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

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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/m^2] included 9 male MZ twin pairs discordant for BMI [intra-pair difference (Δ) in BMI ≥3 kg/m^2]. 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 ΔBMI = 5.9 kg/m^2), 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 17β-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 17β-estradiol (E2) [3,4] compared to leaner men. More recently, population studies have confirmed that body mass index

Abbreviations: 3α-diol, 5α-androstane-3α,17β-diol; BMI, body mass index; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; E1, estrone; E2, 17β-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

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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 5α-androstane-3α,17β-diol (3α-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 $\geq 3\ kg/m^2$], allowing for adjustment of genetic factors and age between the lean and heavy groups. Using Δvalues from both discordant and concordant ($\Delta BMI < 3\ kg/m^2$) 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 ΔBMI, $5.9\ kg/m^2$) and nine BMI-concordant twin pairs (mean ΔBMI, $1.2\ kg/m^2$). 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 −80C 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 $E2 = [10\ exp(-0.003 \times SHBG + 0.389)/100] \times E2$, 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 $\times [2.28 - 1.38 \times \log(SHBG/10)] \times 10$, 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 familiality, 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 Δvalues. 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