



Metabolism of sex steroids is influenced by acquired adiposity—A study of young adult male monozygotic twin pairs



Veera Vihma^{a,b,*}, Jussi Naukkarinen^{c,d}, Ursula Turpeinen^e, Esa Hämäläinen^e, Jaakko Kaprio^d, Aila Rissanen^c, Sini Heinonen^c, Antti Hakkarainen^f, Jesper Lundbom^f, Nina Lundbom^f, Tomi S. Mikkola^{b,g}, Matti J. Tikkanen^{a,b}, Kirsi H. Pietiläinen^{c,h}

^a University of Helsinki and Helsinki University Hospital, Heart and Lung Center, Biomedicum C415, Haartmaninkatu 8, 00290 Helsinki, Finland

^b Folkhälsan Research Center, P.O. Box 63, 00014, University of Helsinki, Finland

^c University of Helsinki, Research Programs Unit, Diabetes and Obesity, Obesity Research Unit, P.O. Box 63, 00014, University of Helsinki, Finland

^d University of Helsinki, FIMM, Institute for Molecular Medicine Finland, P.O. Box 20, 00014, University of Helsinki, Finland

^e Helsinki University Hospital, HUSLAB, P.O. Box 720, 00029 HUS, Helsinki, Finland

^f University of Helsinki and HUS Medical Imaging Center, Helsinki University Hospital, Haartmaninkatu 4, 00290 Helsinki, Finland

^g Helsinki University Hospital, Obstetrics and Gynecology, P.O. Box 140, 00029 HUS, Helsinki, Finland

^h Helsinki University Hospital, Endocrinology, Abdominal Center, P.O. Box 340, 00029 HUS, Helsinki, Finland

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ABSTRACT

Obesity and ageing are associated with lower serum testosterone levels in men. How fat distribution or adipose tissue metabolism, independent of genetic factors and age, are related to sex steroid metabolism is less clear. We studied the associations between adiposity and serum sex hormone concentrations, and mRNA expression of genes regulating sex hormone metabolism in adipose tissue in young adult male monozygotic (MZ) twin pairs. The subjects [$n = 18$ pairs; mean age, 32 years; individual body mass indexes (BMIs) 22–36 kg/m²] included 9 male MZ twin pairs discordant for BMI [intra-pair difference (Δ) in BMI ≥ 3 kg/m²]. Sex steroid concentrations were determined by liquid chromatography–tandem mass spectrometry, body composition by dual-energy X-ray absorptiometry and magnetic resonance imaging, and mRNA expressions from subcutaneous adipose tissue by Affymetrix. In BMI-discordant pairs (mean Δ BMI = 5.9 kg/m²), serum dihydrotestosterone (DHT) was lower [mean 1.9 (SD 0.7) vs. 2.4 (1.0) nmol/l, $P = 0.040$] and mRNA expressions of DHT-inactivating AKR1C2 ($P = 0.021$) and cortisol-producing HSD11B1 ($P = 0.008$) higher in the heavier compared to the leaner co-twins. Serum free 17 β -estradiol (E2) was higher [2.3 (0.5) vs. 1.9 (0.5) pmol/l, $P = 0.028$], and in all twin pairs, serum E2 and estrone concentrations were higher in the heavier than in the leaner co-twins [107 (28) vs. 90 (22) pmol/l, $P = 0.006$; and 123 (43) vs. 105 (27) pmol/l, $P = 0.025$]. Within all twin pairs, i.e. independent of genetic effects and age, 1) the amount of subcutaneous fat inversely correlated with serum total and free testosterone, DHT, and sex hormone-binding globulin (SHBG) concentrations ($P < 0.01$ for all), 2) intra-abdominal fat with total testosterone and SHBG ($P < 0.05$), and 3) liver fat with SHBG ($P = 0.006$). Also, 4) general and intra-abdominal adiposity correlated positively with mRNA expressions of AKR1C2, HSD11B1, and aromatase in adipose tissue ($P < 0.05$). In conclusion, acquired adiposity was associated with decreased serum DHT and increased estrogen concentrations, independent of genetic factors and age. The reduction of DHT could be linked to its increased degradation (by AKR1C2 and HSD11B1) and increased estrogen levels to increased adiposity-related expression of aromatase in adipose tissue.

1. Introduction

Obese men tend to have lower concentrations of serum total

testosterone and sex hormone-binding globulin (SHBG) [1,2] and higher serum 17 β -estradiol (E2) [3,4] compared to leaner men. More recently, population studies have confirmed that body mass index

Abbreviations: 3 α -diol, 5 α -androstane-3 α ,17 β -diol; BMI, body mass index; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; E1, estrone; E2, 17 β -estradiol; FSH, follicle-stimulating hormone; HDL, high density lipoprotein; HOMA-IR, the homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LH, luteinizing hormone; MRI, magnetic resonance imaging; MZ, monozygotic; SHBG, sex hormone-binding globulin

* Corresponding author at: Folkhälsan Research Center, Biomedicum C315a, Haartmaninkatu 8, P.O. Box 63, 00014 University of Helsinki, Finland.

E-mail address: veera.vihma@helsinki.fi (V. Vihma).

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(BMI) or body fatness correlate inversely with serum testosterone, and positively with serum E2 [5,6]. Serum androgen levels also gradually decline during aging, and both general and central obesity in middle-aged men seem to predict a greater decline in serum testosterone and SHBG concentrations with age [7]. In addition to obesity, men with features of the metabolic syndrome such as hypertriglyceridemia, abdominal fat accumulation, or hyperglycemia may present with reduced serum androgen and SHBG concentrations [8–10].

While it is generally accepted that obesity may be associated with alterations in serum sex hormone concentrations, it is less clear how fat distribution is related to serum sex hormone levels, especially in overweight to modestly obese men [11] or in the younger age group. Serum total testosterone has been reported to correlate negatively with both subcutaneous and visceral adiposity in different studies [12–14] or with subcutaneous adiposity only [15] as determined by computed tomography or magnetic resonance imaging (MRI). The association between body composition and serum concentrations of dihydrotestosterone (DHT) [14,15], the most potent androgen, or estrone (E1) [13] has been studied less. Previous reports on serum E2 and body fat distribution have been inconsistent [6,13–16].

Adipose tissue is an important site of steroid hormone metabolism, including peripheral aromatization of androgens to estrogens and inactivation of DHT to 5 α -androstane-3 α ,17 β -diol (3 α -diol) [17]. Therefore, it is of interest to know how obesity affects mRNA expression of genes that are related to metabolism of steroids in adipose tissue. It is also not known whether the associations between sex steroid metabolism and obesity are driven by genetic or acquired factors. A unique model to test these alternative explanations is the study of monozygotic (MZ) twin pairs discordant for obesity. MZ co-twins have identical genome sequences; hence differences between them reflect acquired, non-genetic influences. MZ co-twins are additionally matched on age, sex, and multiple environmental exposures and experiences. We set out to determine the body composition, serum sex hormone concentrations, and expression of genes of interest in subcutaneous adipose tissue in young adult male MZ twin pairs discordant for BMI [intra-pair difference (Δ) in BMI \geq 3 kg/m²], allowing for adjustment of genetic factors and age between the lean and heavy groups. Using Δ values from both discordant and concordant (Δ BMI < 3 kg/m²) pairs, we determined how acquired differences in fat distribution or metabolic markers were related to serum sex steroid or SHBG concentrations and adipose tissue gene expressions.

2. Subjects and methods

2.1. Subjects and study design

This is a cross-sectional study of 36 male MZ twins identified from ten full birth cohorts of Finnish twins of Caucasian ancestry [18]. These 18 twin pairs included nine BMI-discordant (mean Δ BMI, 5.9 kg/m²) and nine BMI-concordant twin pairs (mean Δ BMI, 1.2 kg/m²). The growth patterns did not significantly differ between the leaner and heavier co-twins until the age of 18 years [19]. The clinical characteristics of the twins are described in detail in [20–22]. The subjects were healthy and did not use any regular medications, except for one obese co-twin who had type 2 diabetes and used metformin and insulin. The co-twins were examined at the same visit except for one discordant pair (visit of the co-twin within one month) and two concordant twin pairs (visit of the co-twin within one week). Blood samples, tissue biopsies, and body composition measurements were obtained during the same visit. The study was approved by the Ethical Committee of the Helsinki University Hospital and the study participants gave their written informed consent.

2.2. Body composition

Whole-body fat was measured by dual energy x-ray absorptiometry.

Abdominal subcutaneous and intra-abdominal fat were measured by MRI, and liver fat by magnetic resonance spectroscopy as described earlier [22].

2.3. Quantification of serum hormones and other serum analyses

Blood samples were obtained in the morning between 0700 and 0900 h after an overnight fast and stored at -80°C until assay. Concentrations of serum dehydroepiandrosterone (DHEA) [23], testosterone [24], DHT [25], E2 [26], and E1 [27] were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described previously. Serum SHBG, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were determined by chemiluminescent enzyme immunoassay (Siemens Healthcare Diagnostics) using an Immulite 2000 Xpi analyzer. Circulating free, non-protein-bound E2 was calculated using the following equation: free E2 = $[10 \exp(-0.003 \times \text{SHBG} + 0.389)/100] \times \text{E2}$, where serum concentrations of E2 and SHBG are expressed as nmol/l [28]. Serum free testosterone was calculated according to Anderson's equation as follows: free testosterone = total testosterone $\times [2.28 - 1.38 \times \log(\text{SHBG}/10)] \times 10$, where serum concentration of free testosterone is expressed as pmol/l and concentrations of serum total testosterone and SHBG as nmol/l [29].

Fasting serum insulin and plasma glucose, the homeostasis model assessment of insulin resistance (HOMA-IR), serum high-sensitivity C-reactive protein (hs-CRP), serum high density lipoprotein (HDL) and low density lipoprotein cholesterol, and serum triglycerides were determined as described previously [22].

2.4. Transcriptomics analyses of subcutaneous adipose tissue

Surgical biopsies of subcutaneous adipose tissue from the periumbilical region were obtained under local anesthesia and snap frozen in liquid nitrogen. Total RNA was extracted from adipose tissue as described in [20]. Transcriptomics analyses were performed with Affymetrix U133 Plus 2.0 chips and the raw data were further processed as previously described [21]. We focused on genes involved in metabolism of steroid hormones. The Affymetrix data has been previously validated with quantitative reverse transcription-polymerase chain reaction (RT-qPCR) [21,22].

2.5. Statistical analysis

Data are expressed as mean (standard deviation, SD) for variables with a normal or as median (range, or interquartile range) for variables with a non-normal distribution. Intra-class correlation was used to test the within-pair resemblance; MZ pair intra-class correlations are estimates of familiarity, i.e. the contribution of genetics and shared experiences. Paired *t*-test and Wilcoxon signed ranks tests between the leaner and heavier co-twins were calculated for normally and non-normally distributed data, respectively. Intra-pair difference (Δ) was calculated by subtracting the leaner twin's value from the heavier co-twin's value. Associations between steroid concentrations or gene expression values and clinical characteristics were assessed by Spearman's correlation, partial correlation, and multivariate linear regression using the Δ values. Statistical tests were performed using SPSS Statistics version 22.0 software. The two-tailed level of significance was $P < 0.05$.

3. Results

3.1. Clinical characteristics

The clinical characteristics of the subjects are presented in Table 1. The mean difference in body weight between heavier and leaner co-twin was 18.6 (SD, 6.0) kg for BMI-discordant twin pairs and 4.8 (2.1) kg for BMI-concordant pairs. In the BMI-discordant pairs, the heavier

Table 1

Clinical characteristics of 9 male monozygotic twin pairs discordant for BMI (within-pair difference in BMI ≥ 3 kg/m²) and 9 male monozygotic twin pairs concordant for BMI (within-pair difference in BMI < 3 kg/m²).

Characteristic, median (range)	BMI-discordant, Leaner (n = 9)	BMI-discordant, Heavier (n = 9)	<i>P</i> value for BMI-discordant, Heavier vs. leaner	BMI-concordant, Leaner (n = 9)	BMI-concordant, Heavier (n = 9)
Age, years	32 (25–34)	32 (25–34)		33 (24–36)	33 (24–36)
Height, cm	180 (168–194)	180 (168–193)	0.86	176 (164–193)	177 (170–193)
Weight, kg	88 (66–125)	109 (83–135)	0.008	80 (71–100)	84 (77–109) [†]
BMI, kg/m ²	26.8 (22–33)	33.5 (27–36)	0.008	26.6 (23–32)	26.6 (23–34) [†]
Body fat, %	31 (14–39)	34 (30–41)	0.015	26 (10–33)	30 (9–37) ^{**}
Subcutaneous fat, cm ³	3990 (1100–7280)	4790 (3050–9090)	0.008	2560 (1000–4050)	3020 (830–5200)
Intra-abdominal fat, cm ³	767 (390–4590)	2110 (810–5880)	0.011	1160 (230–2290)	1290 (150–2300)
Ia to Sc fat-ratio, %	25 (19–83)	39 (18–84)	0.038	40 (23–97)	40 (18–73)
Liver fat, %	1.1 (0.3–7.5)	9.1 (0.4–20)	0.021	1.3 (0.5–13)	1.0 (0.4–24)
Smoker	n = 3	n = 3		n = 3	n = 4
Fasting serum insulin, mU/l	5.9 (1.4–9.2)	8.0 (5.2–29)	0.036	5.1 (1.1–17)	6.0 (1.4–8.5)
Fasting plasma glucose, mmol/l	5.4 (4.7–5.8)	5.8 (4.6–9.8)	0.16	5.4 (4.8–6.0)	5.6 (4.9–6.5)
HOMA-IR	1.4 (0.3–2.1)	2.0 (1.1–7.9) ^a	0.050	1.3 (0.2–4.2)	1.5 (0.3–2.5)
LDL-cholesterol, mmol/l	2.7 (1.6–4.4)	2.8 (2.3–4.4)	0.50	2.6 (1.0–4.0)	3.0 (1.1–4.0)
HDL-cholesterol, mmol/l	1.3 (1.0–2.1)	1.1 (0.5–1.4)	0.038	1.2 (0.9–2.1)	1.2 (1.1–1.6)
Triglycerides, mmol/l	1.1 (0.4–2.5)	1.8 (0.7–4.4)	0.038	0.7 (0.4–2.5)	0.9 (0.4–1.2)
Hs-CRP, mg/l	0.5 (0.2–14)	1.3 (0.4–26)	0.26	0.7 (0.3–8.2)	1.1 (0.3–1.8)
Serum LH, IU/l	4.1 (3.1–10.5)	5.9 (2.9–8.8)	0.72	3.2 (2.3–5.9)	5.2 (1.8–6.5)
Serum FSH, IU/l	4.6 (1.2–10.1)	5.2 (1.6–8.3)	0.77	3.0 (1.7–6.9)	3.4 (1.7–9.1)

Statistically significant *P* values are bolded.

* *P* = 0.008.

** *P* = 0.038, heavier vs. leaner co-twin in BMI-concordant twin pairs (Wilcoxon signed ranks test). BMI, body mass index; Sc, subcutaneous; Ia, intra-abdominal; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low density lipoprotein; HDL, high density lipoprotein; Hs-CRP, high-sensitivity C-reactive protein; LH, luteinizing hormone (reference range, 1.7–8.6 IU/l, HUSLAB); FSH, follicle-stimulating hormone (reference range, 1–12 IU/l, HUSLAB).

^a Number of subjects n = 8.

co-twin had a mean of 12.7 (6.6) kg more total body fat compared to the leaner co-twin. In the BMI-concordant pairs, the heavier co-twin had on average 3.2 (2.5) kg more body fat than the leaner co-twin.

3.2. Twin resemblance between the heavier and leaner co-twins

We observed significant intra-class correlations for serum DHEA, androgens, SHBG, LH, and FSH within twin pairs (Supplemental data, Fig. 1). For DHEA, DHT, and FSH, intra-class correlations were significant both within BMI-discordant and -concordant pairs. These results suggest that especially serum levels of gonadotropins, but also those of SHBG and sex hormones are highly familial. In the following section we compare serum hormone levels between the heavier and leaner co-twin, independent of genetic effects.

3.3. Serum hormone concentrations in the heavier and leaner co-twins

Fig. 1 shows the concentrations of serum sex hormones for MZ twin pairs discordant or concordant for BMI. In the discordant pairs, the heavier co-twin had a lower concentration of DHT and SHBG, and a higher concentration of calculated free E2 compared to the leaner co-twin (Fig. 1). If discordant and concordant twin pairs were analyzed together, the median concentrations of serum E2 and E1 were higher in the heavier than in the leaner co-twins (103 vs. 82 pmol/l, *P* = 0.006; and 118 vs. 103 pmol/l, *P* = 0.025, n = 18 pairs, respectively). Altogether six subjects, including two BMI-discordant twin pairs and two heavier co-twins from the BMI-concordant group, had serum E2 values exceeding the normal range (serum E2 reference range, 0–130 pmol/l, HUSLAB). Serum E1 levels were within the normal range in all subjects. Serum total or free testosterone, or DHEA concentrations did not significantly differ between the co-twins (Fig. 1). Total testosterone concentrations in the obese were within the normal range except for one subject (BMI, 36 kg/m²) with a decreased serum total testosterone of 9.5 nmol/l (reference range, 10–38 nmol/l for young adult males; HUSLAB), but a normal calculated free testosterone of 206 pmol/l (reference range, 155–800 pmol/l, HUSLAB). Serum gonadotropin

concentrations did not statistically significantly differ between the heavier and leaner co-twins (Table 1).

3.4. Associations between adiposity and serum hormone and SHBG concentrations

Next, we studied the relation of adiposity to serum hormone concentrations, independent of genetic effects. For these correlations, we calculated the intra-pair differences (Δ values) by subtracting the leaner twin's value from the heavier twin's value. Within twin pairs: 1) percent body fat (*P* < 0.05 for all) and the amount of subcutaneous adipose tissue correlated negatively with serum total and free testosterone, DHT, and SHBG concentrations (Fig. 2); 2) intra-abdominal fat correlated negatively with serum total testosterone and SHBG (Fig. 2); and 3) liver fat correlated negatively with SHBG ($r = -0.622$, *P* = 0.006) but not with any of the serum hormones (data not shown). Serum total testosterone concentration depends on the concentration of its carrier protein, SHBG, and accordingly, we observed a strong positive correlation between serum total testosterone and SHBG within pairs (Fig. 2).

Next, we analyzed in a multivariate model using the Δ values within all 18 pairs (i.e. adjusting for genetic factors), which of the independent measures (subcutaneous fat, intra-abdominal fat, or SHBG) best predicted serum total testosterone, and found that SHBG was the strongest and only significant correlate of total testosterone levels (*P* = 0.024, adjusted $r^2 \times 100\%$ of the whole model, 57%; Supplemental data, Table 1). For serum free testosterone (borderline significant) and DHT, subcutaneous fat was an independent negative correlate (Supplemental data, Table 1). DHT is a metabolite of testosterone and its serum concentration correlated positively with serum total testosterone within twin pairs ($r = 0.881$, *P* < 0.001). When we included serum total testosterone and SHBG as independent variables in the multivariate model, the best predictor of serum DHT concentration was testosterone (*P* = 0.003, adjusted $r^2 \times 100\%$ of the whole model, 64%; Supplemental data, Table 1).

For serum estrogens, there were no statistically significant univariate correlations between adiposity and estrogen concentrations,

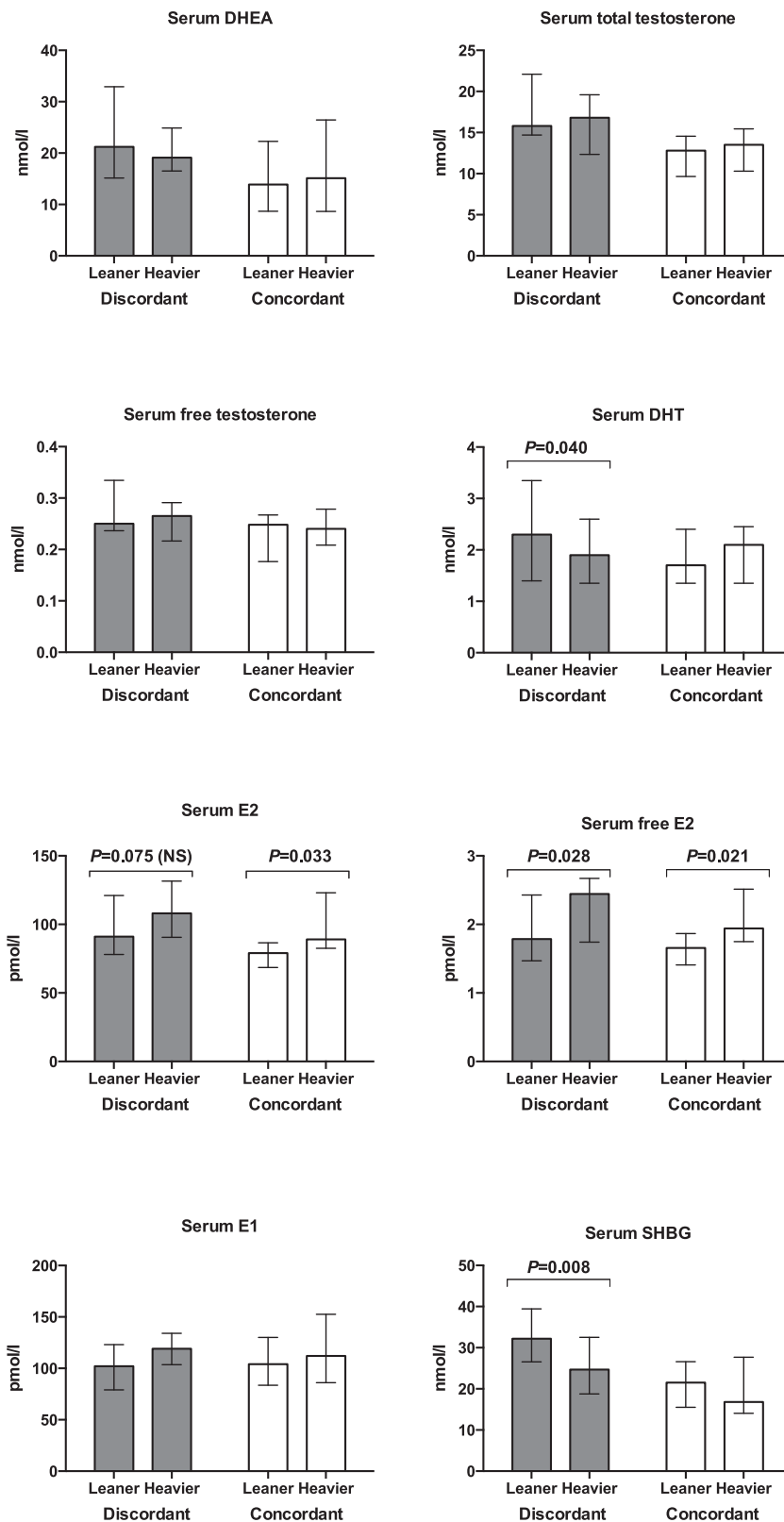


Fig. 1. Serum concentrations of dihydrotestosterone and SHBG were lower and serum free E2 higher in the heavier co-twins in BMI-discordant pairs.

Data are expressed as median, interquartile range. Male monozygotic twin pairs discordant for body mass index (BMI), within-pair difference in BMI ≥ 3 kg/m², n = 9 pairs; BMI-concordant twin pairs, within-pair difference in BMI < 3 kg/m², n = 9 pairs. DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; E2, 17 β -estradiol; E1, estrone, SHBG, sex hormone-binding globulin.

except for serum E2 (a negative correlation with subcutaneous fat) within pairs (Fig. 2). Serum SHBG did not correlate with serum estrogen concentrations either (data not shown). In multivariate analysis with subcutaneous and intra-abdominal fat and SHBG as independent variables, the amount of subcutaneous fat was a negative correlate of E2 ($P = 0.020$, $r^2 \times 100\%$ of the whole model, 21%; Supplemental data, Table 1). Serum E1 was best predicted by serum E2 ($P = 0.001$;

multivariate model including serum E2 and total testosterone as independent variables, adjusted $r^2 \times 100\%$ of the whole model, 52%; Supplemental data, Table 1).

To study whether serum steroid concentrations link with components of metabolic syndrome, independent of genetic factors, we analyzed their correlations within twin pairs. Fasting plasma glucose level was inversely correlated with serum SHBG ($r = -0.602$, $P = 0.008$,

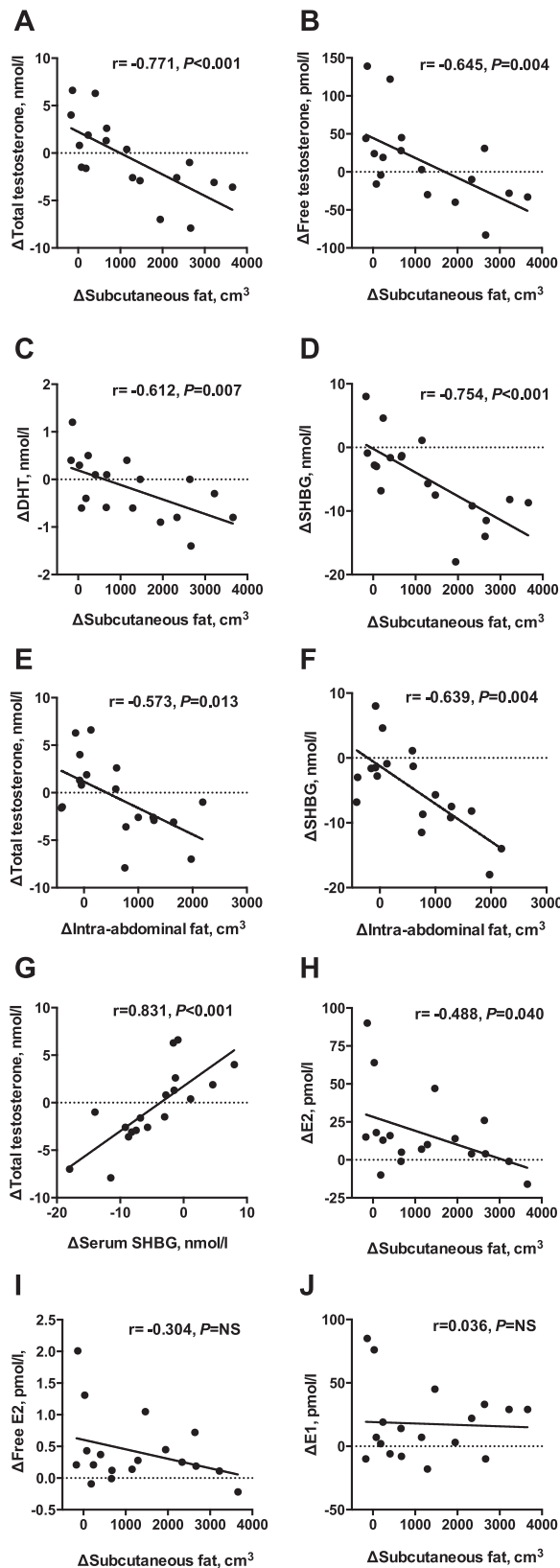


Fig. 2. Subcutaneous or intra-abdominal adiposity correlated inversely with serum androgen and SHBG concentrations within twin pairs ($n = 18$ pairs, Spearman's correlation).

The amount of subcutaneous and intra-abdominal fat and serum hormone concentrations were determined as described in the methods section. The intra-pair differences (Δ values) were calculated by subtracting the leaner twin's value from the heavier co-twin's value. Least squares was the method used for curve fitting. DHT, dihydrotestosterone; E2, 17 β -estradiol; E1, estrone; SHBG, sex hormone-binding globulin.

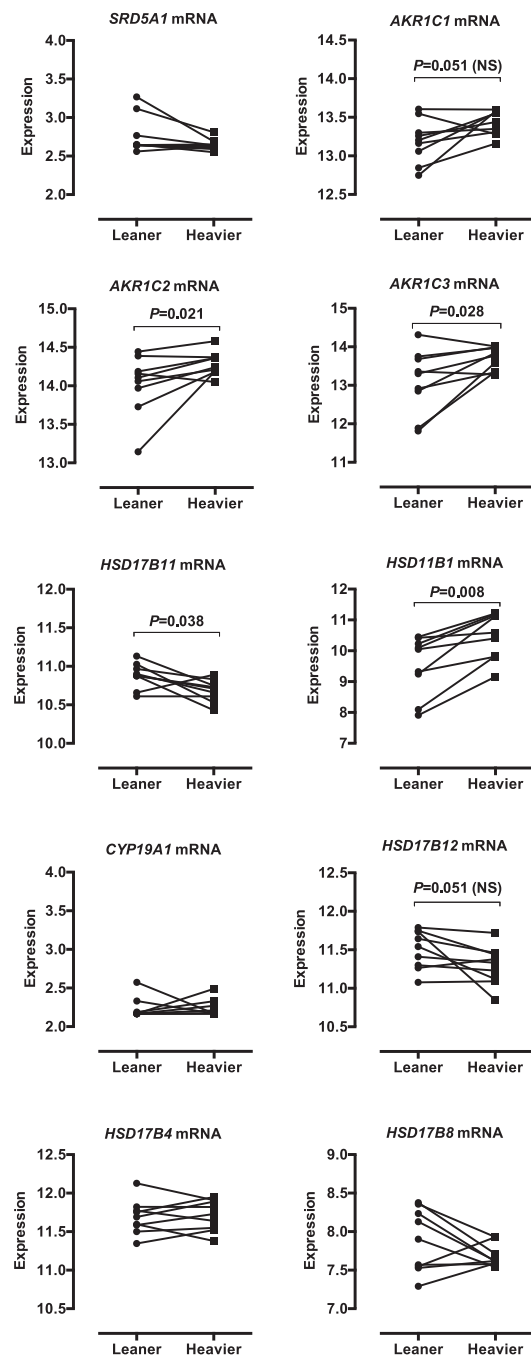


Fig. 3. Gene expressions for androgen and cortisone metabolizing enzymes were higher in adipose tissue from heavier compared to leaner co-twins.

Relative mRNA expressions of selected genes were determined in subcutaneous adipose tissue in male monozygotic twin pairs discordant for body mass index (BMI) (intra-pair difference in BMI ≥ 3 kg/m²; $n = 9$ pairs).

testosterone ($r = -0.589, P = 0.010$), and DHT concentrations ($r = -0.576, P = 0.012$). However, the significant association between plasma glucose and SHBG (or androgens) was lost after correcting for the amount of subcutaneous adipose tissue as a confounding factor ($r = -0.237, P = \text{NS}$, partial correlation). There were no statistically significant correlations between SHBG (or serum androgens or estrogens) and fasting serum insulin, HOMA-IR, serum triglyceride, HDL cholesterol, or hs-CRP concentrations within twin pairs (data not shown).

3.5. mRNA expression of genes for steroid metabolizing enzymes in the heavier and leaner co-twin

Fig. 3 shows the mRNA expression levels of genes for 10 steroid metabolizing enzymes in subcutaneous adipose tissue in the heavier and leaner co-twins discordant for BMI. mRNA expressions of the androgen metabolizing aldoketoreductases (*AKR1C2* and *AKR1C3*) were higher in the heavier co-twins, with similar tendency in *AKR1C1*. *AKR1C1* and *AKR1C3* may play a role in the conversion of androstenedione to testosterone (17 β -hydroxysteroid dehydrogenase type 5 activity), and *AKR1C2* in the conversion of DHT to 3 α -diol (3 α -reductase activity). *HSD17B11*, which may be involved in the further conversion of 3 α -diol to androsterone [30], was less expressed in the heavier co-twin. The heavier co-twins had a higher mRNA expression of *HSD11B1*, responsible for the synthesis of active cortisol from cortisone. mRNA expression of *SRD5A1* (5 α -reductase), which reduces testosterone to DHT, showed no significant difference between the heavier and leaner co-twins. Of the estrogenic genes driving towards the formation of E2, the mRNA expression of *CYP19A1* (aromatase) did not statistically significantly differ between the heavier and leaner co-twins. Gene for *HSD17B12* which reduces E1 to E2, and genes for several of the other 17 β -hydroxysteroid dehydrogenases, which may catalyze the oxidation of E2 to a weaker estrogen E1 including *HSD17B4* and *HSD17B8*, were relatively highly expressed but did not differ between the co-twins (Fig. 3). In BMI-concordant twin pairs, mRNA expression of the genes studied did not differ between the heavier and leaner co-twins (data not shown).

3.6. Associations between adiposity and mRNA expression of genes for steroid metabolizing enzymes in subcutaneous adipose tissue

Within all 18 pairs, i.e. independent of genetic effects, BMI, percent body fat, the amount of intra-abdominal fat, and the intra-abdominal to subcutaneous fat-ratio were positively related to subcutaneous adipose tissue mRNA expressions of *AKR1C2* and *AKR1C3* (Table 2). Moreover, the amount of both subcutaneous and intra-abdominal fat correlated positively with mRNA expressions of *CYP19A1* and *HSD11B1* within pairs (Table 2). Liver fat correlated positively with the expression of *CYP19A1* but not with any other gene studied. mRNA expression of *HSD17B11* was inversely related to the measures of adiposity (Table 2). We next analyzed whether the gene expressions for steroid metabolizing enzymes were associated with body fat distribution (subcutaneous and intra-abdominal fat independent of each other) within pairs. Δ Subcutaneous fat was a predictor of Δ *HSD17B11* in a multivariate model including subcutaneous and intra-abdominal fat as independent variables ($P = 0.042$, adjusted $r^2 \times 100\%$ of the whole model, 42%), but no other significant independent correlations were found (data not shown). This suggests that accumulation of general

adiposity, rather than differences in body fat distribution determine associations for these genes.

4. Discussion

Our carefully phenotyped MZ twins discordant for obesity represent an ideal model to explore the effects of acquired overweight and obesity on serum sex hormone levels, independent of genetic factors. In this study, we show that intra-pair differences in overweight or obesity in young adult male MZ pairs are associated with decreased circulating DHT and SHBG and increased free E2 concentrations, as well as with different expression levels of genes related to sex steroid metabolism in adipose tissue. Our data indicate that familial (genetic and shared exposures) factors affect serum sex hormone and SHBG levels, in line with [31], but that acquired adiposity may significantly influence their serum levels, independent of genetic background and age.

4.1. Adiposity and metabolism of dihydrotestosterone (DHT)

Serum total testosterone inversely correlated with the amount of both subcutaneous and intra-abdominal fat. However, in multivariate analysis, serum SHBG was the single independent predictor of the variability in serum total testosterone within twin pairs. Serum SHBG, in turn, was significantly inversely related to all measures of adiposity. Serum concentration of DHT, the principal metabolite of testosterone, was independently inversely associated with the amount of subcutaneous fat, but when total testosterone and SHBG were included in the multivariate model, serum testosterone remained the strongest predictor of serum DHT. *AKR1C2* enzyme (3 α -reductase activity) is considered important in the inactivation of DHT to the weak androgen 3 α -diol [32]. The heavier co-twins had higher relative mRNA expression of *AKR1C2* in subcutaneous adipose tissue compared to the leaner co-twins, and its expression correlated positively with BMI and measures of adiposity. Thus, our results suggest that the degradation of DHT could be increased in the subcutaneous adipose compartment of the heavier co-twins leading to a decline in serum DHT levels. Previous studies have described a positive correlation between serum and subcutaneous adipose tissue DHT levels [33], and a higher DHT-inactivating 3 α -reductase activity in subcutaneous as compared to omental adipose tissue [17]. In our study, increased adiposity in the heavier co-twin was also associated with a higher mRNA expression of the cortisol-synthesizing *HSD11B1* [34]. Moreover, cortisol production and DHT inactivation are tightly regulated in adipose tissue and *HSD11B1*-induced cortisol can increase the *AKR1C2*-mediated inactivation of DHT in adipose tissue [35]. Thus, the decreased serum DHT level in heavier co-twins could result from its increased degradation related to higher mRNA expressions of *AKR1C2* and *HSD11B1*. However, as serum testosterone was the strongest predictor of serum

Table 2

Correlations between within-pair differences (Δ) in adiposity and mRNA expression of genes in subcutaneous adipose tissue in 18 male monozygotic twin pairs (Spearman's correlation).

Gene		Δ <i>AKR1C1</i>	Δ <i>AKR1C2</i>	Δ <i>AKR1C3</i>	Δ <i>HSD11B1</i>	Δ <i>CYP19A1</i>	Δ <i>HSD17B11</i>
Δ BMI, kg/m ²	r	0.591	0.569	0.542	0.329	0.385	-0.701
	P	0.010	0.014	0.020	0.18	0.12	0.001
Δ % body fat	r	0.440	0.505	0.541	0.427	0.432	-0.576
	P	0.07	0.033	0.020	0.08	0.07	0.012
Δ Sc fat, cm ³	r	0.424	0.284	0.282	0.525	0.576	-0.564
	P	0.08	0.25	0.26	0.025	0.012	0.015
Δ Ia fat, cm ³	r	0.304	0.492	0.474	0.577	0.565	-0.511
	P	0.22	0.038	0.047	0.012	0.015	0.030
Δ Ia to Sc fat-ratio	r	0.356	0.527	0.593	0.362	0.326	-0.511
	P	0.15	0.025	0.009	0.14	0.19	0.030
Δ Liver fat, %	r	0.267	0.307	0.290	0.420	0.482	-0.298
	P	0.28	0.22	0.24	0.08	0.043	0.23

Statistically significant P values are bolded. The within-pair differences (Δ values) were calculated by subtracting the leaner twin's value from the heavier co-twin's value. BMI, body mass index; Sc, subcutaneous; Ia, intra-abdominal; r, correlation coefficient.

DHT we considered the possibility of a reduced synthesis of DHT in the heavier co-twins. DHT is formed from testosterone by 5 α -reductases in peripheral tissues and human preadipocytes *in vitro* [36]. However, the mRNA expression of the 5 α -reductase gene *SRD5A1* in adipose tissue did not differ between the leaner and heavier co-twins, nor did the serum testosterone levels. Thus reduced serum DHT in obesity may be due to its increased AKR1C2-mediated degradation in adipose tissue.

4.2. Adiposity and serum estrogens

For serum estrogens, our study indicates that already a relatively small increase in BMI (a median intra-pair difference in BMI of 2.6 kg/m² in 18 twin pairs) can be associated with higher serum E2 and E1 concentrations in the heavier co-twins. This could result from an increased fat mass and thus increased production of estrogens in the heavier subjects. None of the measures of adiposity were, however, positive correlates of serum estrogen concentrations, in line with [14,15]. One explanation for the higher serum estrogen concentrations might be an increased activity of aromatase converting more testosterone to E2, or androstenedione to E1 [3]. As a possible sign of increased synthesis of E2 in the heavier co-twin, mRNA expression of aromatase gene was positively related to the amount of subcutaneous and intra-abdominal as well as liver fat within twin pairs. Wake et al. have previously reported a positive association between BMI and aromatase mRNA expression in subcutaneous fat from middle-aged men [37]. Thus, the larger fat mass associated with greater expression of the aromatase mRNA, might contribute to the higher serum estrogen levels associated with male obesity.

4.3. SHBG

General adiposity and the concomitant decrease in serum SHBG seem to explain the inverse correlation between serum total and free testosterone and acquired obesity in our study in young men, in line with other studies [13,16]. Our observation that SHBG was the strongest predictor of serum total testosterone concentration, independent of fat distribution, is supported by a previous population study in young adult men [14]. Lower serum SHBG levels in obese men have been explained by inhibitory effects of insulin on hepatic SHBG production [38], and some previous studies have reported inverse associations between serum insulin levels and SHBG [39] or between serum insulin and testosterone concentrations [12,40]. While fasting serum insulin levels were significantly higher in the heavier compared to the leaner co-twins in the present study, SHBG or androgen concentrations did not correlate with serum insulin or measures of insulin resistance (HOMA-IR) within pairs. These results are in agreement with two other studies in men [16,41].

To the best of our knowledge, this is the first study comparing body fat distribution, serum sex steroid concentrations including DHT and estrogens and mRNA expression of genes for steroid metabolizing enzymes in subcutaneous adipose tissue in lean and overweight to obese men. The strengths of the study include a study population of MZ twin pairs. Accordingly, the heavier and leaner subjects were completely matched for sex, age, and multiple environmental and developmental factors in addition to genetic background. As all twins were studied fasting in the morning and either at the same visit or within a few weeks, the possible diurnal or seasonal effects on serum hormone levels were minimized. The blood sampling and adipose tissue biopsies as well as the measurement of body composition were contemporaneous. Another strength is the use of LC–MS/MS as the analytical method for measuring serum sex steroids. Limitations of the study include the use of a single sample for hormone measurements, discounting the pulsatile rhythm of hormone secretion which might vary between the co-twins. Moreover, due to the cross-sectional design it is not possible to infer causality. Because of the extreme rarity of young, healthy monozygotic BMI-discordant pairs, our sample size is small. This introduces a type 2

error (false negatives) in the within-pair analyses.

In conclusion, acquired modest obesity was associated with a decrease in circulating DHT and an increase in serum free E2 concentrations in the heavier co-twins in young male MZ twin pairs. Overexpression of mRNA for the DHT-inactivating AKR1C2 and cortisol-producing HSD11B1 in adipose tissue from the heavier co-twins lends support to the possibility that increased AKR1C2-mediated degradation of DHT in fat tissue reduces its serum concentration. General adiposity associated with greater mRNA expression of aromatase might contribute to the higher serum estrogen concentrations associated with male obesity.

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Declaration of interest

The authors have nothing to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jsbmb.2017.06.007>.

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