (CT) or magnetic resonance imaging (MRI) have been used to assess visceral and subcutaneous adipose tissue areas or volumes specifically. Other methods to assess visceral and subcutaneous fat accumulation also may include ultrasonography or DXA. Addressing the strengths and limitations of each measurement technique is obviously beyond the scope of the

present article. However, as a general rule, imaging techniques assessing either body composition (i.e., DXA) or body fat distribution (i.e., CT, MRI, ultrasound) usually are considered superior measurements compared to anthropometric indices such as body weight or BMI (for body composition), and WC or the WHR (for body fat distribution). Limitation in the ability to adequately assess body fat distribution is one of the factors possibly contributing to discrepancies among available studies on the relationship between body fat distribution and androgens in men and women. From a lexical standpoint, the present article uses the term “visceral obesity” when visceral fat tissue was examined specifically. The term “abdominal obesity” is used when anthropometric measurements were used.

Additional methodological issues pertain to the assessment of androgens levels. With the initiative of the Endocrine Society, a committee has been formed to define objectives and develop a consensus on the issue of testosterone assay standardization (226). A full discussion of steroid measurement methods is beyond the scope of this article, but other authoritative papers have examined this issue (43, 224, 225, 261). Briefly, testosterone concentration may be assessed by immunoassay or liquid chromatography tandem-mass spectrometry (LC-MS/MS) (225). Radioimmunoassays and chemiluminescent immunoassays can be performed directly in the matrix examined (direct assays) or with prior extraction or chromatography (indirect immunoassays) (225). Immunoassay disadvantages are attributed mainly to specificity issues, usually problems with the antiserum or antibody. Sensitivity may also be lacking because of the low affinity of antibodies. Cross-reactivity may occur with structurally related compounds and interference of steroid-binding proteins. These issues were largely improved with the use of indirect immunoassays (261). The use of LC-MS/MS methodology has become increasingly prevalent in steroid hormone analysis because of its high analytical specificity (43). LC-MS/MS methods for testosterone and other steroid hormones were reviewed previously (43). The conclusion is that LC-MS/MS methods report values that are close to true testosterone concentrations. Unfortunately, no reference methods are available for androstenedione and dehydroepiandrosterone (DHEA), so the trueness of these methods could not be estimated (43). Some authors suggested that if the steroid assayed is in high concentration or specific, a well-validated immunoassay method is completely reliable. Testosterone in men is a good example (261). Taylor et al. (261) conducted a large comparative study on testosterone measurements by a well-standardized immunoassay compared to mass spectrometry in serum samples of more than 3000 men. They concluded that testosterone measurements by immunoassay offered good accuracy at all concentrations found in both eugonadal and hypogonadal men. However, in children and women, where low levels of androgens are seen or when structurally similar steroids may interfere, the LC-MS/MS method offered more accurate and reliable quantitation (261). Despite the generally accepted superiority of LC-MS/MS for measuring

testosterone in females, a recent study comparing total testosterone assays in women concluded that various LC-MS/MS methods still showed variability and poor precision at low levels, and that the results obtained by immunoassays and LC-MS/MS were comparable (163). LC-MS/MS has been proposed as the preferable method in the Endocrine Society Position Statement (163). Consequently, the majority of experts in the field emphasize the importance of assay validation and quality control, independently of the assay technology (261). Also, the majority of circulating testosterone is bound to sex hormone binding globulin (SHBG) (60-65%) or albumin (35-40%) (72). The fraction bound to SHBG is not accessible to tissues as opposed to testosterone bound to albumin (72). This physiological situation led to the employment of various means for expressing circulating testosterone levels: total testosterone, bioavailable testosterone (free and bound to albumin), and free testosterone. Free testosterone may be assayed directly or indirectly, or calculated with law of mass equations (225). The free androgen index (FAI) is also widely used, which is a testosterone/SHBG ratio (225). In general, reliability of androgen assessments will depend partly on the appropriateness of the methodology used (225), which may have contributed to discrepancies in the literature. The importance of the methodological issues pertaining to the measurement of body composition, body fat distribution, or androgen levels will be emphasized where relevant.

Regarding study design, the term “observational study” used in the section on androgens in men refers to intervention protocols (testosterone replacement therapy, TRT) in a single cohort follow-up. Outcomes are obtained by comparing the baseline and final values within the same individuals. Randomized controlled studies are based on the baseline random attribution of patients to two separate groups, a group receiving the treatment and the other receiving no intervention or a placebo (the control group). Outcomes are generated by comparing the intervention group values to the control group values. The term “observational study” in the section on androgens in women refers to cross-sectional or longitudinal data. It is not linked to any intervention protocol.

Androgens and Body Fat Distribution in Men

Endogenous androgens

Studies on androgens and body fat distribution in men generally are consistent in showing that obesity as well as increased accumulation of abdominal, visceral fat and the metabolic syndrome are associated with proportionally lower levels total testosterone, as evidenced from both cross-sectional and longitudinal studies (26, 27, 105, 162, 200). There is a linear age-related decrease in serum testosterone with concomitant increases in SHBG concentrations starting around age 20-30 years in men (151) that may, in some individuals, create a relative state of hypogonadism which is commonly

associated with abdominal obesity and the metabolic syndrome (162, 233). Convincing evidence for a higher risk of abdominal obesity and metabolic syndrome in men with lower total testosterone levels also was provided in a meta-analysis of 20 published studies where the presence of metabolic syndrome features predicted low total testosterone levels even after statistical adjustment for age and BMI (59).

The association between circulating levels of other androstane C19 steroids and body fat distribution is less convincing. In a review on DHEA and obesity (270), we found that in men, most studies reported a significant negative association between levels of unesterified DHEA and abdominal fat accumulation (62, 95, 268). When focusing on CT measurements of VAT area, a negative correlation was also observed with DHEA (62, 268). However, regarding the sulfate ester (DHEAS), negative associations were reported in some studies (62, 95), whereas others reported the opposite (219, 268). The same is true for studies using CT (62, 268). Some of the discrepancies observed in available studies on DHEA and body fat distribution may partly be due to variation in the ability of peripheral sites such as adipose tissues to convert DHEA into more potent steroids, as discussed later in this article.

The physiological mechanisms underlying the association between low testosterone and (abdominal) obesity have been reviewed elegantly elsewhere and this relationship appears to be bidirectional (103). The first causal direction is illustrated by the fact that variation in body weight relates to concomitant changes in total testosterone, SHBG, and free testosterone levels. For example, a review of 15 weight loss studies (103) indicated that the increase in circulating total testosterone was directly proportional to the amount of weight lost (or to the decrease in BMI) during the intervention. Not all studies found significant increases in free testosterone with weight loss, but positive findings apparently required more substantial weight loss (103). In a study where male sedentary monozygotic twins were required to achieve an energy deficit of 1000 kcal per day through standardized submaximal cycle-ergometer exercise during 93 days, a 5.0 ± 2.2 kg weight loss and a 4.9 ± 2.3 kg fat mass loss were observed (213). This intervention led to significant increases in total testosterone (from 12.3 ± 4.1 to 17.4 ± 5.1 nmol/L). Interestingly, males that lost the most visceral fat in response to the intervention had higher baseline testosterone levels and significant correlations were observed between changes in visceral fat or total BFM on the one hand and changes in testosterone on the other. Moreover, twin resemblance observed in baseline testosterone levels was not maintained after the intervention, suggesting that important changes in body composition and fat distribution superseded genetic determinism in this case (213).

The second causal direction is that low testosterone levels may underlie fat mass accretion and the development of abdominal obesity and concomitant metabolic alterations (103). As one example, we investigated the impact of baseline hormone concentrations on the susceptibility to gain weight and BFM in response to a standardized 840 kcal per day

overfeeding protocol over 100 days, leading to an average gain of 5.4 ± 1.9 kg in a sample of 24 young men (36). High baseline testosterone was one of the significant predictors of lower BFM accretion in response to the intervention. Other markers of androgenic status also predicted the response to overfeeding including baseline DHEA levels, which were negatively related to body weight and BFM gain, and concentrations of androgen metabolite androstene- $3\alpha,17\beta$ diol-glucuronide, which were positively related to these responses (36). The relevance of androgen metabolism in abdominal obesity is addressed later in this review.

Overall, a low androgenic status is reflected not only by reduced total testosterone but also by lower concentrations of free testosterone or other androstanes such as DHEA or DHEAS. In most reports, low androgenic status is a frequent feature in men with abdominal obesity and the metabolic syndrome. From the causality standpoint, this relationship seems to be bidirectional because body fatness fluctuation modulates testosterone levels and differences in testosterone predict modulation of fatness over time or in various weight-modifying intervention protocols.

Androgen replacement therapy in men

Abundant evidence is available in the scientific and clinical literature regarding the effect of androgen replacement therapy on body composition and fat distribution in various patient populations. Two meta-analyses examining data from available randomized clinical trials (RCTs) and observational studies showed that TRT was associated with a significant decrease in BFM and an increase in LBM (57,58). Within these meta-analyses, study sizes, variability in patient population, treatment dosing, and methods to measure body composition conferred small to medium treatment effect for BFM and LBM, respectively. This was consistent with a meta-analysis published 11 years before on RCTs in pooled hypogonadal and eugonadal middle-aged and aging men using testosterone, testosterone ester and dihydrotestosterone (DHT) preparations (138). Yet, the effect of TRT on total weight, BMI and WC is not as consistent in the literature. The RCT meta-analysis by Corona et al. revealed no TRT effect on these outcomes (58), whereas the meta-analysis of observational studies reported a significant time-dependent effect, which may account for these discrepancies (57). The pooled baseline BMI values of patients enrolled in the studies included in both meta-analyses differed significantly (57). The observational studies tended to have a longer duration of treatment (mean 18 months) whereas available RCTs were limited to shorter duration (<12 months) and may not have captured the effect of TRT on WC and weight (57,58). Indeed, with long term TRT, a 10.6 to 14.3 cm decrease in WC was reported and total weight loss averaged 17.4 to 30.5 kg, when stratifying by baseline class of obesity in a registry cohort of obese men treated with testosterone undecanoate over 5 years (249). This compares to a change in WC of 6.23 cm and a weight loss of 3.5 kg reported in the observational

meta-analysis (57). Other baseline characteristics of the study populations differed, with observational studies enrolling younger patients (average of 51.7 ± 6.1 years) with more severe hypogonadism as determined by baseline testosterone levels (average of 7.2 nmol/L vs. 11.6 nmol/L in pooled RCTs) and higher rates of T2D (57). This may reflect more rigid entry criteria for the RCTs in which the effect of TRT on body composition, WC, and total weight were not the primary outcomes. However, these meta-analyses still demonstrated a greater treatment effect in terms of WC, weight loss, and BMI in younger patients (<60 years) with lower baseline testosterone measurements and in patients with T2D or obesity (BMI >30 kg/m²) at baseline (57,58).

Regarding indirect markers of visceral adiposity and the MetS, the same meta-analyses reported changes in blood lipid parameters, glucose metabolism, and blood pressure. There were no statistically significant changes in total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol in the pooled population of patients receiving TRT within RCTs (58). However, when only placebo-controlled TRT trials in hypogonadal patients are considered (baseline total T < 12 nmol/L), triglycerides and total cholesterol decreased significantly (58). This finding was also supported in observational studies, which showed a significant decrease in total cholesterol (-0.83 mmol/L, $P < 0.0001$), triglycerides (-0.47 mmol/L, $P < 0.0001$), and a significant increase in HDL cholesterol (0.12 mmol/L, $P < 0.01$) (57). Glucose metabolism is improved as well with TRT in studies including normoglycemic and hyperglycemic patients. Fasting glucose (44,57,58) and HOMA-IR (57,58) were reduced in the two RCT meta-analyses and in pooled observational studies with greater effect among patients with MetS and obesity. These findings are supported by another meta-analysis and systematic review of TRT effect in men with T2D and metabolic syndrome (MetS) that also reported a significant decrease in insulin resistance with studies using HOMA1, but not with studies using HOMA2 (116). Glycated hemoglobin A1c did not differ significantly in the meta-analysis specifically examining patients with T2D, but this may reflect the relative short duration of the trials examined (44,116). Discrepancies on the effect of TRT on blood pressure appeared between study designs with no changes in systolic or diastolic blood pressure reported in pooled RCTs (58), but a decrease in both parameters was shown in observational studies (57).

There was no association between BFM and LBM outcomes and final total testosterone level, although within the studies represented in the RCT meta-analysis, testosterone levels in the treatment groups normalized and were significantly higher when compared to placebo and baseline testosterone measurements (58). It was noted also that intramuscular (IM) and transdermal preparations were related to higher testosterone levels at follow-up compared to oral preparations. Improvements of body composition were significant with the use of transdermal and parenteral formulations, but transdermal gel preparations produced significantly better results than patches in short-term studies (274). Among the numerous

types of parenteral preparations, IM testosterone undecanoate generated the largest effect. No improvements in BFM or LBM were noted in RCTs using oral testosterone formulations (58).

Large RCTs in various hypogonadal populations examining the effect of TRT on body composition have not been reported and are less likely to occur in the future as the focus of TRT has now shifted to cardiovascular safety and mortality outcomes. RCTs and observational studies with similar baseline patient characteristics and treatment formulation are lacking, making the broad interpretation of outcomes more challenging. As a result, it is currently difficult to draw formal conclusions to guide clinical practice in using TRT to alter body composition in the treatment of obesity and metabolic disease. A thorough analysis may be useful to understand the variable and conflicting results within existing literature, specifically in terms of WC, which is a recognized clinical marker of VAT accumulation and has the largest effect on MetS. The next sections present additional analyses of existing literature to specifically assess the effect of TRT on WC and VAT accumulation.

Scientific literature on TRT, weight, BMI, and abdominal obesity in men

To delineate the effects of TRT on body composition and fat distribution, we conducted an extensive PubMed search with the following key words and MESH terms: “visceral/adipose tissue,” “body composition,” “fat mass,” “testosterone/treatment/replacement therapy/supplementation,” “clinical trial,” “randomized clinical trial,” and “observational” to the end of August 2017. We also screened the references in the three previously cited meta-analyses (57,58,138) and the bibliographies of all other additional single studies. Studies were included after 1992 due to variability of testosterone assay reporting and lack of data on WC, VAT, and BFM in older trials. English-language observational studies and RCTs were included, and case reports and case series were excluded. Studies met inclusion if they comprised the following criteria: (i) available baseline total testosterone measurement; (ii) outcome reporting of WC, weight or BMI as a primary or secondary outcome; (iii) presence of a testosterone-only subgroup in studies using other androgen formulations (such as DHT) or concomitant therapies with other androgen-modifying agents (finasteride, clomiphene, hCG, and anabolic steroids). Studies reporting the effects of TRT on female or transgendered patients and patients with HIV were excluded from this section of the article.

Baseline patient characteristics including age, BMI, WC, total testosterone, comorbidities, testosterone formulation, and treatment duration as well as study design are summarized in Tables 1 and 2. Hypogonadal status was determined by a baseline total testosterone level below the lower limit of the normal range in the testosterone assay used if available, or less than 11 nmol/L if not reported, per Endocrine Society Guidelines (22). Measurements of free or bioavailable

testosterone and SHBG were not included due to limited reporting among studies. Our primary outcome was WC with additional analysis of treatment effect on weight, BMI, SAT and VAT. Outcomes were described qualitatively as “decrease,” “no change,” or “increase.” To establish a qualitative change, a statistically significant difference was required post-treatment between the TRT group and the placebo group for RCTs. For observational studies, the effect was determined based on results compared to baseline as determined in each individual study.

A total of 84 studies that tested the effect of TRT on body composition met all our selection criteria. Forty-seven of these studies were RCTs (Table 1) including 38 double-blind randomized placebo-controlled studies, 5 double-blind randomized placebo-controlled crossover studies, and 3 studies of single-blind randomized placebo-controlled crossover, single-blind randomized placebo-controlled and open-label randomized controlled protocols. Thirty-seven were observational studies, of which 34 had prospective designs (Table 2). The studies varied widely with respect to testosterone preparation, delivery method and dose as well as protocol design, measured endpoints and baseline characteristics of patients including age (range of mean 20.8–77.6 years). Baseline testosterone levels ranged from 2.5 to 21.6 nmol/L and various testosterone assays were used. Represented patients were heterogeneous in terms of medical comorbidities and indications for TRT with 61 studies including patients meeting criteria for hypogonadism and 29 studies including patients with features of MetS or T2D (Tables 1 and 2). One study was conducted in patients treated long term with exogenous glucocorticoids for respiratory conditions (74), three studies included patients with established cardiac disease (64, 176, 182) and two studies were conducted in patients with chronic obstructive pulmonary disease (COPD) (49, 252).

Effect of TRT on body weight and BMI

Baseline mean reported weight and BMI for patients were included in the 34 RCTs and 28 observational studies that documented weight outcomes. Patients were within the overweight and obese categories with the exception of two RCTs, including one that enrolled patients with COPD (252, 274) and one observational study (189). With respect to measured weight, 2 RCTs reported a decrease after TRT (39, 146), 25 reported no change (2, 17, 35, 37, 45, 64, 76, 94, 108, 120, 132, 135, 175, 179, 180, 182, 190, 195, 240, 242, 248, 249, 252, 253, 303), and 7 reported an increase (49, 101, 102, 169, 176, 238, 274). In contrast, a higher proportion of observational studies reported weight loss, with 13 studies showing a decrease from baseline (5, 99, 100, 122–124, 228–230, 232, 275, 309, 310). No changes in weight were reported in nine studies (71, 143, 150, 187, 221, 227, 247, 296, 319) and weight gain was observed in six (23, 41, 189, 297, 298, 312). Similar results were seen with respect to BMI. Among the 35 RCTs that documented BMI, only 2 reported a decrease (146, 182). The majority of RCTs reported no change in BMI

Table 1 Randomized Controlled Trials Examining the Effect of TRT on Body Composition

Ref.	Baseline characteristics of the participants					TRT formulation/duration		Outcomes			
	N	Comorbidities	Age (years)	BMI (kg/m ²)	WC (cm)	TTL (nmol/L)	Formulation	Duration (months)	WC (Δ)	Weight (Δ)	BMI (Δ)
Tenover 1992	13	-	66.7 ± 5.4	22.0 ± 9.0	NA	11.7 ± 1.5	IM TE	3	NA	↑	NA
Sih 1997	17	-	67 ± 7	29.1 ± 5.2	NA	9.2 ± 0.9 ^a	IM TCY	12	NA	↔	↔
Snyder 1999	54	-	≥ 65	27.1 ± 2.9	NA	12.7 ± 2.7	TP	36	NA	↔	↔
Kenny 2001	24	-	76 ± 4	27 ± 3	NA	13.5 ± 6.0	TP	12	NA	NA	↔
Ferrando 2002 ^a	7	-	68 ± 3	NA	NA	9.6-15.9	IM TE	6	NA	↔	NA
Boyanov 2003	24	T2D	57.5 ± 4.8	31.08 ± 4.79	NA	9.56 ± 2.33	Oral TU	3	NA	↓	↔
Liu 2003 ^a	17	-	67.5 ± 0.8	NA	NA	NA	IM ME	1	NA	↑	↑
Steidle 2003	106 102	-	56.8 ± 10.6 60.5 ± 9.7	29.9 ± 3.3 29.9 ± 3.8	NA	8.1 ± 2.2 8.3 ± 2.4	Gel TP	3	NA	↔	NA
Wittert 2003	39	-	69 ± 6	27.9 ± 4.1	NA	17.0 ± 4.4 ^a	Oral TU	12	NA	↔	NA
Casaburi 2004	12	COPD	66.6 ± 7.5	NA	NA	10.5 ± 3.1	IM TE	2.5	NA	↑	NA
Malkin 2004 ^a	13	CHF	74.1 ± 2.3	25.9 ± 1.2	NA	14.3 ± 2.1	IM ME	1	NA	↑	NA
Svarberg 2004	15	COPD	64.5 ± 6.5	23.8 ± 3.2	NA	21.6 ± 5.7	IM TE	6	NA	↔	NA
Page 2005	24	-	71 ± 4	28.7 ± 3.6	NA	9.9 ± 1.6	IM TE	36	NA	↔	NA
Giannoulis 2006 ^a	23	-	70.3 ± 0.6	26.9 ± 0.7	NA	17.2 ± 1.2	TP	6	NA	NA	↔
Nair 2006 ^b	27	-	66.2 (61.8-72.3)	28.4 (25.7-30.3)	NA	12.4 (9.8-16.1)	TP	24	NA	↔	↔
Emmerlot-Vonk 2008	120	-	67.1 ± 5.0	27.4 ± 3.8	NA	11.0 ± 1.9	Oral TU	6	NA	NA	↔
Caminiti 2009	35	CHF	71	26.4 ± 3.7	NA	8.0 ± 6.2	IM TU	3	NA	↔	↑
Mathur 2009	7	CA	62.1 ± 5.2	30.4 ± 4.7	NA	9.8 ± 1.9	IM TU	12	NA	↔	↓
Sheffield-Moore 2011	8	-	73 ± 8	27 ± 2	NA	11.8 ± 2.9	IM TE	5	NA	↑	↔
Behre 2012	183	-	61.9 ± 6.6	28.5 ± 3.3	NA	10.4 ± 2.6	Gel	6	NA	↔	NA
Glinborg 2013 ^b	20	-	68 (62-72)	NA	107 (103-115)	NA	Gel	6	NA	NA	NA
Borst 2014 ^a	14	-	69.2 ± 8.0	29.4 ± 4.6	NA	8.5 ± 2.5	IM TE	12	NA	↔	↔
Marin 1992 ^a	11	-	51.9 ± 2.0	29.3 ± 0.8	106.0 ± 2.6	16.0 ± 1.2	Oral TU	8	↔	↔	↔
Marin 1993 ^a	9	-	56.7 ± 2.2	29.4 ± 0.7	107.3 ± 1.5	15.1 ± 0.8	Gel	9	↔	↔	↔
Munzer 2001 ^a	17	-	70.8 ± 0.7	26.4 ± 0.8	94.3 ± 2.2	15.3 ± 0.8	IM TE	6	↔	NA	↔
Simon 2001 ^a	6	-	52.8 ± 4.2	29.9 ± 0.9	NA	8.3 ± 0.3	Gel	3	↔	↔	NA
Crawford 2003 ^a	18	ITGCT	58.7 ± 4.9	26.7 ± 1.6	98.9 ± 3.9	13.8 ± 0.4	IM ME	12	↔	↔	↔
Agladahl 2008	13	-	68.9 ± 5.4	30.6 ± 3.9	109.1 ± 9.8	8.5 ± 1.7	IM TU	12	↔	NA	↔
Allan 2008 ^a	31	-	62.1 ± 1.0	26.1 ± 0.4	94.7 ± 1.4	13.6 ± 0.5	TP	12	↔	↔	↔
Svarberg 2008	17	-	69 ± 5	30.6 ± 3.8	110 ± 10	8.4 ± 1.7	IM TU	12	↔	↔	↔

(Continued)

Table 1 (Continued)

Ref.	Baseline characteristics of the participants						TRT formulation/duration		Outcomes		
	N	Comorbidities	Age (years)	BMI (kg/m ²)	WC (cm)	TTL (nmol/L)	Formulation	Duration (months)	WC (Δ)	Weight (Δ)	BMI (Δ)
Gopal 2010	22	T2D	44.23 ± 3.29	23.94 ± 4.46	89.09 ± 11.4	10.2 ± 3.7	IM TCY	12	↔	NA	↔
Jones 2011	108	MetS or T2D	59.9 ± 9.1	32.87 ± 6.58	112.70 ± 13.22	9.2 ± 2.6	Gel	12	↔	NA	↔
Frederiksen 2012	23	–	68	30.2 ± 3.6	109.0 ± 8.2	12.5 ± 4.0	Gel	6	↔	↑	↑
Hoyos 2012	33	OSA	48.0 ± 1.6	34.9 ± 4.3	115.7 ± 8.8	13.2 ± 5.3	IM TU	4.5	↔	↔	↔
Bouloux 2013	237	–	58.7 ± 5.8	27.3 ± 3.4	100.3 ± 10.1	12.8 ± 4.2	Oral TU	12	↔	↔	NA
Frederiksen 2013 ^b	20	–	68 (62.72)	29.8 (27.5–32.9)	107 (103–115)	12.2 (9.4–15.8)	Gel	6	↔	↑	↑
Hildreth 2013	47	–	66.5 ± 5.8	29.2 ± 3.3	106.3 ± 19.2	10.3 ± 1.5	Gel	12	↔	↔	↔
Tan 2013 ^c	56	–	53.8 ± 6.9	30.5 ± 5.3	103.1 ± 12.5	8.9 ± 2.0	IM TU	12	↔	NA	↔
Gianatti 2014 ^b	45	T2D	62 (58–68)	32.5 (28.3–35.5)	110.0 (104.0–120.8)	10.6 (9.0–13.0)	IM TU	7.5	↔	↔	↔
Dhindsa 2016	20	T2D	56.4 ± 7.9	39.0 ± 7.6	128.0 ± 20	8.98 ± 2.95	IM TCY	6	↔	↔	↔
Magnussen 2016	20	T2D	61 ± 6	30.6 (28.9–32.3) ^b	106 (102–111) ^b	7.1 (6.6–11.9) ^b	Gel	6	↔	↔	↔
Kapoor 2007 ^a	20	T2D	63.15 ± 1.5	33.28 ± 1.02	115.95 ± 2.72	7.54 ± 0.55	IM ME	3	↓	NA	↔
Aversa 2010 ¹	32	MetS	58 ± 10	30.2 ± 4.5	105 ± 10	NA	IM TU	6	↓	NA	↔
	10		57 ± 8	32.5 ± 5.2	NA	NA	Oral TU		↔		
Aversa 2010 ²	40	MetS	58 ± 10	30.2 ± 4.5	105.5 ± 8.0	9.0 ± 1.7	IM TU	12	↓	NA	↔
Kalinchenko 2010	113	MetS	51.6	35.3	118.0	6.7	IM TU	7.5	↓	↓	↓
Hackett 2014	92	T2D	61.2 ± 10.5	33.0 ± 6.1	115.1 ± 13.1	9.2 ± 3.1	IM TU	7.5	↓	↔	↔

The data are presented as mean ± SD unless otherwise indicated.

^aMean ± SEM.

^bMedian (interquartile range).

^cMedian ± SD.

Non-SI unit values of testosterone were converted to nmol/L by a factor of 0.03467.

↑, increase; ↔, no change; ↓, decrease; NA, nonavailable; WC, waist circumference; TTL, total testosterone level; CA, chronic angina; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; Gel, testosterone gel; IM, intramuscular; LTGCT, long-term glucocorticoid treatment; ME, mixed esters; MetS, metabolic syndrome; OSA, obstructive sleep apnea; PHH, postsurgical hypogonadotropic hypogonadism; SL, sublingual; T2D, type 2 diabetes; TCY, testosterone cypionate; TCYD, testosterone cyclodextrin; TE, testosterone enanthate; TP, testosterone patch; TU, testosterone undecanoate.

Study design:

(1, 2, 10, 17, 35, 37, 45, 64, 76, 85, 94, 101, 102, 108, 109, 120, 132, 135, 144, 146, 155, 175, 179, 180, 182, 188, 190, 195, 238, 240, 242, 248, 252, 253, 260, 303): Double-blind randomized placebo-controlled study

(11, 249): Double-blind randomized placebo-controlled crossover study

(113, 147, 148, 169, 274): Double-blind randomized placebo-controlled crossover study

(176): Single-blind randomized placebo-controlled crossover study

(49): Single-blind randomized placebo-controlled study

(39): Open-label randomized controlled study

Table 2 Observational Studies Examining the Effect of TRT on Body Composition

Ref.	Baseline characteristics of the participants					TRT formulation/ duration		Outcomes			
	N	Comorbidities	Age (years)	BMI (kg/m ²)	WC (cm)	TTL (nmol/L)	Formulation	Duration (months)	WC (Δ)	Weight (Δ)	BMI (Δ)
Morley, 1993	8	–	77.6±2.3	NA	NA	NA	IM TE	3	NA	↔	NA
Young, 1993 ^a	13	–	30.2±1.5	NA	NA	20.7±1.7	IM TE	6	NA	↑	NA
Brodsky, 1996 ^a	5	–	NA	NA	NA	3.7±1.9	IM TCY	6	NA	↑	NA
Katznelson, 1996	29	–	57 ^c	28.2±0.8 ^a	NA	6.4±0.6 ^a	IM TE	18	NA	↔	↔
Zgliczynski, 1996 ^a	22	–	58.5±2.6	28.1	NA	4.4	IM TE	12	NA	NA	↔
Bhasin, 1997	7	–	36	24.7	NA	2.5±1.0 ^a	IM TE	2.5	NA	↑	NA
Snyder, 2000	18	–	51 ^c	NA	NA	2.7±2.7	TP	36	NA	↔	NA
Wang, 2000 ^a	78	–	(19-67)	NA	NA	8.60±0.55	Gel	6	NA	↑	NA
	76					8.22±0.55	TP				
Wang, 2004 ^a	123	–	51.5±0.9	29.00±0.34	NA	9.2	Gel	42	NA	↔	NA
Dean, 2005	371	–	58.5±10.0	NA	NA	8.1±2.1	Gel	12	NA	↔	NA
Naharci, 2007	24	–	20.75±0.74	20.93±2.12	NA	5.8±1.3	IM ME	6	NA	↑	↑
Minnemann, 2008	20	–	18-65	28.1±4.5	NA	NA	IM TE	7	NA	NA	↔
	20			26.6±4.4			IM TU				
Moon, 2010	133	–	54.0±9.6	25.0±2.7	NA	8.7±7.3	IM TU	6	NA	NA	↔
Schwarz, 2011	56	–	52.3±7.8	33.2±3.3	NA	15.2±6.8	IM TCY	6-18	NA	↓	↓
Arafa, 2012	56	T2D	55.5±7.8	30.7±4.5	NA	8.9±1.7	IM TU	3-6	NA	↓	↓
Jo, 2013	18	–	35.9±3.3	25.6±5.1	NA	3.12±2.2	IM TU	12	NA	↔	↔
Ko, 2013 ^b	246	–	58.5 (52.0-64.2)	24.91 (23.24-26.55)	NA	8.7 (6.8-10.6)	IM TU	14.7	NA	NA	↓
Rodriguez-Tolra, 2013	712	345 MetS and 151 T2D	59.1±5.6	29.0±3.8	NA	10.2±3.6	Gel and IM TU	24	NA	↔	↔
Wang, 1996 ^a	67	–	19-60	28.0±0.5	98.7±1.4	4.13±0.40	SL TCYD	6	↑	↑	↑
Saad, 2008	27	–	60	NA	107.8±9.4	7.6±1.4	Gel	9	↓	↔	NA
	28		61		102.0±11.0	7.6±2.1	IM TU			↔	
Heufelder, 2009 ^a	16	MetS or T2D	57.3±1.4	32.1±0.5	107.9±1.3	10.5±0.2	Gel	12	↓	NA	NA
Permpongkosol, 2010	161	93 MetS	60.4±9.27	26.0±3.7	93.34±9.20	9.4 (8.1-11.4) ^b	IM TU	23 ^c	↓	NA	↔
Bhattacharya, 2011	213	MetS	53.0±11.3	34.6±6.6	114.3±16.0	9.0	Gel	12	↓	NA	NA
	368	–	50.9±12.2	28.7±6.4	95.5±13.7	10.9			↔		

(Continued)

Table 2 (Continued)

Ref.	N	Comorbidities	Age (years)	Baseline characteristics of the participants			TRT		Outcomes		
				BMI (kg/m ²)	WC (cm)	TTL (nmol/L)	Formulation	Duration (months)	WC (Δ)	Weight (Δ)	BMI (Δ)
Aversa, 2013	40	MetS	58 ± 10	30.0 ± 4.5	NA	8.3 ± 2.4	IM TU	36	↓	NA	↔
Saad, 2013	255	80 T2D	58.02 ± 6.30	33.90 ± 5.51	107.24 ± 9.14	9.93 ± 1.38	IM TU	60	↓	↓	↓
Tirabassi, 2013	15	PHH	55.66 ± 8.64	NA	95.7 ± 10.3	5.31 ± 1.8	IM TU	18.5-21	↓	↓	NA
Yassin, 2013	261	80 T2D	59.5 ± 8.4	31.7 ± 4.4	107.7 ± 10.0	7.7 ± 2.1	IM TU	60	↓	↓	↓
Zitzmann, 2013	1493	14% T2D	49.2 ± 13.9	NA	99.50 ± 15.25	9.6 ± 7.5	IM TU	9-12	↓	↔	NA
Francomano, 2014 ¹	20	MetS or T2D	57 ± 8	31 ± 6	NA	8.3 ± 2.4	IM TU	60	↓	↓	↓
Francomano, 2014 ²	12	71% MetS	53 ± 8	42.6 ± 5.2	134 ± 12	8.5 ± 1.8	IM TU	13.5	↓	↓	↓
Haider, 2014 ²	181	178 MetS and 72 T2D	59.11 ± 6.06	36.72 ± 3.72	111.20 ± 7.54	10.06 ± 1.30	IM TU	60	↓	↓	↓
Haider, 2014 ¹	156	T2D	61.17 ± 6.18	36.31 ± 3.51	114.00 ± 8.69	8.9 ± 1.99	IM TU	72	↓	↓	↓
Pexman-Fieth, 2014	712	345 MetS and 151 T2D	53 ± 12	31 ± 4	107 ± 12	NA	Gel	6	↓	NA	↓
Saad, 2015	450	–	56.10 ± 6.29	32.58 ± 5.08	106.54 ± 9.03	8.96 ± 1.95	IM TU	72	↓	↓	↓
	111	–	68.45 ± 2.91	32.84 ± 4.86	108.95 ± 10.75	8.48 ± 2.26					
Haider, 2016	77	CVD and 41 T2D	60.65 ± 4.98	37 ± 4	112 ± 8	9.8 ± 1.6	IM TU	96	↓	↓	↓
Saad, 2016	411	173 T2D	59.46 ± 7.05	35.43 ± 3.48	110.6 ± 8.4	9.13 ± 1.9	IM TU	96	↓	↓	↓
Yassin, 2016	115	–	62.28 ± 7.34	30.81 ± 4.33	106.47 ± 8.72	7.84 ± 2.34	IM TU	102.9	↓	↓	↓

The data are presented as mean ± SD unless otherwise indicated.

^aMean ± SEM.

^bMedian (interquartile range).

^cMedian ± SD.

Non-SI unit values of testosterone were converted to nmol/L by a factor of 0.03467.

↑, increase; ↔, no change; ↓, decrease; NA, nonavailable; WC, waist circumference; TTL, total testosterone level; CA, chronic angina; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; Gel, testosterone gel; IM, intramuscular; LTGCT, long-term glucocorticoid treatment; ME, mixed esters; MetS, metabolic syndrome; OSA, obstructive sleep apnea; PHH, postsurgical hypogonadotropic hypogonadism; SL, sublingual; T2D, type 2 diabetes; TCY, testosterone cypionate; TCYD, testosterone cyclodextrin; TE, testosterone enanthate; TP, testosterone patch; TU, testosterone undecanoate.

Study design:

[2,6,7,12,15,39,45,54,61,63,64,78,88,93,94]: Prospective study

(159,232,275): Retrospective study

(122-124,228-230,309,310): Cumulative prospective registry study

(9,100,187,189,312): Controlled prospective study

(131): Single-blind randomized parallel study

(184,296,298): Open-label randomized parallel study

(99,227): Prospective parallel study

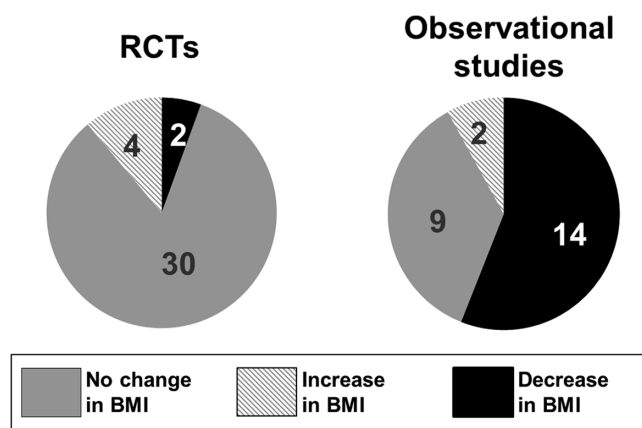


Figure 2 Contrasting effects of available randomized control trials (RCTs) and observational studies describing the effect of testosterone replacement therapy (TRT) on the body mass index (BMI). Studies included in this figure were identified as described in the text. Most RCTs reported a nonsignificant effect of TRT on BMI whereas a higher proportion of observational studies reported a significant decrease in BMI following TRT. Numerical values on the charts indicate the number of study treatment groups in each category.

(1, 2, 10, 11, 35, 39, 64, 76, 85, 108, 109, 113, 120, 132, 135, 144, 147, 148, 155, 175, 179, 180, 188, 190, 238, 240, 248, 253, 260) and a small number reported an increase (45, 101, 102, 169). Among the 24 observational studies that documented BMI, the majority of them reported a reduction in BMI (5, 99, 100, 122-124, 159, 207, 228-230, 309, 310) and a small number reported no change (9, 143, 150, 184, 186, 206, 221, 316). Two studies reported an increase in BMI (189, 297). Where both postintervention weight and BMI were reported, the results were largely consistent. Figure 2 illustrates the contrast between RCTs and observational studies. Interestingly, decreases in weight or BMI were observed, independent of study design, in studies recruiting men with a combination of a baseline BMI in the obesity range and a low baseline total testosterone (Tables 1 and 2). Corona et al. previously reported no effect of TRT on weight or BMI among RCTs; however, the studies included in the analysis generated pooled data of BMI in the overweight range and/or eugonadism at initiation of therapy (58).

As mentioned, duration of therapy also may influence weight outcomes. Treatment duration of studies that showed weight gain or increased BMI did not exceed 6 months (41, 101, 102, 189, 238, 297, 298, 312) and most of these studies lasted 3 months or less (23, 49, 169, 176, 274). In contrast, observational studies reporting weight loss and reduced BMI ranged in treatment duration from 3.0 to 102.9 months (5, 99, 100, 122-124, 228-230, 232, 275, 309, 310). This observation may be explained by the fact that weight and BMI cannot differentiate between LBM and BFM and that increases in LBM may occur before the decrease of BFM, thus causing a transient increase or neutral effect on overall measured weight. For example, Corona et al. postulated that opposing effects of TRT causing an increase in LBM and reduction in BFM to the same degree could lead to a null effect on weight

and BMI (58). Observational studies showing a decrease in weight had a mean treatment duration of 56 months, suggesting that the RCTs included in Corona et al. did not generate significant results due to insufficient treatment duration. Indeed, both in our analysis and that of Corona et al. the mean treatment duration of RCTs where a weight neutral or gain effect was demonstrated was 9.6 months. The null effect on weight might occur between 6 and 12 months. Therefore, extending trial duration is needed to observe decreases in BFM that are superior to LBM increases. This hypothesis is further supported by a study from Hackett et al. (120). Treatment with testosterone undecanoate in a patient population with T2D demonstrated no significant change in body weight or BMI after 7.5 months; however, after treatment extension to 13 months, a significant decrease in both body weight and BMI was reported. The concomitant changes in LBM and BFM with TRT also suggest that a more specific measurement of body composition may be required to assess the metabolic effects of treatment. Consideration of the WC and changes in VAT through imaging methods could logically differentiate the effects of TRT on body composition from those more specifically affecting body fat distribution and metabolic outcomes.

Effect of TRT on waist circumference

WC is used frequently as a surrogate marker for VAT (267). Measurements of WC were documented in 25 RCTs and 19 observational studies, the majority of which included patients with a WC value meeting MetS criteria. The majority of RCTs displayed a neutral effect (1, 2, 37, 64, 76, 101, 102, 108, 113, 132, 135, 144, 175, 179, 180, 188, 242, 253, 260), but a small number of studies showed a decrease in WC (10, 11, 120, 146-148). Among observational studies, all reported that TRT induced a decrease in WC (9, 24, 99, 100, 122-124, 131, 206, 207, 227-230, 275, 309, 310, 319), with the exception of one study reporting a WC increase (297) and one group within a parallel study that had a WC within normal limits prior to treatment showing no change (24). These observations are consistent with previous meta-analyses (57, 58). The contrast in treatment effects on WC between RCTs and observational studies is illustrated in Figure 3.

To further investigate the importance of baseline levels of obesity and testosterone, we plotted the values obtained from RCTs and observational studies and found a significant negative correlation between baseline BMI and baseline testosterone (Fig. 4). This association with data obtained from many independent studies is consistent with the one usually observed in cross-sectional samples. Interestingly, the studies that reported a significant loss of WC in response to TRT generally segregated to the left of the regression, suggesting that independent of trial design, studies enrolling obese men with low baseline total testosterone are more likely to report a decrease in WC in response to TRT (Fig. 4). A very similar relationship is observed when plotting values of baseline WC and baseline testosterone level (Fig. 5). In both analyses, it

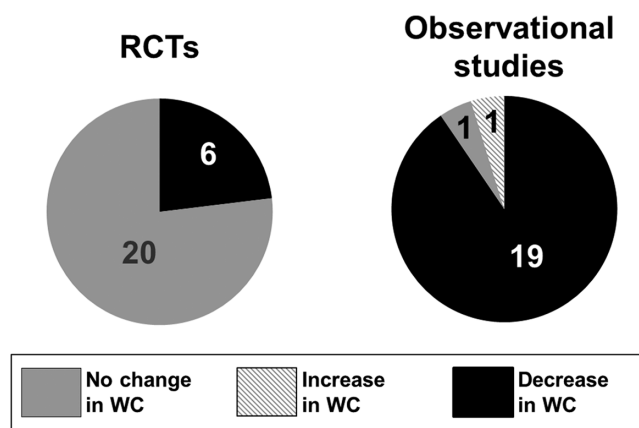


Figure 3 Contrasting effects of available randomized control trials (RCTs) and observational studies describing the effect of testosterone replacement therapy (TRT) on waist circumference (WC). Studies included in this figure were identified as described in the text. Most RCTs reported a nonsignificant effect of TRT on WC whereas a much higher proportion of observational studies reported a significant decrease in WC following TRT. Numerical values on the charts indicate the number of study treatment groups in each category.

is striking that none of the studies involving men with baseline testosterone levels above 11 nmol/L reported a significant changes in WC (Figs. 4 and 5). TRT appears to be effective only in men with the combination of (abdominal) obesity and hypogonadism. Relatively similar results were obtained when testing this association in RCTs and observational studies separately. For example, when only RCTs are considered, the studies reporting a significant WC decrease consisted of patients with BMI in the obesity range ($>30 \text{ kg/m}^2$) and baseline total testosterone suggestive of hypogonadism. Again, none of the studies reporting a favorable outcome on WC had baseline testosterone levels above 10 or 11 nmol/L. In this case, however, some of the trials examining patients with low

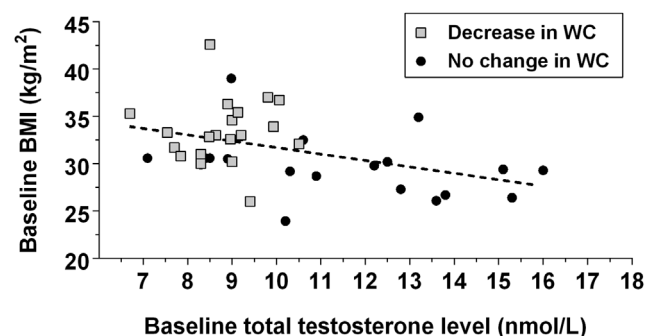


Figure 4 Correlation between average baseline total testosterone concentration and initial BMI in trials on testosterone replacement therapy (TRT) that observed either a decrease in waist circumference (WC) (gray squares), or no change in WC (black circles). Data were extracted from randomized control trials and observational studies as described in the text. Statistical significance of the change in WC was used as described in each publication. The correlation was significant (Spearman rank correlation coefficient -0.41 , $P < 0.01$). None of the studies reporting a significant effect of TRT on WC had average baseline total testosterone values above 11 nmol/L.

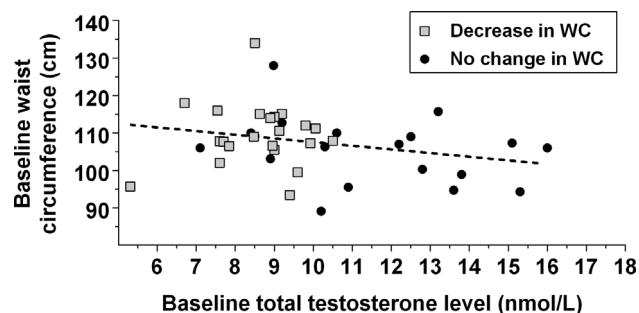


Figure 5 Correlation between average baseline total testosterone concentration and initial waist circumference (WC) in trials on testosterone replacement therapy (TRT) that observed either a decrease in WC (gray squares), or no change in WC (black circles). Data were extracted from randomized control trials and observational studies as described in the text. Statistical significance of the change in WC was used as described in each publication. The correlation was close to significance (Spearman rank correlation coefficient -0.30 , $P < 0.06$). None of the studies reporting a significant effect of TRT on WC had average baseline total testosterone values above 11 nmol/L.

testosterone at baseline reported no impact of the treatment. It is important to note that these studies also enrolled a large proportion of nonobese men, which could have skewed pooled WC outcomes. No relationship between age and baseline total testosterone level was observed despite the known decline in testosterone levels with age (not shown). This likely is due to a selection bias of younger patients with hypogonadism in available studies. Therefore, firm conclusions cannot be reached as to whether older age is a contributing factor to the absence of effect on WC seen in RCTs. Overall, findings of these analyses indirectly suggest that enrolling obese men or abdominally obese men with low initial testosterone levels likely increases the probability of detecting a significant effect of TRT on WC.

As opposed to what is observed with body weight and BMI, short-term studies are sufficient to detect a significant decrease in WC with TRT. In fact, many short-term RCTs lasting 9 months or less (11, 120, 146–148) reported significant statistical differences in WC between the treatment and control/placebo groups while a considerable number of RCTs showed no effect with longer follow-up periods up to 12 months (1, 2, 37, 64, 113, 132, 144, 253, 260). Studies examining longer treatment duration would contribute to a greater effect size of TRT on WC. However, extending placebo-based treatments in hypogonadal patients would increase the likelihood of side effects on reproductive function or the risk of osteoporosis, which is not feasible. The differences in the kinetics of the body composition vs. WC response to TRT further suggests that WC and other imaging modalities may offer more information in assessing the impact of TRT.

Effect of TRT on visceral and subcutaneous adipose tissue

There were 13 RCTs and one observational study (150) that assessed the effect of TRT on SAT and on VAT directly

or in addition to other anthropometric markers such as WC. Measurements were performed by multiple modalities including MRI (2, 101, 108, 111, 188), DXA (135, 190), CT (135, 150, 179, 180, 190, 253), or ultrasonography (85). Among these studies, results are equivocal with respect to the effect of TRT on SAT with studies showing either a reduction (101, 108, 111, 150, 188, 253) or no change (2, 85, 135, 179, 180). With VAT, the effect of TRT appears to be neutral as 75% of studies reported no change (85, 101, 108, 111, 135, 150, 188, 190, 253). Although three studies did find a correlation between TRT and decreased VAT, they did not reach statistical significance with respect to the observed decrease in WC (2, 179, 180). Furthermore, decreases in VAT were in the order of -0.2 , -0.4 , and -0.6 kg, respectively, and may only have been significant due to demonstrated increases in VAT in the placebo groups. It is difficult to base conclusions on these data due to the limited number of studies assessing this outcome and the heterogeneity among the reports. The studies differed not only in the measurement method of adipose tissue, but also in treatment formulations and age of the subjects. Furthermore, most studies enrolled eugonadal, nonobese subjects, whom as discussed previously may be less responsive to TRT regarding WC. Further studies assessing the effect of TRT on VAT and SAT are needed as these measurements previously have been shown to be more accurate in assessing visceral obesity than WC (267).

Effect of TRT on body composition in specific patient subgroups

In addition to evidence of a treatment effect in obese, hypogonadal subjects, our analysis revealed that all RCTs showing a significant decrease in WC enrolled men with metabolic impairments, either T2D (120, 147, 148) or MetS (10, 11, 146) and the majority of studies reporting no effect of TRT on WC included subjects without metabolic complications (1, 2, 37, 64, 101, 102, 132, 135, 179, 180, 188, 242, 253, 260). Only five studies with MetS or T2D subjects showed no effect (76, 108, 113, 144, 175). A similar result is noted among observational studies, where 10 publications involved a patient sample with a form of metabolic impairment and at least half had a complication of metabolic disease (9, 24, 99, 100, 122–124, 131, 206, 207). Three studies did not quantify the number of patients with a metabolic complication (227, 230, 309) and one study enrolled men with postsurgical hypogonadotropic hypogonadism (275). Furthermore, three studies had a considerable percentage (~ 30 – 42%) of patients with T2D in their sample, but did not document presence of MetS in the absence of T2D (228, 229, 310). Only a single observational study reporting an effect did not include a large proportion of metabolic disease (14% T2D) (319). The Testim registry based in the US population has assessed the effect of TRT in a patient population with MetS and non-MetS. Concordant with the observations presented here, they reported a decrease in WC in the MetS group but no change in the non-MetS group (24).

Impact of androgen formulation

The various formulations of testosterone replacement used across studies introduce further heterogeneity that could impact the conclusion on the effect of testosterone. Among the RCTs examined, many formulations were utilized including oral testosterone undecanoate, transdermal testosterone gel or patches as well as IM testosterone undecanoate, testosterone enanthate, testosterone cypionate, and mixed esters. The same formulations were used within observational studies in addition to IM testosterone cyclodextrin. In both RCTs and observational studies, IM routes of therapy were associated more frequently with decreases in WC compared to oral or transdermal formulations, despite potential concerns about medication nonadherence that could occur in observational studies with formulations that are self-administered. These findings are substantiated further by direct comparison of oral and IM testosterone undecanoate whereby the latter was superior in decreasing WC and BFM in hypogonadal patients with the MetS (11). Corona et al. also noted no improvement in LBM or BFM with oral formulations using pooled data from RCTs but significant improvements were noted with parenteral and IM formulations with testosterone undecanoate leading to larger reductions (58). Further heterogeneity is likely found in studies testing IM formulations because target testosterone levels differed among publications and not all reported peak levels achievement with injections. Based on the limited results available, we suggest that although oral, parenteral, and IM testosterone formulations are all able to yield therapeutic testosterone levels, IM testosterone undecanoate results in greater changes in LBM and BFM.

Summarizing data on TRT, body composition, and body fat distribution in men

The present review indicates that controlled studies on TRT report a decrease in BFM and an increase in LBM in hypogonadal men (total testosterone level < 11 nmol/L) with obesity (BMI > 30 kg/m²) and metabolic impairments. These findings were more prominent when considering WC, which is a marker of body fat distribution. However, the limited number of studies and differing protocols impair our ability to draw similar conclusions with respect to the effect of TRT on VAT and SAT measured by imaging techniques. Our findings are consistent with several conclusions within three previous meta-analyses (57, 58, 138), which included RCTs and observational studies that were analyzed as separate pooled populations. Our analysis provides a novel perspective suggesting that the effects of TRT on body composition in observational studies but not in RCTs are likely the result of treatment duration (> 12 months) rather than specific protocol design issues. A few limitations may be pointed out in our analysis. Our identification of articles, although inclusive, was not conducted in a systematic manner. Moreover, due to limited availability of patient characteristics in many studies, it is also possible that some patient samples overlapped, particularly in the prospective registry studies. In many cases, the studies evaluated

did not include anthropomorphic indices or body composition as a primary outcome and, therefore, may not have been sufficiently powered to achieve statistical significance. Most studies did not include weight loss as a targeted outcome or initial selection criterion, which also may have affected the results. Finally, due to the heterogeneity of studies, our analyses were limited mostly to qualitative assessments of significance. Further studies are required prior to considering TRT as a specific, sustainable treatment for obesity or abdominal obesity, even in hypogonadal populations (228). In addition to inconsistent literature, safety concerns over TRT in obese patients and complications associated with the MetS warrant caution of its use in these populations. Four studies published within the last 6 years reported an increase of cardiovascular events among men who were treated with testosterone, prompting the issuance of a black box warning on testosterone formulations by the Food and Drug Administration (FDA) citing increased risk of myocardial and other adverse cardiac events (15,96,293,305). The Androgen Study Group has since challenged the conclusions of these studies over concerns of statistical analysis and study methodology. A large retrospective observational case-control study reported no association between the risk of myocardial infarction and all current or past use of testosterone (86). Furthermore, TRT actually may reduce all-cause mortality, risk of myocardial infarction and stroke in hypogonadal patients (237). Testosterone is known to induce erythropoiesis, with increases in hemoglobin and hematocrit occurring in a linear, dose-dependent manner (63). Because obesity is also a known risk factor for venous thromboembolism, thorough monitoring is required with TRT in this population (236). Despite these ongoing controversies and concerns, the high prevalence of overweight and obesity in North American, European and South Asian populations require scientific and medical attention. Our analysis further contributes to the literature examining the role of testosterone as a potential treatment for abdominal obesity in select male populations.

Regarding supraphysiological levels of androgens, a recent literature review reported on the effects of anabolic-androgen steroids (AAS) use on human health (210). Briefly, AAS use has been associated with cardiovascular side effects such as cardiomyopathy, hypertension, and an increased risk of myocardial infarction, stroke, sudden death as well as conduction and coagulation abnormalities (210). AAS is reported to cause changes in heart morphology and histology including cardiomegaly, fibrosis, and myocytolysis (210). AAS is also related to altered blood lipids mainly involving increased LDL-C and a decrease in HDL-C (210). Endothelial dysfunction may be implicated as well because a study demonstrated increased nitrous oxide production and oxidative stress when supraphysiological testosterone levels were reached (245). Polycythemia is a frequent adverse event, as androgens stimulate erythropoiesis by increasing sensitivity to erythropoietin, suppressing hepcidin transcription, and increasing iron availability for erythropoiesis (210). Psychiatric symptoms are also reported in AAS abuse groups

including increases in hypomanic or manic syndromes, the latter which are characterized by irritability, aggressiveness, exaggerated self-confidence, hyperactivity, reckless behavior, and occasional psychotic symptoms (210). Other adverse effects such as hypothalamic-pituitary-thyroid axis suppression and increased risk of tendon rupture are associated with AAS (210). Deleterious effects on the liver and kidneys may also be observed but the association remains to be clearly established. Imperlini et al. (137) have shown that supra-physiological T or DHT affected many molecular pathways related to inflammation, atherosclerosis, calcium homeostasis, and apoptosis (137). In a cross-sectional study, AAS users had significantly higher VAT measured by DXA and lower insulin sensitivity compared to the control group (218).

Role of Androgens in Female Body Fat Distribution

As previously discussed, women preferentially tend to accumulate fat in the gluteo-femoral area compared to men who tend to have more adipose deposition in the abdominal region (196). Differences in sex steroid hormone levels, mainly androgens and their ratios to estrogens, are thought to be the cause of differences in fat distribution, although fat accumulation also may influence hormone levels conferring a more androgenic profile (185). This is supported by hormonal and physical changes that occur during menopause, where reductions in estrogen production in the face of preserved androgen secretion from the ovaries and a shift toward increased adrenal androgen production likely cause increases in the testosterone/estrogen ratio. These hormonal shifts are accompanied by changes in body composition and body fat distribution consistent with accelerated visceral fat accumulation following menopause (171). Furthermore, in both pre- and postmenopausal women, abdominal obesity has been associated with increases in free testosterone levels and lower sex hormone binding globulin (SHBG), conferring a more androgenic profile (88). Low circulating levels of SHBG also are associated with accumulation of visceral abdominal fat and a pattern of metabolic complications similar to those in men (66, 121, 152, 269, 272). Along with other evidence from women with polycystic ovary syndrome (PCOS, see section 3.3 below), the documented sexual dimorphism in fat distribution and the menopause-related increases in abdominal adiposity led to a widespread assumption that high androgens increase abdominal fat and VAT accumulation in women. However, reports on this relationship are far from unanimous. This section of the article reviews studies that focused on androgens and body fat distribution in women.

Scientific literature on androgens and body fat distribution in women

An extensive PubMed search using the following keywords and MESH terms was performed: “visceral/adipose tissue,”

“body composition,” “women/female,” “pre-/peri-/post-menopause,” “testosterone/androgen,” and “polycystic ovary syndrome/PCOS” up to August 2017. We also screened and selected articles from each of the references of reports selected in this query. Publications included were English-language RCTs or observational studies with case reports and case series excluded. To be included, studies needed to meet the following criteria: (i) available assessment abdominal fat or VAT accumulation using anthropomorphic measurements (WC or WHR) or imaging modalities; (ii) report on the relationship between body composition and total testosterone level, free testosterone level, androstenedione, DHEA or SHBG concentration. Studies describing the effects of androgens in transgendered patients or patients with androgen excess syndromes other than PCOS such as isolated hirsutism, congenital adrenal dysplasia, or androgen-producing tumors were excluded.

Study characteristics including cohort size, methodology to measure body fat distribution, correlation coefficient between hormone levels and adiposity variables as well as statistical significance for total testosterone, free testosterone and androstenedione, in women with PCOS or normal androgens are summarized in Tables 3 to 7. Androgen measurements were interpreted in the context of normal range values for the specific assays used in each study. PCOS status was confirmed by criteria outlined in each study or through Rotterdam Criteria (90) unless explicitly stated. We further cross-referenced average BMI (per WHO classification) (46) to determine if this value influenced study outcomes. Our primary goal was to determine whether a correlative relationship exists between circulating androgen levels and abdominal fat accumulation in women.

Androgens and body fat distribution in women with no indication of androgen excess

As shown in Table 3, a total of 21 cross-sectional and 2 longitudinal studies tested the association between total testosterone level and abdominal fat with variable results. Of the smaller cross-sectional studies, 9 (47, 51, 79, 88, 152, 153, 199, 203, 208, 314) found no significant correlation between testosterone levels and body fat distribution indices. In contrast, four studies were able to demonstrate a positive association between total testosterone level and body fat distribution measurements (48, 54, 70, 87, 106, 199) across different measurement modalities, whereas four reported negative correlations (6, 67, 69, 281). The data from cross-sectional measurements in larger studies also are equivocal. Indeed, in 359 women from the Study on Woman's Health Across the Nation (SWAN) cohort, Janssen et al. reported no correlation between total testosterone level and VAT measured by CT (141). Furthermore, cross-sectional data from the Mammary Carcinoma Risk factor Investigation (MARIE) cohort that included 1180 women, showed no association between total testosterone level and WC (168). On the other hand, the Shanghai Breast Cancer Study (SBCT), which included 420 women, showed a

positive association between total testosterone level and WC (40). In the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, Vaidya et al. found a positive association between total testosterone level and WHR in the cross-sectional baseline data analysis, but they did not find any significant correlation between the changes in those parameters over a 1- to 3-year follow-up (284). Goss et al. also found no correlation between testosterone and VAT changes in 53 women enrolled in a 2-year study (114). Taken together, combined evidence from multiple studies with varied protocols do not allow a firm conclusion on the relationship between total testosterone level and abdominal fat or VAT accumulation at this time.

Table 4 lists the 21 studies examining free testosterone levels and abdominal fat measurements. Of these, seven small cross-sectional studies found free testosterone levels to be positively associated with abdominal fat or VAT accumulation in women (47, 54, 88, 119, 129, 141, 161, 168, 203, 208, 234) but seven studies found no correlation (6, 47, 67, 139, 140, 152, 281). Interestingly, the study by Cao et al. evaluated the correlation between free testosterone levels and abdominal fat in an early postmenopausal group and a late postmenopausal group and a significant positive correlation was seen only in the former group (47).

All cross-sectional studies using large cohorts (MARIE, SWAN, MESA, and Seidell et al.) showed a positive association between free testosterone level and either WC (168, 234) or VAT accumulation (141, 185). In 2015, Janssen et al. examined the relationship between the changes of free testosterone level and the changes of VAT in the SWAN cohort over a 4-year follow-up and were able to demonstrate a positive correlation (141). Furthermore, two smaller longitudinal studies found positive associations between free testosterone level and VAT accumulation (2-year follow-up) as well as abdominal fat measured by DXA (5-year follow-up), respectively (114, 119). Because longitudinal studies and cross-sectional samples in both large cohorts and most small studies show a positive correlation, the conclusion that free testosterone level is positively associated with abdominal fat and VAT in women seems accurate. Menopausal status did not seem to influence the correlation between free testosterone and VAT, suggesting that even the lower free testosterone level seen in premenopausal, nonhyperandrogenic women may predict abdominal adiposity. Differences in the results across multiple studies and inconsistencies in measuring free testosterone and total testosterone levels in women illustrate the need for additional studies confirming these findings.

With respect to androstenedione, almost all studies found no significant association between circulating levels of this steroid and abdominal adiposity indices (51, 61, 67, 69, 88, 139, 152, 153, 199, 203, 281) (Table 5). Cross-sectional data from the MARIE cohort generated a nonsignificant association (168), as did the longitudinal study by Goss et al. (114). Only one cross-sectional study found androstenedione to be a positive correlate of WC (106).

Data on DHT is limited due to its being sparsely measured in research studies compared to other androgens. However, in

Table 3 Selected Studies That Tested the Relation Between Total Testosterone and Body Fat Distribution Indices in Women Without Androgen Excess

Study	n	Menopausal status	Age	Body fat distribution measurement	Correlation coefficient	P value
Nonsignificant correlation						
<i>Cross-sectional studies</i>						
Evans et al., 1983	80	Premenopausal	19-49 ^b	WHR	−0.22	NS
Kaye et al., 1991	88	Postmenopausal	61.0 ± 3.3	WHR	−0.11	NS
Pasquali et al., 1993	138	Premenopausal	27.9 ± 8.1	WHR	−0.064	NS
Zamboni et al., 1994	19	Premenopausal	37.1 ± 13.8	CT - VAT	0.23	NS
Pedersen et al., 1995	25	Premenopausal	33.2 ± 1.5 ^a	DXA- Trunk fat	−0.02	NS
Phillips et al., 2008	78	58 Premenopausal	32.9 ± 1.2 ^a	MRI - VAT	0.11	NS
		20 Postmenopausal	61.4 ± 2.4 ^a		0.42	NS
Casson et al., 2010	29	Postmenopausal	60.1 ± 1.0 ^a	CT - VAT	−0.11	NS
Janssen et al., 2010	359	48 Premenopausal 177 Perimenopausal 134 Postmenopausal	50.6 ± 3.9	CT - VAT	0.062	NS
Keller et al., 2011	30	Premenopausal	27.3 ± 0.8 ^a	CT - VAT	−0.38	NS
Liedtke et al., 2012	1180	Postmenopausal	64.1 ± 5.8	WC	0.01	NS
Cao et al., 2013	212	105 Early Postmenopausal	54.58 ± 2.98	DXA - Trunk/leg fat	0.076	NS
		107 Late Postmenopausal	69.41 ± 7.28		−0.055	NS
<i>Longitudinal studies</i>						
Vaydia et al., 2012	1678	Postmenopausal	65.6 ± 9.2	WHR	NS	NS
Goss et al., 2012	53	Postmenopausal	45-55 ^b	CT - VAT	NS	NS
Significant correlation						
<i>Cross-sectional studies</i>						
Armellini et al., 1994	36	Premenopausal	34 ± 11	CT - VAT	−0.513	<0.01
De Pergola et al., 1994	40	Premenopausal	29.5 ± 8.1	Sonography - IAT	−0.324	<0.05
Cigolini et al., 1996	18	Premenopausal	38 ± 0	CT - VAT	0.48	<0.05
De Pergola et al., 1996	28	Premenopausal	33.8 ± 9.61	CT - VAT	−0.401	<0.05
Turcato et al., 1997	41	26 Premenopausal	33.7 ± 10.2	CT - VAT	−0.41	<0.01
		15 Postmenopausal	57.9 ± 5.9			
Garavulet et al., 2000	55	22 Premenopausal	38 ± 8	WC	0.31	<0.05
		33 Postmenopausal	61 ± 6			
De Simone et al., 2001	29	Premenopausal	14.19 ± 1.05	MRI - VAT	0.993	<0.00001
Boyapati et al., 2004	420	Postmenopausal	56.6	WC	0.29	<0.05
Carranza-Lira et al., 2006	125	Postmenopausal	53.0 ± 6.5	WHR	0.297	<0.005
Vaydia et al., 2012	1678	Postmenopausal	65.6 ± 9.2	WHR	+	S

The data are presented as mean ± SD unless otherwise indicated.

^aMean ± SEM.

^bMin-Max range.

CT, computed tomography; VAT, visceral adipose tissue; DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging; WHR, waist-to-hip ratio; WC, waist circumference; IAT, intra-abdominal thickness; NS, nonsignificant; S, significant; +, positive correlation.

Table 4 Selected Studies That Tested the Relation Between Free Testosterone and Body Fat Distribution Indices in Women Without Androgen Excess

Study	n	Menopausal status	Age	Body fat distribution measurement	Correlation coefficient	P value
Nonsignificant correlation						
<i>Cross-sectional studies</i>						
Kaye et al., 1991	88	Postmenopausal	61.0 ± 3.3	WHR	0.2	NS
Armellini et al., 1994	36	Premenopausal	34 ± 11	CT - VAT	−0.18	NS
De Pergola et al., 1994	40	Premenopausal	29.5 ± 8.1	Sonography - IAT	0.286	NS
Turcato et al., 1997	41	26 premenopausal 15 postmenopausal	33.7 ± 10.2 57.9 ± 5.9	CT - VAT	−0.16	NS
Ivancic et al., 1998	49	Premenopausal	34.6 ± 7.7	WHR	0.179	NS
Ivancic et al., 2002	74	Premenopausal	NA	WHR	0.195	NS
Cao et al., 2013	107	Late postmenopausal	69.41 ± 7.28	DXA - Trunk/leg fat	−0.05	NS
Significant correlation						
<i>Cross-sectional studies</i>						
Evans et al., 1983	80	Premenopausal	19-49 ^b	WHR	0.44	<0.001
Seidell et al., 1990	434	Premenopausal	38 ± 0	WC	0.21	<0.01
Pedersen et al., 1995	25	Premenopausal	33.2 ± 1.5 ^a	DXA- Trunk fat	0.46	<0.05
Cigolini et al., 1996	18	Premenopausal	38 ± 0	CT - VAT	0.58	<0.01
Guthrie et al., 2003	102	53 perimenopausal 49 postmenopausal	NA	DXA - Trunk fat	+	S
Korhonen et al., 2003	63	Premenopausal	44	WC	0.259	<0.001
Phillips et al., 2008	78	58 premenopausal 20 postmenopausal	32.9 ± 1.2 ^a 61.4 ± 2.4 ^a	MRI - VAT	0.41 0.53	<0.001 <0.05
Janssen et al., 2010	359	48 premenopausal 177 perimenopausal 134 postmenopausal	50.6 ± 3.9	CT - VAT	0.345	<0.001
Liedtke et al., 2012	1180	Postmenopausal	64.1 ± 5.8	WC	0.1	<0.01
Cao et al., 2013	105	Early postmenopausal	54.58 ± 2.98	DXA - Trunk/leg fat	0.339	<0.001
Mongraw-Chaffin et al., 2015	855	Postmenopausal	NA	CT - VAT	+	S
<i>Longitudinal studies</i>						
Guthrie et al., 2003	102	53 perimenopausal 49 postmenopausal	NA	DXA - Trunk fat	+	S
Goss et al., 2012	53	Postmenopausal	45-55 ^b	CT - VAT	+	S
Janssen et al., 2015	243	28 premenopausal 127 perimenopausal 88 postmenopausal	51.1 ± 3.7	CT - VAT	+	S

The data are presented as mean ± SD unless otherwise indicated.

^aMean ± SEM.

^bMin-Max range.

CT, computed tomography; VAT, visceral adipose tissue; DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging; WHR, waist-to-hip ratio; WC, waist circumference; IAT, intra-abdominal thickness; NS, nonsignificant; S, significant; +, positive correlation; NA, not available.

the three cross-sectional studies that measured DHT, a significant, inverse correlation was noted between DHT and WHR, trunk fat and VAT accumulation respectively (61, 198, 203). Further studies are needed to address the significance of the apparently consistent negative correlation between circulating

levels of the most potent natural androgen and abdominal, visceral fat accumulation in women.

Body fat distribution indices of which SHBG concentration has been found to be a negative correlate include: WHR (88, 152), WC (79), trunk fat measured by DXA (47, 203)

Table 5 Selected Studies That Tested the Relation Between Androstenedione and Body Fat Distribution Indices in Women Without Androgen Excess

Study	n	Menopausal status	Age	Body fat distribution measurement	Correlation coefficient	P value
Nonsignificant correlation						
<i>Cross-sectional studies</i>						
Evans et al., 1983	80	Premenopausal	19-49 ^b	WHR	-0.13	NS
Kaye et al., 1991	88	Postmenopausal	61.0 ± 3.3	WHR	-0.07	NS
Pasquali et al., 1993	100	Premenopausal	27.9 ± 8.1	WHR	0.117	NS
De Pergola et al., 1994	40	Premenopausal	29.5 ± 8.1	Sonography - IAT	0.01	NS
Pedersen et al., 1995	25	Premenopausal	33.2 ± 1.5 ^a	DXA- Trunk fat	0.02	NS
De Pergola et al., 1996	28	Premenopausal	33.8 ± 9.61	CT - VAT	NA	NS
Turcato et al., 1997	41	26 premenopausal 15 postmenopausal	33.7 ± 10.2 57.9 ± 5.9	CT - VAT	-0.15	NS
Ivandic et al., 2002	74	Premenopausal	NA	WHR	0.008	NS
Casson et al., 2010	29	Postmenopausal	60.1 ± 1.0 ^a	CT - VAT	-0.19	NS
Keller et al., 2011	30	Premenopausal	27.3 ± 0.8 ^a	CT - VAT	-0.41	NS
Cote et al., 2012	60	50 premenopausal 10 postmenopausal	47.1 ± 5.1	CT - VAT	-0.18	NS
Liedtke et al., 2012	1180	Postmenopausal	64.1 ± 5.8	WC	0.01	NS
<i>Longitudinal studies</i>						
Goss et al., 2012	53	Postmenopausal	45-55 ^b	CT - VAT	0.05	NS
Significant correlation						
<i>Cross-sectional studies</i>						
Garaulet et al., 2000	55	22 premenopausal 33 postmenopausal	38 ± 8 61 ± 6	WC	0.34	<0.05

The data are presented as mean ± SD unless otherwise indicated.

^aMean ± SEM.

^bMin-Max range.

CT, computed tomography; VAT, visceral adipose tissue; DXA, dual-energy X-ray absorptiometry; WHR, waist-to-hip ratio; WC, waist circumference; IAT, intra-abdominal thickness; NS, nonsignificant; NA, not available.

and VAT area measured by CT (69, 281) or by MRI (70, 208). Only two of the selected studies found no association between SHBG and VAT accumulation (51, 153). Furthermore, cross-sectional data from major longitudinal studies (SWAN, MARIE, MESA, and SBCT cohorts) also found SHBG to be a consistent negative correlate of abdominal fat and VAT accumulation (40, 141, 168, 284). The body of evidence available allows for the conclusion that SHBG is a consistent negative correlate of abdominal fat and VAT accumulation in women. Discussion of the pathophysiological basis of this complex association is beyond the scope of the present article.

Androgens and body fat distribution in women with PCOS

Women presenting with pathologic androgen excess, such as patients with PCOS, have a high prevalence of overweight and obesity that is associated with insulin resistance and metabolic

disorders. Increased activity of steroid-converting enzymes in women with PCOS suggests that adipose tissue function is influenced at the tissue level by these sex steroid hormones (see section 5.2 below). The prevalence of abdominal obesity and high VAT in the PCOS population and their relation with the hyperandrogenic state, however, are not completely defined (90). Several studies have assessed the relationship between androgen levels (total testosterone, free testosterone, and androstenedione) and abdominal fat or VAT accumulation in women with PCOS using various anthropomorphic and imaging modalities (Table 6).

Within the PCOS population, most studies found that total testosterone level was correlated positively with the presence and amount of abdominal adiposity (82, 83, 87, 133), with only two studies reporting that WC or WHR measurements were not related to total testosterone level (81, 199). For VAT specifically, only two studies (34, 170) have quantified this relationship with discordant results. Borruel et al.

Table 6 Selected Studies That Tested the Relation Between Androgens and Body Fat Distribution Indices in Women with PCOS

Study	n	Age ^b	BMI category	Body fat distribution measurement	Correlation coefficient	P value
<i>Total testosterone</i>						
Pasquali et al., 1993	100	20.8 ± 5.9	Overweight	WHR	0.091	NS
Holte et al., 1994	67	NA	Overweight	DXA - trunk/leg fat	0.5	<0.001
Douchi et al., 1995	40	25.8 ± 6.2	Normal	DXA - trunk/leg fat	0.585	<0.05
Douchi et al., 2001	67	28.8 ± 6.6	Normal	DXA - trunk/leg fat	0.5	<0.001
Lord et al., 2006	40	29.1 ± 5.0	Severely obese	CT - VAT	−0.15	NS
Dong et al., 2012	408	27 (23-29) ^a	Normal	WC	NA	NS
Borrueal et al., 2013	55	26.0 ± 6.0	Obese	Sonography – P-VC	0.297	<0.01
<i>Free testosterone</i>						
Glintborg et al., 2006	51	NA	Overweight	DXA - Trunk fat	0.305	<0.05
Lord et al., 2006	40	29.1 ± 5.0	Severely obese	CT - VAT	0.14	NS
Yucel et al., 2006	33	27.6 ± 3.9	Overweight	DXA - trunk fat	0.227	NS
Godoy-Matos et al., 2009	24	28.3 ± 8.4	Obese	DXA - trunk/leg fat	0.411	NS
Dong et al., 2012	408	27 (23-29) ^a	Normal	WC	0.162	<0.05
Aydin et al., 2013	28	21.4 ± 4.2	Normal	BIA - Trunk fat	0.402	<0.05
Borrueal et al., 2013	55	26.0 ± 6.0	Obese	Sonography – P-VC	0.32	<0.001
Jin et al., 2015	90	26.3 ± 6.3	Normal	CT - VAT	0.326	<0.05
Tosi et al., 2015	116	24.3 ± 5.3	Overweight	DXA - trunk/leg fat	0.367	<0.001
<i>Androstenedione</i>						
Pasquali et al., 1993	100	20.8 ± 5.9	Overweight	WHR	+	<0.05
Douchi et al., 1995	40	25.8 ± 6.2	Normal	DXA - Trunk/leg fat	0.253	NS
Borrueal et al., 2013	55	26.0 ± 6.0	Obese	Sonography – P-VC	0.099	NS

The data are presented as mean ± SD unless otherwise indicated.

^aMedian (interquartile range).

CT, computed tomography; VAT, visceral adipose tissue; DXA, dual-energy X-ray absorptiometry; WHR, waist-to-hip ratio; WC, waist circumference; P-VC, peritoneum-vertebral column; NS, nonsignificant; +, positive correlation; NA, not available.

BMI category description: Normal: 20.0-24.9 kg/m²; overweight: 25.0-29.9 kg/m²; obese: 30.0-34.9 kg/m²; severely obese: ≥ 35.0 kg/m².

^bMenopausal status is considered to be premenopausal in PCOS groups.

demonstrated a positive correlation between total testosterone or free testosterone level and ultrasonography-measured VAT accumulation in a cross-sectional study involving women with or without PCOS and men with similar BMI values (34). Women with PCOS were found to have intermediate thickness of intraperitoneal and mesenteric fat depots compared to women without PCOS or men ($P < 0.01$). However, Lord et al. were only able to demonstrate a correlation between DHEAS levels and CT-measured VAT areas (170).

Free testosterone level also appears to be positively correlated with abdominal fat and VAT accumulation in women with PCOS, with the majority of studies reaching this conclusion (12, 34, 81, 110, 142, 254, 278) and only three studies using CT or DXA reporting no association (112, 170, 313). Interestingly, Jin et al. found that free testosterone level was associated positively with VAT but not with the visceral and subcutaneous abdominal fat ratio (142), suggesting

that both VAT and SAT may be increased in women with PCOS (see the succeeding text). BMI category did not seem to influence the outcome for VAT. No large cohort or longitudinal studies have been conducted to test the relationship between androgen levels and body fat distribution indices, but the data from available cross-sectional studies indicate that total testosterone and free testosterone level are generally positive correlates of abdominal fat accumulation in women with PCOS.

Only three studies assessed the association between androstenedione levels and abdominal fat or VAT accumulation. Results are discordant, with two studies using ultrasonography and DXA finding no significant correlation (34, 82), and one study using WHR and reporting a positive association (16, 199). Additional studies are needed to determine whether androstenedione is a significant correlate of abdominal fat accumulation in women with PCOS.

Table 7 Selected Studies That Compared Abdominal or Visceral Fat Accumulation in Women with PCOS Versus Women Without Androgen Excess

Study	PCOS group ^a		Body fat distribution measurement	Group with higher value of abdominal fat	P value
	n	Mean BMI category			
No significant difference					
Holte et al., 1994	67	Overweight	WHR	–	NS
Yildirim et al., 2003	30	Normal	WHR	–	NS
Glintborg et al., 2006	51	Overweight	DXA - Trunk fat	–	NS
Svendsen et al., 2008	18	Obese	DXA - Trunk/leg fat ratio	–	NS
Barber et al., 2008	50	Obese	MRI - VAT	–	NS
Oh et al., 2009	39	Normal	CT - VAT	–	NS
Dolfing et al., 2011	10	Normal	MRI - VAT	–	NS
Manneras-Holm et al., 2011	31	Obese	MRI - VAT	–	NS
Penaforte et al., 2011	30	Severely obese	CT - VAT	–	NS
Karabulut et al., 2012	46	Overweight	WHR	–	NS
Aydin et al., 2013	28	Normal	BIA – Trunk fat	–	NS
Jin et al., 2015	90	Normal	CT - VAT	–	NS
Significant difference					
Evans et al., 1988	84	NA	WHR	PCOS	<0.001
Hauner et al., 1988	20	Severely obese	WC	PCOS	<0,05
Pasquali et al., 1993	100	NA	WHR	PCOS	<0.05
Douchi et al., 1995	40	Normal	DXA - Trunk/leg fat ratio	PCOS	<0.01
Kirchengast et al., 2001	10	Normal	DXA - Trunk fat	PCOS	<0.001
Dixon et al., 2002	30	Severely obese	WC	PCOS	<0.001
Puder et al., 2005	20	Overweight	WHR	PCOS	<0.01
Yucel et al., 2006	33	Overweight	WHR	PCOS	<0.05
Cosar et al., 2008	31	Overweight	WHR	PCOS	<0.05
Svendsen et al., 2008	17	Normal	DXA - Trunk/leg fat ratio	PCOS	<0.05
Borrueal et al., 2013	55	Normal	WC	PCOS	<0.05

MRI, magnetic resonance imaging; VAT, visceral adipose tissue; DXA, dual-energy x-ray absorptiometry; CT, computed tomography; WHR, waist-to-hip ratio; WC, waist circumference; BIA, bioelectrical impedance analysis; NS, nonsignificant; NA, not available.

BMI category description: Normal: 20.0–24.9 kg/m²; overweight: 25.0–29.9 kg/m²; obese: 30.0–34.9 kg/m²; severely obese: ≥35.0 kg/m².

^aMenopausal status is considered to be premenopausal in PCOS groups.

Much like in women with non-pathologic levels of androgens, the type of androgen assay did not appear to influence the outcome of the studies in women with PCOS. SHBG, however, again was found to be a consistent negative correlate of abdominal fat and VAT in this population (12, 16, 34, 81, 112, 170).

Body fat distribution in women with vs. those without PCOS

As discussed in the sections above, PCOS is characterized by androgen excess and the correlation between levels of

testosterone or other androgens and abdominal adiposity seems to be fairly consistent in women with PCOS. We further tested if there was a higher prevalence of abdominal or visceral obesity in this population (Table 7). Previous studies have found increased abdominal adiposity in women with PCOS compared to control groups when matched for potential confounders (34, 60, 79, 82, 87, 129, 158, 199, 214, 254, 313) but several studies also reported no significant difference (12, 16, 110, 133, 149, 254, 311). Similar patterns occur within specific BMI subgroups, where patients with PCOS in the normal, overweight, obese, and severely obese BMI categories were found to have more abdominal fat than controls in some,

but not all studies. Among specific fat distribution indices, trunk fat mass, the trunk-and-leg fat ratio, WC and WHR were increased more often in PCOS vs. controls compared to findings with specific measurements of VAT (Table 7). This discrepancy could be due to methodology itself, as trunk fat measurements do not exclude breast mass which could overestimate abdominal obesity prevalence and WHR or WC include SAT. All studies specifically examining VAT found no significant difference (14, 80, 142, 177, 194, 204) between the PCOS and the control group. BMI category did not seem to influence the outcome for VAT differences (Table 7). Taken together, combined evidence from multiple studies with varied protocols do not allow a firm conclusion that PCOS women have higher abdominal and especially VAT accumulation compared to non-hyperandrogenic controls.

Summarizing the data on androgens and body fat distribution in women

Because of limited data and variability among studies, broad conclusions on the relationship between physiological states of androgen excess in women and preferential VAT accumulation are premature at this time. The discrepancies among existing studies may be multifactorial. Methodological issues with the quantitative measurement of androgens and lack of normative data on total and free testosterone levels in women may confound these data. Assays have been developed to allow for sensitivity and specificity in measuring androgens in small quantities in the presence of similar structures, but are less accurate in measuring the low levels of testosterone found in premenopausal and postmenopausal women (225). Variability in measurements among similar samples using different assays also makes defining differences at low concentrations less reliable within patient populations (258, 295). However, comparing the androgen assays used in the studies presented within this review, we were unable to define a specific effect of assay type on study outcome (data not shown).

Our analysis demonstrates that free testosterone level generally correlates with abdominal adiposity and VAT accumulation. However, it is not the case with androstenedione in patients in the menopausal state where the ratio of estrogens-to-adrenal or ovarian androgens changes. Similarly, free testosterone level appeared to be a better correlate of abdominal adiposity in patients with PCOS. Free testosterone level is modulated by numerous factors, such as SHBG, the latter being modulated by many factors (125). It is, therefore, not surprising that we were able to demonstrate that free testosterone level, which is believed to be a better marker of androgenic status in women, shows a stronger correlation with abdominal adiposity and VAT accumulation compared to total testosterone level. The relationship of fat distribution indices with SHBG is generally much more consistent than that with androgens, with observation of a negative correlation between SHBG and central fat accumulation in the vast

majority of cross-sectional studies (26, 266). The fact that total and free testosterone levels have more predictive value for abdominal adiposity in PCOS than in nonhyperandrogenic menopausal women may reflect increases in both overall adiposity and androgen levels in the PCOS, which respectively will cause lower levels of SHBG altering androgen dynamics (125) and facilitating more accurate androgen measurements (302). Further evidence for the role of androgens on body fat distribution changes in the menopause has been provided by a small sample of women with premature ovarian failure and earlier onset form of menopause, where women did not have elevated levels of total testosterone or SHBG compared to controls and did not demonstrate significant differences in SAT, VAT, or preperitoneal fat thickness despite increased WC (>88 cm) (8). The change in body fat distribution in the menopausal period is likely multifactorial and at this time recommendations regarding interventions targeting androgens or their receptors with respect to abdominal adiposity cannot be made due to a paucity of evidence.

Androgenic Impact on Selected Aspects of Adipose Tissue Function

For a very long time, physiologists have known that androgens, androgen receptor, and androgen binding are detectable in adipose tissue (33, 68, 74, 91, 92, 145, 257). The most abundant androstane steroids in human adipose tissues are DHEA, androstenedione, and testosterone (18, 91) but the most potent natural androgen DHT, though present at lower levels, also may be measured with sensitive techniques (18). Many studies have tested the impact of androgens on various aspects of adipose tissue function. We have reviewed this topic in other articles (32, 192, 290). The present section provides an overview of the effects of androgens on selected aspects of adipose tissue function including adipogenesis (preadipocyte differentiation), lipolysis and lipid storage.

Androgens and adipogenesis

Adipose tissue expansion takes place through: (i) adipocyte hypertrophy, which corresponds to an increase in the size of existing cells; (ii) adipocyte hyperplasia, corresponding to an increase in cell number through differentiation of preadipocytes to lipid-storing, mature cells; or (iii) a combination of both phenomena (65, 172). Differentiation of preadipocytes into adipocytes, or adipogenesis, is a complex and tightly regulated process controlled by a cascade involving two major transcription factors, PPAR γ and C/EBP α and many other proteins [reviewed in (65, 115, 172, 222)].

A number of studies have demonstrated that androgens inhibit preadipocyte differentiation [reviewed in (315)]. Testosterone and DHT inhibited *in vitro* differentiation of 3T3-L1 and C3H10T1/2 murine preadipocytes through an

androgen receptor-mediated pathway (78, 243, 244). This effect was partially blocked by receptor antagonists flutamide or bicalutamide (243, 244). In another report, the effects of testosterone and DHEA were examined in the 3T3-L1, 3T3-F442A, and 3T3-A31 murine preadipocyte cell lines; it was shown that both steroids decrease 3T3-L1 proliferation and adipogenic differentiation (104). Although DHEA decreased 3T3-F442A cell proliferation in that study, its effects were not detected in the presence of Trilostane, an inhibitor of 3 β -hydroxysteroid dehydrogenase, suggesting that enzymatic conversion of DHEA to androgens or other steroids is necessary to observe an effect of this steroid in adipocytes (104). It is important to note that even if the murine 3T3-L1 cell line has been used extensively, it does not allow any conclusion on sex-dependent and fat compartment-dependent adipogenesis.

Regarding human adipogenesis, both testosterone and DHT have been reported to inhibit differentiation of preadipocytes from the subcutaneous, mesenteric, and omental (OM) fat depots (118). Most interestingly, this effect does not appear to be sex-specific and is also linear with androgen concentration in both sexes (29). For example, adipogenic differentiation measured by the activity of glycerol-3-phosphate dehydrogenase or oil red staining of lipids was inhibited by both testosterone and DHT, in men as well as in women, and in both the subcutaneous and visceral fat compartments (29). Although cells from women appeared to be slightly more sensitive to androgens than cells from men, we were unable to demonstrate a biphasic effect of androgens on adipogenesis in cells from women. In fact, even when increasing the androgen dose to 1 μ mol/L *in vitro*, we were unable to show a stimulation of adipogenesis by androgens in cells from women. Quite the opposite, we found a clear inhibitory effect (29). Hence, a putative stimulatory effect of androgens on abdominal fat accumulation in women would logically have to occur through pathways other than stimulated adipogenesis.

Lipolysis

Lipolysis is the pathway leading to triglyceride hydrolysis in adipocytes, providing energy to tissues in the form of fatty acids; it is a complex process, which is tightly regulated (160). Two major enzymes in the lipolytic cascade are responsible for triacylglycerol degradation in the adipocyte: hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) (52). Under stimulation of β -adrenergic receptors and a G protein-coupled activation of adenylate cyclase and the protein kinase (PKA) pathway, HSL undergoes phosphorylation and translocates to the fat droplets of adipocytes (52). Many agents regulate lipolysis including catecholamines, acting not only on β - but also α -adrenergic receptors, as well as insulin, which has an inhibitory effect on lipolysis, with a more pronounced effect in subcutaneous than in visceral adipocytes (264, 318). Reports on the role of androgens in lipolysis are not unanimous. Treatment with testosterone increased

norepinephrine-stimulated lipolysis in SAT obtained in men (220), a finding that was reported also in testosterone- or DHEA-treated human and rodent adipocytes (130, 306). Results obtained in female rhesus macaques suggest that the regulation of lipolysis by sex hormones is complex. Animals in the luteal phase of the menstrual cycle (high estradiol and progesterone production) had higher basal lipolysis and HSL expression in VAT but not in SAT compared to menstruating controls (288). Testosterone supplementation starting in the prepubertal period blunted the effect of the luteal phase on basal lipolysis, but these hormonal effects were not apparent when lipolysis was stimulated by β -adrenergic agonist isoproterenol (288). Other groups described testosterone inhibition of catecholamine-induced lipolysis in primary subcutaneous preadipocytes obtained from women and differentiated *in vitro* (77). These effects appeared to occur through androgen receptor-mediated (3) regulation of HSL, adenylate cyclase and β -adrenergic receptors (77, 201, 202, 306, 307), although aromatization to estrogens cannot be excluded as a potential mediator of these effects. DHEAS was found to stimulate lipolysis in adipocytes obtained from the subcutaneous but not the visceral compartment of women, but no effect was found in adipocytes from either the visceral or the subcutaneous compartment of men (130). Finally, we reported a positive association between androgen concentrations in plasma or in VAT and the responsiveness of isolated adipocytes to positive lipolytic stimuli such as isoproterenol, dibutyryl cyclic AMP, and forskolin (18). In sum, although available studies are not unanimous, androgens (testosterone was studied most) appear to have sex-specific stimulatory effects on adipocyte lipolysis. Such regulation may involve the androgen receptor, but may also be mediated in part by aromatization to estrogens.

Lipid accumulation

Regarding lipid uptake and triglyceride synthesis in adipocytes, also described as lipogenesis, the majority of studies seem to indicate that androgens decrease lipid uptake and storage in adipose tissue. In men, testosterone treatment has been shown to reduce both lipoprotein lipase (LPL) activity and triglyceride uptake in the abdominal fat compartments (167, 220). Divergent effects were described in isolated mature adipocytes (3) and in simian adipose tissue from animals that were castrated and testosterone-replaced (289). Specifically, castration of Japanese macaques stimulated formation of small and multilocular adipocytes, which was reversed by testosterone (289). *In vitro* DHT treatment of adipose tissues obtained from the retroperitoneal fat compartment of female rhesus macaques inhibited fatty acid uptake in insulin-stimulated conditions, but uptake was stimulated in the basal state (289). In other studies, interaction with the menstrual cycle was described (288). As an example, chronic testosterone treatment increased fatty acid uptake and insulin signaling in omental adipose tissue during the menstrual period but this effect was not observed during the luteal phase (288).

In a study of whole adipose tissue explants obtained from the visceral and subcutaneous compartments of men and women, we tested the effects of DHT and testosterone on LPL activity (29). Both androgens appeared to inhibit LPL activity, but the effect was especially apparent in explants from the males (29). The inhibitory effects of testosterone on LPL activity in visceral and subcutaneous explants of women were, at best, modest and were detected at supraphysiologic dose (1 $\mu\text{mol/L}$). Consistent with what was described in the section on adipogenesis, no biphasic effect of androgens were observed on adipose tissue LPL activity in women, again suggesting that a putative stimulatory effect of androgens on abdominal fat accumulation in women would have to occur through mechanisms other than LPL activity regulation. More studies are needed to establish the depot-specific impact of androgens on the pathways of lipid storage in adipose tissues from both men and women.

Overall, active androgens testosterone and possibly DHT seem to favor fat mass reductions that manifest through inhibition of adipogenesis and lipogenesis and a possible stimulation of lipolysis. The effects have been reported to vary according to the fat compartment examined and also as a function of the nature and dose of the androgen tested. Considering the sex-specific effects of androgens on adipose tissue metabolism and their dimorphic impact on adipose tissue distribution patterns, local synthesis or inactivation of active androgens could logically contribute to a depot-specific and a sex-specific effect of these hormones, affecting androgen availability and possibly adipose tissue accumulation. This has been one of our central working hypotheses of the past years. The next section addresses the potential importance of adipose tissue steroid-converting enzymes on androgen dynamics and body fat distribution patterns.

Local Androgen Metabolism in Adipose Tissues

Regional differences in adipose tissue steroid content have been identified by many investigators. For example, we examined steroid content of SAT and VAT in men (18) and reported that although similar testosterone levels were observed in these adipose tissue compartments, DHEA, androstenedione and DHT levels were higher in omental compared to SAT. These differences may result from depot-specific differences in steroid-converting enzyme activities, which may in turn contribute to the local availability of active androgens in any given compartment. These results, along with many other studies, support the notion of a depot-specific regulation of androgen availability in adipose tissue by steroid-converting enzymes. A detailed review of all the adipose tissue enzymes with activity toward steroids has been published a few years ago (265). Only enzymes relevant to androgen dynamics in adipose tissue are described in this section (Fig. 6).

17 β -hydroxysteroid dehydrogenases

17 β -HSD type 2 is a 387 amino acid protein that has a molecular weight of 42.7 kDa and is encoded by a 1.4-kb cDNA (304). It catalyzes the conversion of active 17 β -hydroxysteroids into less active 17-ketosteroids using NAD⁺ as a cofactor (301). In a specific manner, it catalyzes the transformation of testosterone to androstenedione, of estradiol (E_2) to estrone (E_1) and of 20 α -dihydroprogesterone to progesterone (304). It also was shown to have 3 β -HSD activity in HEK 293 cells stably overexpressing this isoenzyme (251). It has been detected in the liver, the placenta and the endometrium (50) as well as in human fetal liver, and urinary tract at 20 weeks of gestation, surface epithelial cells of the stomach, small intestine, colon, and renal medulla (259). Its activity has been suggested to play a possible role in maintaining progesterone levels in pregnancy by inactivating placental androgens and estrogens (304). The enzyme also possibly has a role in decreasing E_2 secretion rates toward the fetal blood circulation (84).

In adipose tissue of men, we reported that 17 β -HSD type 2 activity was higher in VAT compared to SAT using both whole tissue homogenates as well as explant cultures. Similar differences were found regarding 17 β -HSD type 2 mRNA expression (97). In adipose tissues from females, 17 β -HSD type 2 mRNA expression also was higher in visceral compared to SAT (28). We reported that the enzyme appears to be localized in the vasculature of adipose tissue (97), suggesting that fat-depot differences in expression and activity possibly may result from differences in the vascularization of adipose tissue compartments (136). The cellular localization of 17 β -HSD type 2 is shown in Figure 7. We described that the enzyme seems to be localized in the endoplasmic reticulum of CD31-positive endothelial cells (Fig. 8) (97). Consistent with this finding, Wu et al., (304) have shown that 17 β -HSD type 2 protein contains a carboxyl-terminal endoplasmic reticulum retention motif. This was confirmed by our demonstration that 17 β -HSD type 2 exhibited high expression and strong activity in Human Adipose Microvascular Endothelial Cultures (HAMEC) (97) (Fig. 8). The oxidative activity on testosterone was significantly inhibited by specific 17 β -HSD type 2 inhibitor EM-919, indicating that 17 β -HSD type 2 is, indeed, responsible for this activity (97) (Fig. 8). Finally, 17 β -HSD type 2 mRNA and activity are also high in endothelial cell cultures from umbilical artery (HUAEC) and vein (HUVEC) (241).

In vivo studies are consistent with these previous findings. Boulton et al. (38) examined arterio-venous concentration differences in human SAT and reported that a fraction of testosterone was removed from the circulation when passing through the vascular bed of fat tissue and, most interestingly, that the rates of removal were correlated with arterial testosterone levels. 17 β -HSD type 2-mediated inactivation of testosterone in adipose tissue vasculature may affect the availability of testosterone and its impact on various aspects of

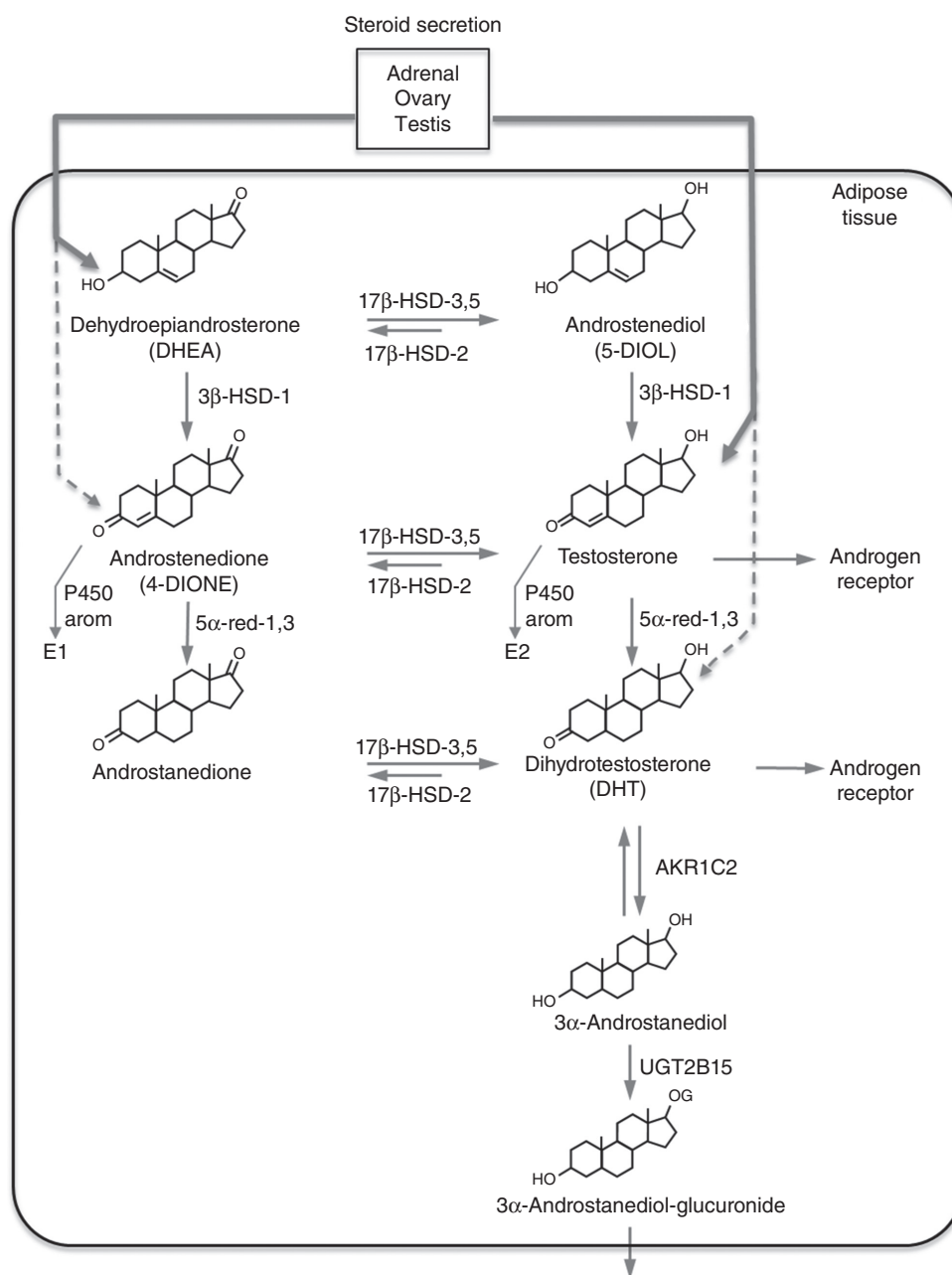


Figure 6 Schematic representation of the pathways of androgen synthesis and inactivation in adipose tissue. This is a partial version of the figure in our review article (265). HSD, hydroxysteroid dehydrogenase; P450 arom, P450 aromatase; E1, estrone; E2, estradiol; 5α-red, 5α-reductase; UGT2B15, UDP-glucuronosyltransferase 2B15; G, glucuronide (two isomers of the glucuronide derivative are formed, 3α and 17β).

adipose tissue function or metabolism (97), including body fat distribution patterns, preadipocyte differentiation (29,53) and lipolysis (26).

Positive associations were detected between BMI and 17β-HSD type 2 activity in OM or SAT. The association was also positive between 17β-HSD type 2 activity in SAT and subcutaneous adipocyte cell size (97). Obesity-related changes in vascularization may explain this association (191)

although some studies reported lower adipose tissue capillary density (197) and reduced perfusion in obesity (134). In other words, 17β-HSD type 2 activity increases in obesity may be due either to increased vascularization or to specific up-regulation of the enzyme in endothelial cells in the absence of increased vascularization (97). Various 17β-HSD type 2 inhibitors may prove relevant for osteoporosis treatment in situations of low circulating androgens and estrogens, for

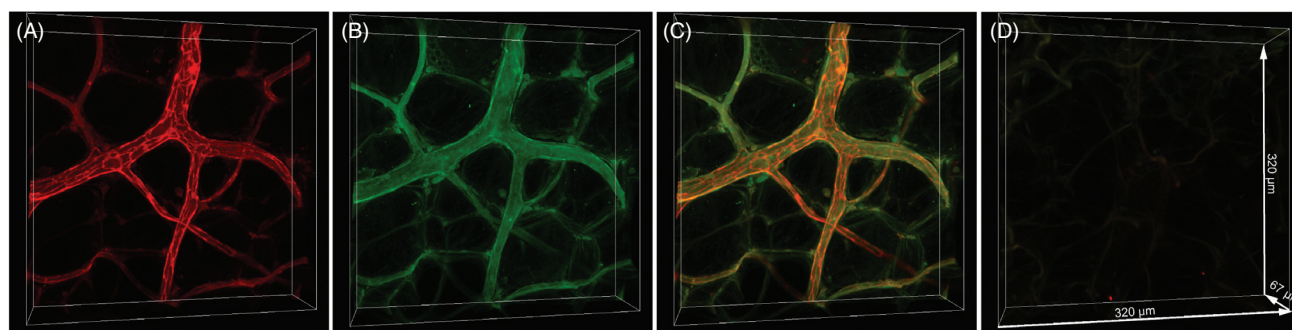


Figure 7 Tridimensional confocal imaging of 17 β -HSD type 2 in human adipose tissues. (A) CD31 labelling (endothelial cell marker); (B) 17 β -HSD type 2 labelling; (C) merging of the labelings; and (D) isotype controls. The experiment shows a clear colocalization of CD31 and 17 β -HSD type 2 in the blood vessels of the tissue (97). Reprinted with permission.

example in postmenopausal women or elderly men (107, 178, 301). In adipose tissue, we tested the EM-919 inhibitor (209) and found that the majority of the conversion of testosterone into androstenedione detected in this tissue is, indeed, mediated by 17 β -HSD type 2. However, the impact of this inhibitor on adipose tissue function remains to be established.

17 β -HSD type 3 is expressed in human SAT and VAT (28, 56, 174) and converts androstenedione to testosterone (173). In preadipocyte cultures, differentiation tends to increase expression levels of this enzyme (28, 216), but its specific role in modulating availability of androgens in fat tissue remains to be established. The ratio of VAT 17 β -HSD3-to-aromatase mRNA ratio was associated with BMI in one study (55). However, 17 β -HSD type 5, another enzyme converting androstenedione to testosterone, is expressed at higher levels in adipose tissue than the type 3 isoenzyme (see the succeeding text).

As mentioned, 17 β -HSD type 5 (AKR1C3) also mediates the conversion of androstenedione to testosterone and adipose tissue expression levels of this isoenzyme were associated positively with several adiposity indices (294). *In vitro*, adipocyte differentiation substantially upregulates expression and activity of this enzyme. Specifically, testosterone formation is stimulated fivefold in differentiated adipocytes from the subcutaneous and visceral fat compartments, and mRNA expression follows the same pattern (20, 28, 30). Consistent with increased expression in differentiated adipocytes, mRNA of the enzyme is more abundant in the subcutaneous fat compartment (28, 30) and it is expressed also at higher levels in larger than in smaller adipocytes from the same tissue donor (255). Whether obesity-related increases in expression levels and activity of this isoenzyme contribute to androgen availability remains to be formally established. As is the case with many steroid-converting enzymes, other activities of the isoenzyme could mediate its relationship with obesity. 17 β -HSD type 5 is known to be involved in the synthesis of prostaglandins, which are known modulators of PPAR γ (215). However, we reported that AKR1B1 was likely more important for adipose tissue synthesis of prostaglandin F_{2 α} compared to AKR1C3 (183).

5 α -reductases

DHT can be generated directly by 5 α -reduction of testosterone, or from 5 α -reduction of androstenedione and subsequent 17-oxoreduction by available 17 β -HSD isoenzymes. In general, it is assumed that the testosterone-to-DHT conversion is predominant (13). Yet in the sebaceous gland, our group previously has demonstrated that the formation of DHT likely results from androstenedione reduction (231). Enzymology data support that this may be related to enzyme characteristics and, therefore, the substrate preference observed in sebaceous gland may apply to other cell types or tissue (4).

Three 5 α -reductase isoenzymes, which are generated from three different genes, have been identified: SRD5A1, SRD5A2, and SRD5A3 (166). The type 1 and 2 isoenzymes have low homology and they differ in their chromosomal localizations, kinetics, and tissue distributions (193). The third type of 5 α -reductase (282) was detected in prostatic tissue and was reported *in vitro* to be poorly inhibited by dutasteride at high androgen concentrations (276). This isoenzyme was detected also in other tissues and organs (282).

We reported recently that only SRD5A1 and SRD5A3 are expressed in human adipose tissue and that SRD5A3 is the most highly expressed subtype (98). Upreti et al. (283) previously had reported that SRD5A1, not SRD5A2, was expressed in human SAT. *In vitro*, mRNA of SRD5A1 and SRD5A3 were not modulated dramatically when inducing differentiation of primary preadipocytes (98). Blouin et al. (28), also reported that SRD5A1 expression was not modulated during preadipocyte differentiation in cells from both the subcutaneous and visceral fat compartments. A positive correlation was found between adipose tissue SRD5A1 mRNA expression and BMI (98). Tsilchorozidou et al. (280) also demonstrated that 5 α -reductase activity toward cortisol was associated positively with BMI in a sample of PCOS women. Conversely, Wake et al. (294) reported that SRD5A1 mRNA level in human SAT did not predict the amount or distribution of body fat.

As mentioned, in many tissues, the majority of DHT production likely results from the transformation of androstenedione (4, 231, 250). As one example, Perel et al. (205) showed

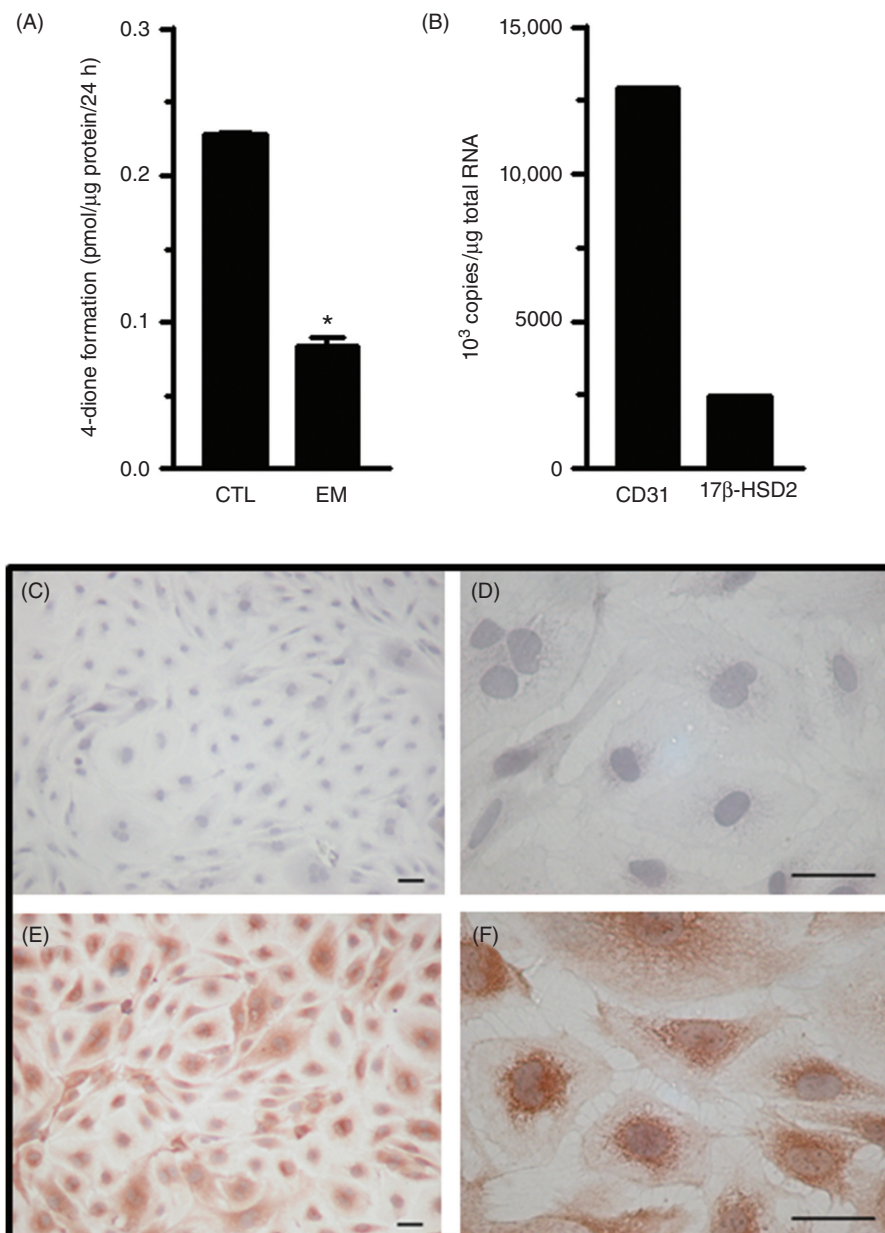


Figure 8 Activity, expression, and localization of 17β-HSD type 2 in human adipose microvascular endothelial cells. (A) Androstenedione formation rate after 24 h incubation with 0.03 μmol/L ¹⁴C-testosterone and inhibition with EM-919 (EM); (B) mRNA expression level of CD31 and 17β-HSD type 2 expressed as number of copies/μg total RNA; (C and D) immunohistochemical localization of 17β-HSD type 2; and (E and F) rabbit antiserum. Scale bar 20 μm. Mean ± SEM are shown. **P* < 0.05 (97). Reprinted with permission.

that 5α-reduced metabolites such as androstenedione, androstosterone, and DHT were formed in stromal cells from breast adipose tissue incubated with androstenedione, and that formation of 5α-reduced metabolites exceeded E₁ formation by 100-fold. DHT formation in preadipocyte cultures showed higher DHT production from androstenedione over 24 h compared to equimolar treatment with testosterone (98). DHT formation was slightly higher in subcutaneous compared to

visceral preadipocyte cultures (98) but regional differences remain uncertain. No statistical difference in 5α-reductase activity between flank and abdominal adipose tissue cell cultures was reported in another study (157).

We previously tested the effects of 5α-reductase inhibitors 4-MA and finasteride on 5α-reductase isoenzymes using HEK-293 cultures stably transfected with each subtype (98) (Fig. 9). Cells overexpressing 5α-reductase type 1 showed

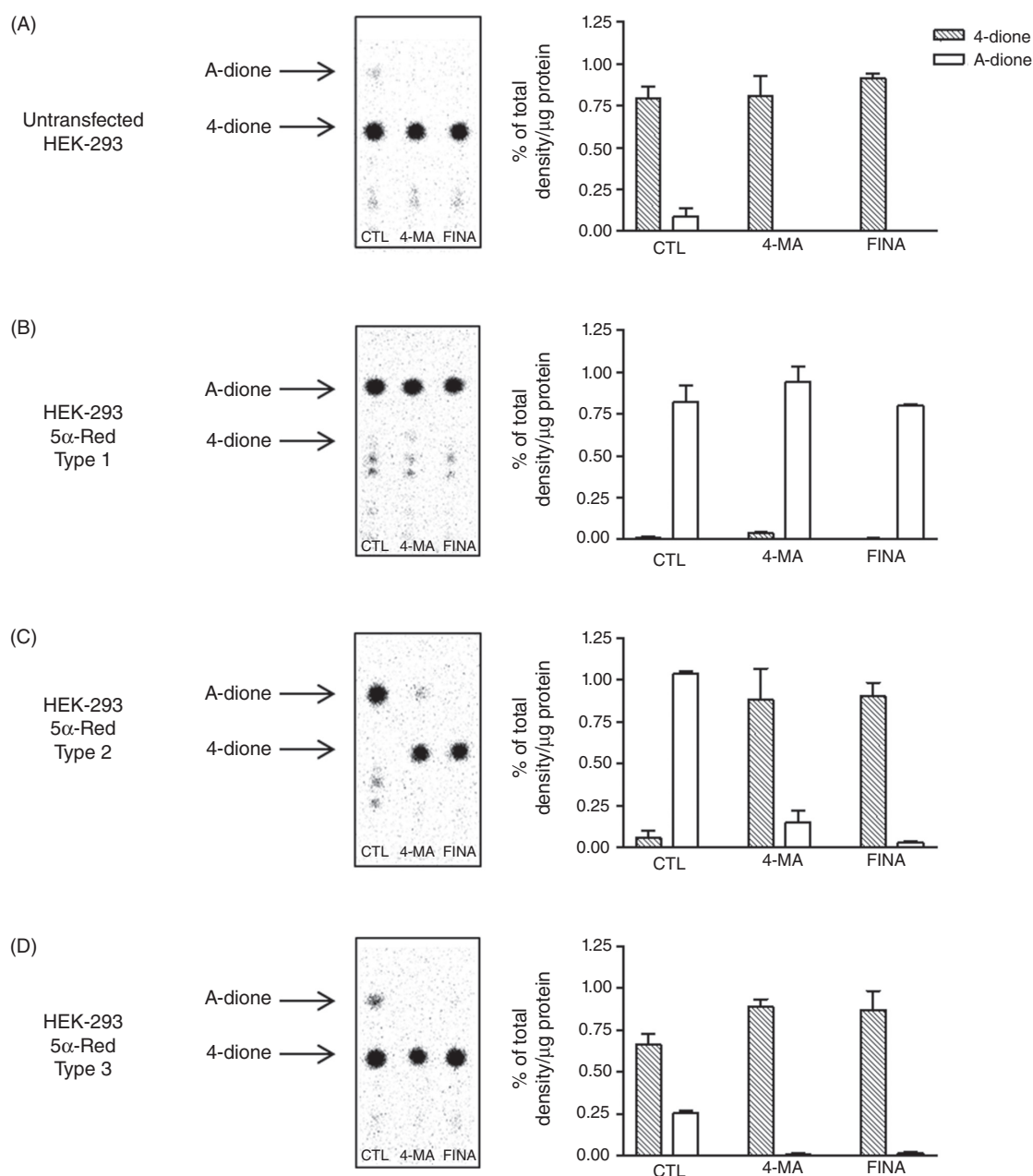


Figure 9 Activity of 5 α -reductases type 1, 2, or 3 and inhibitory effects of 4-MA or finasteride in HEK-293 stably overexpressing each isoenzyme. (A) Untransfected cells; (B) 5 α -reductase type 1-expressing cells; (C) 5 α -reductase type 2-expressing cells; and (D) 5 α -reductase type 3-expressing cells. Thin-layer chromatography images and corresponding densitometric analyses are shown for each cell line. A-dione, androstenedione; 4-dione, androstenedione; FINA, finasteride. Mean \pm SEM (98). 4-MA corresponds to 17 β -N,N-diethylcarbamoyl-4-methyl-4-aza-5 α -androstan-3-one. Reprinted with permission.

very strong androstenedione-to-androstenedione activity that was slightly blunted by 4-MA, but not by finasteride (Fig. 9). Strong activity was also detected in the 5 α -reductase type 2 cell line, but was inhibited by both inhibitors (Fig. 9). Finally, cells overexpressing 5 α -reductase type 3 had lower activity, which was blocked completely by both 4-MA and finasteride (Fig. 9) (98). With the exception of SRD5A2, which is not expressed in adipose tissue, inhibitors were effective against the type 3 isoenzyme, but not against type 1 (98). Considering

that most of the DHT produced by primary preadipocyte cultures was also blunted by both inhibitors (98), we suggest that this provides indirect indication that the type 3 isoenzyme may be relevant for DHT formation in human preadipocytes (98).

The impact of 5 α -reductase inhibition was also tested in human primary preadipocytes undergoing differentiation (98). The 5 α -reductase inhibitors completely reversed the inhibitory effect of androstenedione and testosterone on preadipocyte differentiation (Fig. 10). As described earlier

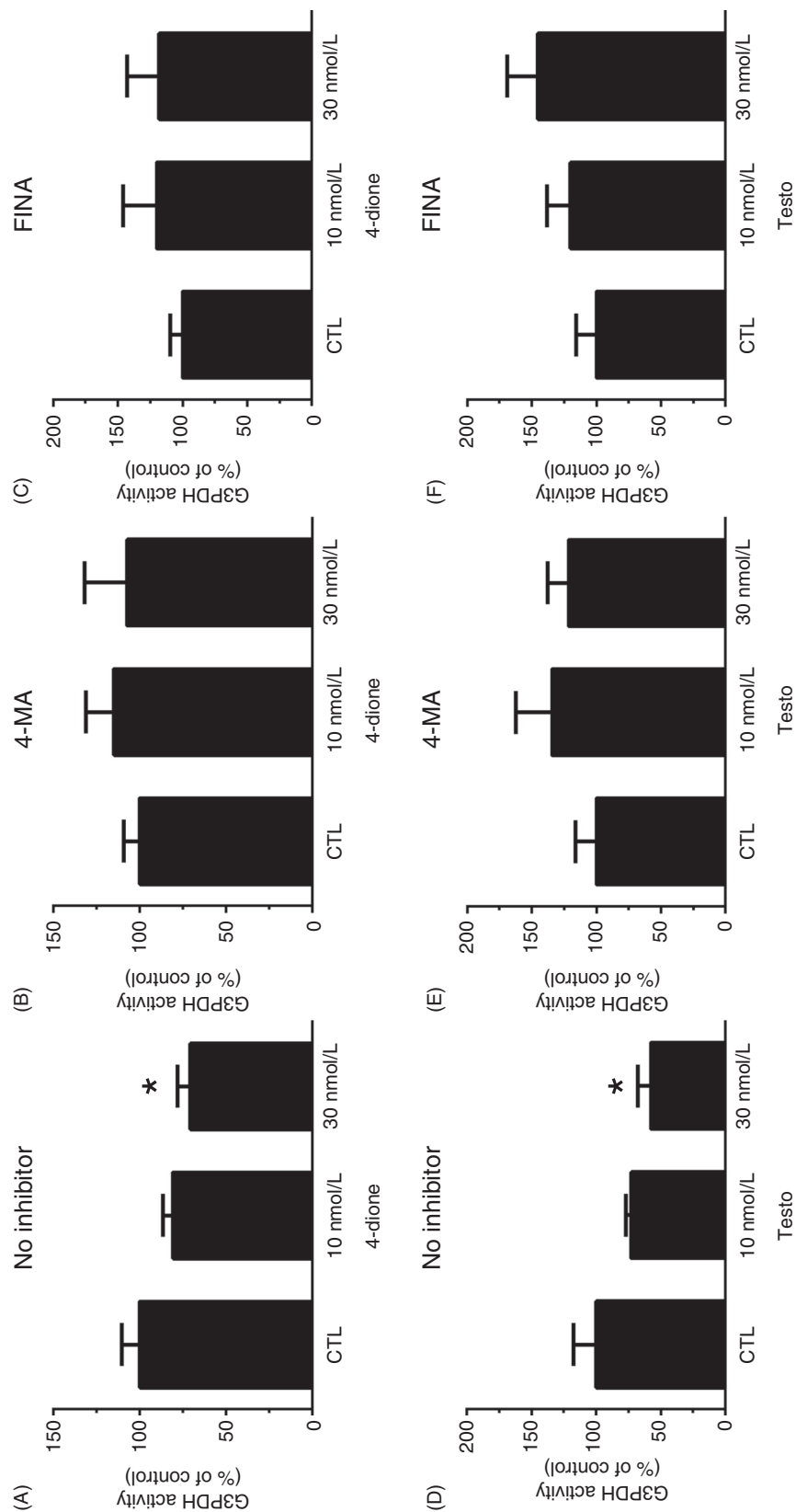


Figure 10 Effect of 5 α -reductase inhibitors on preadipocyte differentiation. G3PDH activity in differentiating subcutaneous preadipocytes treated with (A) androstenedione [4-dione, $n=7$] and (B) 500 nmol/L of 4-MA or (C) finasteride (FINA) over 14 days. G3PDH activity in differentiating subcutaneous preadipocytes treated with (D) testosterone [Testo, $n=5$] and (E) 500 nmol/L of 4-MA or (F) finasteride (FINA) over 14 days. G3PDH activity expressed as % of control (CTL). Mean \pm SEM. * $P<0.05$ [98]. Reprinted with permission.

in this article, we previously had shown that testosterone and DHT both inhibit preadipocyte differentiation in visceral and subcutaneous primary preadipocyte cultures of both sexes (29). Our findings support the notion that DHT generated through 5 α -reductase action may be responsible for an important portion of the effect of both androstenedione and testosterone on preadipocyte differentiation (98).

Production of 5 α -reduced metabolites of other steroids may also be relevant in adipose tissue. Our group reported that 5 α -pregnane-3 α / β -ol-20-one, 5 α -pregnanedione, and 5 α -pregnane-20 α -ol-3-one were major metabolites of progesterone in visceral and subcutaneous preadipocyte cultures (317). Tomlinson et al. (277) also reported lower 5 α -reductase activity after weight loss based on the ratio of circulating 5 α -THF over THF. The liver was likely the major contributor to this change, but a contribution of adipose tissue is not impossible. The relevance of 5 α -reductase isoenzymes to a local, depot-specific modulation of the availability of active androgens, progesterone, and glucocorticoids requires further investigation.

3 β -HSD

The conversion of DHEA to androstenedione and of androst-5-ene-3 β ,17 β -diol (5-diol) to testosterone is catalyzed by 3 β -HSD. This enzyme was found to be more highly expressed in SAT than in VAT (28). Expression of 3 β -HSD is also higher in SAT of women with PCOS compared to controls (299). Messenger RNA abundance of 3 β -HSD (HSD3B1) were found to be decreased in VAT in mice that gained weight under a high-fat diet, suggesting reduced androgen synthesis (286). Fujioka et al. (104) tested the effect of testosterone and DHEA on preadipocyte differentiation in the 3T3-L1 murine preadipocyte cell line and found that these steroids decreased adipogenic proliferation and differentiation. Interestingly, the effects of DHEA were blocked in the presence of the 3 β -HSD inhibitor Trilostane, suggesting that conversion to active androgens or other steroids is required to observe an effect of DHEA in this cell culture model (104). 3 β -HSDs may also convert 17-OH-pregnenolone into 17-OH-progesterone and pregnenolone into progesterone (19,217) which may be relevant for adipocyte function. The specific impact of 3 β -HSD for adipose tissue steroid hormone synthesis remains to be determined.

3 α -HSD type 3 (AKR1C2) and UDP-glucuronosyltransferases

Several years ago, we published original studies showing that circulating concentrations of the 3 α -reduced, glucuronide conjugate of DHT (3 α -androstane 3 β ,17 β -glucuronide) were increased in men with abdominal obesity and were modulated by weight gain or loss (212,213,271). These initial results were confirmed in a large cohort study by another group (287). This led to our interest in adipose tissue enzymes that

may be involved in androgen inactivation. We have detected significant expression of UDP-glucuronosyltransferase in adipose tissue (263). Our work further has shown that the conversion of DHT to the inactive androgen metabolite 3 α -androstenediol (the precursor of the glucuronide conjugated metabolite described above) (Fig. 6) is detectable in adipose tissue of both men and women (25,30,31). This activity appears to be higher in SAT compared to VAT, and, most importantly, DHT inactivation rates in VAT are correlated positively with adiposity indices such as BMI, adipocyte size and VAT area assessed by CT (25,30,31).

We have shown that the enzyme responsible for most of the DHT-to-3 α -diol conversion in humans is 3 α -HSD type 3, also known as AKR1C2 (291) (Fig. 6). Higher expression and activity of AKR1C2/3 α -HSD3 in subcutaneous compared to visceral fat of both men and women suggested that cell composition of the tissue might affect the enzyme. Accordingly, we found that mature adipocytes had higher rates of DHT inactivation compared to preadipocytes (31). Further experiments showed that induction of fat cell differentiation increased both androgen inactivation rates and AKR1C2 mRNA expression (28). Interestingly, AKR1C2 expression was increased in SAT of PCOS women compared to non-hyperandrogenic controls, which was observed in conjunction with a pattern of enzyme expression potentially reflecting increased testosterone but lower DHT levels in this condition (299).

When we examined factors that could modulate DHT inactivation rates in preadipocytes, we were intrigued by the finding of a dose-dependent inactivation of DHT by dexamethasone (28). This effect was apparent after only 24 h; it did not require additional lipogenic factors (insulin or PPAR- γ agonist) and was completely reversed by glucocorticoid receptor antagonist RU486. Active glucocorticoids stimulate adipogenesis and are synthesized locally by 11 β -HSD type 1 in proportion to mature adipocyte size and number (42,181). As mentioned previously, DHT inhibits adipogenesis, but we now learned that it may be inactivated locally by an enzyme that is responsive to glucocorticoids. We have suggested that the stimulation of AKR1C2 expression and DHT inactivation by glucocorticoids in preadipocytes may remove some of the inhibitory effect of androgens and allow adipogenesis. Other interactions have been noted in adipose tissue between the androgen and glucocorticoid signaling pathways (128,165). Our working model postulates that interaction of these hormonal signals at the local level may represent significant modulators of human body fat distribution patterns.

Other Mechanisms

SHBG

As mentioned, SHBG is a consistent correlate of adiposity and body fat distribution in both men and women and could mediate part of the association between androgens and abdominal obesity. Discussing the role of SHBG regulation is

beyond the scope of this article and has been done thoroughly elsewhere (125). Many endogenous factors including metabolic agents and hormones indirectly influence hepatic expression and circulating levels of human SHBG. Such modulation is directly linked to HNF4- α activity modulation (125). Pro-inflammatory cytokines (TNF- α , IL-1 β), which are generally increased in the low-grade, pro-inflammatory state of obesity, contribute to reduced expression and low plasma levels of SHBG (125). Adiponectin has been showed to increase SHBG production by reducing hepatic lipid production and to increase the level of HNF4- α (125). Additional modulators include thyroid hormones (125), monosaccharides, especially fructose (125) and cortisol (223).

Gut microbiota

Emerging data support the idea that steroid hormone levels may regulate composition of the gut microbial community and that changes in the latter would be associated with metabolic disorders. Some authors suggested that the gut microbiota may be implicated in the PCOS physiopathology (117, 154). Two studies used letrozole (a nonsteroidal aromatase inhibitor) to induce PCOS in female mice/rats and reported significant changes in the composition of the fecal microbiome in PCOS animals compared to the control group (117, 154). Species abundance and phylogenetic diversity of the gut microbiome was considerably reduced in the PCOS group (154). Testosterone levels were correlated negatively with alpha-diversity in that study (154). In another study, prevalence of *Lactobacillus*, *Ruminococcus* and *Clostridium* were lower and *Prevotella* was higher in PCOS rats compared with a control group (117). When PCOS rats were treated with fecal microbiota transplant from healthy rats, *Lactobacillus* and *Clostridium* were increased in both groups to levels similar to that of the control group. The prevalence of *Prevotella* was decreased in transplant animals compared to untreated PCOS animals, but the changes were more significant in the fecal transplant group (117). Improved estrus cycle, ovarian morphology, and significant increases in estradiol and estrone levels were seen, whereas testosterone and androstenedione levels were decreased compared to untreated rats (117). Overall, gut microbiota dysbiosis was associated with hyperandrogenemia in PCOS models (117, 154). However, whether alterations in the gut microbiome cause the PCOS metabolic phenotype or result from it remains to be determined (154). Androgen deprivation via castration altered cecal and fecal microbiota in a high-fat diet-dependent manner in male mice. Indeed, castration increased the Firmicutes/Bacteroidetes phyla ratio and *Lactobacillus* species (126, 127). In another study, Harada et al. showed that castrated mice fed with a high-fat diet exhibited abdominal obesity, impaired fasting glucose, excess accumulation of liver triglycerides, and thigh muscle loss. Interestingly, these effects were not observed after castration when antibiotics were administered (127). It is important to point out that changes in microbiota in animal models may not be directly

applicable to humans. For example, even the gut microbiota association with and impact on obesity is still unclear in humans (256). More studies are obviously needed.

Conclusion

In this review on androgens and body fat distribution in men and women, we confirm that low androgen levels including reduced total testosterone but also free testosterone or adrenal C19 steroids in some reports, are frequently observed in men with abdominal and/or visceral obesity and the metabolic syndrome. Data on TRT are, however, much less consistent in showing a significant favorable effects when considering specifically body fat distribution. Our analysis lends support to the notion that, independent of study design, trials involving patients with initially low baseline testosterone (<11 nmol/L) and a high BMI are more likely to lead to lower WC in response to TRT. In women, the positive association between total testosterone or free testosterone levels and abdominal adiposity indices seems to be fairly consistent but only in women with androgen excess. Studies remain equivocal in women without androgen excess. At the functional level, testosterone and DHT inhibit adipogenesis and LPL activity in both men and women, pointing toward other mechanisms to explain the positive association between androgens and visceral fat accumulation in women with androgen excess. A stimulatory effect of androgens on lipolysis may be present, but is not unanimous in the literature. In addition, at the tissue level, many steroid-converting enzymes are expressed and active in the various cell types of adipose tissues including 17 β -HSDs, 5 α -reductases and aldoketoreductases, which may contribute to alter androgen dynamics in a depot-specific manner and in so doing, may contribute to explain the effect of androgens on body fat distribution in humans.

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