Steroid sulfatase activity in subcutaneous and visceral adipose tissue: a comparison between pre- and postmenopausal women

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Abstract

Objective: Adipose tissue is an important extragonadal site for steroid hormone biosynthesis. After menopause, estrogens are synthesized exclusively in peripheral tissues from circulating steroid precursors, of which the most abundant is dehydroepiandrosterone sulfate (DHEAS). Our aim was to study activity of steroid sulfatase, an enzyme hydrolyzing DHEAS, and expression of steroid-converting enzyme genes in subcutaneous and visceral adipose tissue derived from pre- and postmenopausal women.

Design: Serum and paired abdominal subcutaneous and visceral adipose tissue samples were obtained from 18 premenopausal and seven postmenopausal women undergoing elective surgery for non-malignant reasons in Helsinki University Central Hospital.

Methods: To assess steroid sulfatase activity, radiolabeled DHEAS was incubated in the presence of adipose tissue homogenate and the liberated dehydroepiandrosterone (DHEA) was measured. Gene mRNA expressions were analyzed by quantitative RT-PCR. Serum DHEAS, DHEA, and estrogen concentrations were determined by liquid chromatography–tandem mass spectrometry.

Results: Steroid sulfatase activity was higher in postmenopausal compared to premenopausal women in subcutaneous (median 379 vs 257 pmol/kg tissue per hour; $P=0.006$) and visceral (545 vs 360 pmol/kg per hour; $P=0.004$) adipose tissue. Visceral fat showed higher sulfatase activity than subcutaneous fat in premenopausal ($P=0.035$) and all ($P=0.010$) women. The mRNA expression levels of two estradiol-producing enzymes, aromatase and 17 β -hydroxysteroid dehydrogenase type 12, were higher in postmenopausal than in premenopausal subcutaneous adipose tissue.

Conclusions: Steroid sulfatase activity in adipose tissue was higher in postmenopausal than in premenopausal women suggesting that DHEAS, derived from the circulation, could be more efficiently utilized in postmenopausal adipose tissue for the formation of biologically active sex hormones.

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Introduction

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are the most abundant steroid hormones in the circulation. These prohormones, secreted principally by

the adrenal glands, can be converted to active androgens and estrogens in peripheral tissues (1). After the cessation of ovarian estrogen production at menopause, all

estrogens are synthesized locally in peripheral target tissues from the circulating steroid precursors DHEAS, DHEA, and androstenedione (1).

Adipose tissue is an active endocrine organ that plays an important role in extragonadal steroid hormone biosynthesis and metabolism. Adipose tissue expresses steroidogenic enzymes required for the conversion of precursor hormones to active estrogens and androgens (2, 3). DHEAS, which circulates in women at 1000–100 000 times higher concentrations than 17 β -estradiol (E₂) (4), provides a large reservoir of substrate for conversion into biologically active androgens and estrogens in peripheral intracrine tissues. It has been shown that human adipocytes can internalize and hydrolyze DHEAS, and human subcutaneous adipose tissue is able to convert DHEAS to DHEA (5). Steroid sulfatase catalyzes hydrolysis of its two major substrates DHEAS and estrone sulfate (E₁S) into DHEA and estrone (E₁), respectively (6, 7). The gene and protein expression, as well as enzyme activity of steroid sulfatase have been detected in human abdominal subcutaneous adipose tissue (5). However, the activity of steroid sulfatase in human visceral adipose tissue is unknown. Moreover, the possible differences in steroid sulfatase activity between pre- and postmenopausal adipose tissue have not been studied. As steroid sulfatase hydrolyzes inactive steroid sulfates derived from the circulation, it may play an important role in regulating the formation of biologically active steroids in adipose tissue.

There is substantial heterogeneity between different adipose tissue depots. Subcutaneous and visceral adipose tissues exhibit different profiles in the expression of enzymes, hormone receptors, and adipokines (8, 9, 10). Gene expression of steroid sulfatase appears to be higher in subcutaneous compared to visceral adipose tissue in women (8, 11) and in obese men (11). Similarly, aromatase and hormone-sensitive lipase genes are more expressed in subcutaneous than in visceral fat (11).

We have previously investigated DHEA in a form of inactive fatty acyl ester and shown that it can be bound by lipoprotein particles in the circulation and taken up by lipoprotein receptors into peripheral cells and hydrolyzed to biologically active DHEA to be used as a precursor for other steroids (12, 13, 14). In the present study, we investigated hydrolysis of DHEA sulfate ester in adipose tissue samples obtained from premenopausal and postmenopausal women. As postmenopausal women are dependent on extragonadal estrogen synthesis, we explored the possibility that the steroid sulfatase activity could be different in

pre- and postmenopausal adipose tissue. Furthermore, we compared the enzyme activity in subcutaneous and visceral adipose tissue depots. Apart from the steroid sulfatase activity, adipose tissue mRNA expression of steroid sulfatase (*STS*) and other enzymes related to the formation of active estrogens, namely aromatase (*CYP19A1*); 17 β -hydroxysteroid dehydrogenase types 1, 7, and 12 (*HSD17B1*, *HSD17B7*, *HSD17B12*); and hormone-sensitive lipase (*LIPE*) were analyzed. Furthermore, we determined serum concentrations of DHEAS, DHEA, E₁, and E₂ by liquid chromatography–tandem mass spectrometry (LC–MS/MS).

Subjects and methods

Subjects and study design

We studied 18 premenopausal and seven postmenopausal women undergoing elective gynecological surgery for non-malignant reasons in the Department of Obstetrics and Gynecology in Helsinki University Central Hospital. The operations performed were as follows: abdominal hysterectomy ($n=16$), abdominal hysterectomy with salpingo-oophorectomy ($n=5$), laparoscopic hysterectomy ($n=2$), salpingo-oophorectomy ($n=1$), and enucleation of uterine myomas ($n=1$). Reasons for surgery were: uterine myomas ($n=23$), dysmenorrhea ($n=1$), and ovarian cyst ($n=1$). None of the women received systemic estrogen or progestin treatment. At the preoperative visit, we obtained detailed clinical information, including menopausal status, medical history, medication, height, weight, as well as waist and hip circumferences.

Blood samples were drawn before the operation. Serum was isolated by centrifugation and stored at -20°C until the analyses. During the operation, paired abdominal subcutaneous and visceral adipose tissue samples were taken and snap-frozen in liquid nitrogen. The adipose tissue samples were stored at -80°C until further processing. The study was approved by the ethics committee of Helsinki University Central Hospital, and all subjects signed written informed consent.

Assay for steroid sulfatase activity in adipose tissue

Radiolabeled DHEAS (1,2,6,7-³H(N)-DHEAS; Perkin Elmer, Boston, MA, USA; specific activity 2.33 TBq/mmol) was purified with DEAE-Ac ion exchange column (15) (DEAE Sephadex A-25, Amersham Biosciences). ³H-DHEAS was dissolved in 0.5 ml of 70% methanol and applied to the

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column. The first fraction containing free DHEA and other impurities was eluted with 29 ml of solvent (15) and discarded. ^3H -DHEAS was then eluted with 15 ml of 0.4 mol/l formic acid and 0.25 mol/l KOH in 70% methanol, followed by removal of KOH with Sep-Pak C18 cartridge (Waters Corp., Milford, MA, USA). Frozen adipose tissue sample (200 mg) was homogenized in 1 ml of distilled water with Ultra Turrax (T8; IKA-Werke, Staufen, Germany) on ice. Purified ^3H -DHEAS (total of 2.1×10^5 dpm; 1.5 pmol/200 mg adipose tissue) was added in 6 μl of ethanol to the adipose tissue homogenate, and the mixture was incubated at $+37^\circ\text{C}$ for 3 h. Incubation of ^3H -DHEAS with water served as control. To observe the conversion of ^3H -DHEAS to ^3H -DHEA as a function of time, we incubated ^3H -DHEAS (2.8×10^5 dpm/200 mg adipose tissue) with pooled, homogenized subcutaneous and visceral adipose tissue obtained from two premenopausal women for 0, 1, 2, 3, 6, and 24 h at $+37^\circ\text{C}$. Following incubation, the mixture was extracted four times with 4.5 ml of diethyl ether. After quick freezing of the ^3H -DHEAS-containing water phase, the organic phase was removed and evaporated under nitrogen flow. The oily residue was dissolved in 1 ml of hexane and subjected to Sephadex LH-20 chromatography (column dimension 1×6 cm; Amersham Pharmacia Biotech AB) to purify ^3H -DHEA formed during the incubation. The sample tube was rinsed twice with 0.5 ml of hexane, which was also applied to the column. The hydrophobic fraction was collected by further eluting with 7 ml of hexane, after which the column was washed with 6 ml of hexane, and DHEA fraction was eluted with 17 ml of chloroform/hexane (1:1, v/v). Following evaporation under nitrogen, the DHEA fraction was dissolved in hexane and the radioactivity was determined by liquid scintillation counting (Rack-beta, Wallac, Turku, Finland). The results were calculated by subtracting the amount of radioactivity in the control sample from the radioactivity in the adipose tissue-containing samples.

Preparation and quantification of mRNA

Frozen adipose tissue samples (~ 200 mg) were homogenized in 1 ml of TRIzol, and total RNA was isolated and purified as previously described (11). RNA (1.0 μg) was reverse transcribed into cDNA, and mRNA expression levels of *STS*, *LIPE*, *CYP19A1*, *HSD17B1*, *HSD17B7*, and *HSD17B12* were quantified by real-time RT-PCR as described (11). The efficiency ranged between 93% and 114%. The data were normalized to the geometric mean of the two most stable control genes, importin 8 (*IPO8*)

and lysine (K)-specific demethylase 2B (*KDM2B*) (11). Gene expressions of other potentially important $17\beta\text{-HSD}$ enzymes, such as types 2, 5, and 8, were not investigated in this study.

Quantification of DHEAS, DHEA, E_1 and E_2 by LC-MS/MS

DHEAS ► To 25 μl of serum, 25 μl of deuterated DHEAS (15 $\mu\text{mol/l}$; D_6 -DHEAS- Na , Sigma-Aldrich Co., St. Louis, MO, USA) was added as internal standard (IS) in 50% methanol (v/v), followed by precipitation with 0.2 ml of methanol. After mixing for 30 s and allowing to stand for 5 min, the samples were centrifuged for 3 min at 13 000 g . To an aliquot of 25 μl of the supernatant, 50 μl of water and 425 μl of 40% methanol were added. Calibrators containing 0.1–20 $\mu\text{mol/l}$ of DHEAS- Na (Sigma-Aldrich Co.) were prepared in 40% methanol. The sample extracts and calibrators were diluted 1:200 with 40% methanol, and a 5- μl aliquot was analyzed on an LC-MS/MS system equipped with an API 4000 triple quadrupole mass spectrometer (ABSciex, Concord, Canada). Peripherals included an Agilent series 1200 HPLC system with a binary pump. Separation was performed on a SunFire C18 column (2.1×50 mm; 3.5 μm ; Waters Corp.). The mobile phase was a linear gradient consisting of methanol (B) and 5 mmol/l ammonium acetate in water (A), at a flow rate of 300 $\mu\text{l/min}$. The gradient was: 0 min, 40% B; 1.0 min 100% B; 4.0 min 100% B; and 4.1–10 min 40% B. DHEAS was detected in the negative mode with the following transitions: m/z 367.2 to m/z 96.8 and 79.9, and IS m/z 373.5 to m/z 97.8. Data were acquired and processed with the Analyst Software (version 1.4.2; ABSciex). The mass calibration and resolution adjustments (at 0.7 atomic mass units at full width and half height) on both the resolving quadrupoles were optimized using a polypropylene glycol solution with an infusion pump. The limit of quantification was 50 pmol/l ($S/N=10$).

DHEA ► Serum DHEA was quantified as previously described (16) with some modifications. To 200 μl of serum, 40 μl of $^{13}\text{C}_3$ -DHEA as IS (0.2 $\mu\text{mol/l}$; IsoSciences, King of Prussia, PA, USA) in 50% methanol was added, followed by the extraction with 3 ml of diethyl ether. After mixing for 3 min, the upper layer was collected and evaporated to dryness. The residues were dissolved in 3 ml of water and mixed for 1 min. After adding 3 ml of hexane and mixing for 3 min, the organic phase was evaporated to dryness and dissolved in 200 μl of 50% methanol, and

a 25- μ l aliquot was analyzed by LC–MS/MS. The mobile phase was a linear gradient consisting of methanol (B) and 2 mmol/l ammonium acetate in water (A), at a flow rate of 300 μ l/min. The gradient was: 0 min, 50% B; 3.0–9.0 min 100% B; and 9.5–15 min 50% B. DHEA was detected as ammonium adduct in the positive mode with the following transitions: m/z 306.2 to m/z 253.1 and 213.1, and IS m/z 309.2 to m/z 256.1.

E₁ and E₂ ► In sera from premenopausal women, E₁ and E₂ were quantified in one run. To 250 μ l of serum, 25 μ l of IS containing ¹³C₃-estradiol (5 nmol/l; IsoSciences) and ¹³C₃-estrone (2.5 nmol/l; IsoSciences) in 50% methanol was added, followed by 250 μ l of water, 500 μ l of 50 mmol/l ammonium acetate/NH₃ (pH 9), and 2.5 ml of diethyl ether. After mixing for 3 min, the organic layer was collected and evaporated to dryness. The residue was dissolved in 100 μ l of 50% methanol, and a 40- μ l aliquot was analyzed by LC–MS/MS. Calibrators containing 25–1000 pmol/l of estradiol (Sigma–Aldrich Co.) and estrone (Vetranal, Sigma–Aldrich Co.) were prepared in 50% methanol. The mobile phase was a linear gradient consisting of methanol (B) and water (A), at a flow rate of 300 μ l/min. The gradient was: 0 min, 50% B; 5–8.5 min 100% B; and 9–15 min 50 % B. Ions were detected in the negative mode with the following transitions: estradiol m/z 271.2 to m/z 145.2, estrone m/z 269.1 to m/z 145.0, estradiol-IS m/z 274.2 to m/z 148.2, and estrone-IS m/z 272.1 to m/z 148.0. The limit of quantification for E₁ was 5 pmol/l, and for E₂, 15 pmol/l (S/N=10).

In sera from postmenopausal women, E₁ and E₂ concentrations were determined separately. To quantify E₁, ¹³C₃-estrone IS was added to 250 μ l of serum, and the sample was processed as previously described (17). Serum E₂ concentration was determined as described (18), with some modifications. To 250 μ l of serum, 25 μ l of ¹³C₃-estradiol IS (5 nmol/l) in 50% methanol was added, followed by 250 μ l water, 500 μ l of 50 mmol/l ammonium acetate/NH₃ (pH 9), and 2.5 ml diethyl ether. After mixing for 3 min, the organic layer was collected, and 500 μ l of water was added. The tubes were mixed for 1 min and the organic phase was evaporated and dissolved in 100 μ l of 50% methanol, and a 40- μ l aliquot was analyzed by LC–MS/MS. The mobile phase was a linear gradient consisting of methanol (B) and water (A), at a flow rate of 300 μ l/min. The gradient was: 0 min, 50% B; 5–8.5 min 100% B; and 9–15 min 50% B. Estradiol was detected in the negative mode with the following transitions: m/z 271.2 to m/z 145.2 and 183.0, and IS m/z 274.2 to m/z 148.2.

Other analyses

Serum follicle-stimulating hormone (FSH) was determined to confirm the menopausal status (reference value in postmenopausal women, 26–135 IU/l; competitive electrochemiluminescence immunoassay, Roche Modular Analytics E170 system, Roche Diagnostics). Serum sex hormone-binding globulin (SHBG) was measured as described (19).

Statistical analysis

Statistical analyses were performed using SPSS Statistics 22.0 software. Data are expressed as median (range) or median (interquartile range). The Wilcoxon signed rank test was performed for pairwise comparisons, and the Mann–Whitney *U* test for comparisons between the groups. Correlation analyses were performed using Spearman's nonparametric correlation. The level of significance was *P*<0.05. When multiple correlation analyses between hormone concentrations and gene expression levels were carried out, the correlations were considered significant at *P*<0.01.

Results

Clinical characteristics and hormone concentrations

Body mass index (BMI), waist to hip ratio (WHR), and serum SHBG concentrations did not significantly differ between the pre- and postmenopausal women (Table 1). BMI correlated positively with WHR (*r*=0.429, *P*=0.037) and negatively with SHBG (*r*=−0.517, *P*=0.008). Serum DHEAS, DHEA, E₁, and E₂ concentrations were higher in premenopausal than in postmenopausal women (Table 1). All these hormone concentrations correlated negatively with age when all women were analyzed together (DHEAS *r*=−0.581; DHEA, *r*=−0.719; E₁, *r*=−0.513; E₂, *r*=−0.467; *P*<0.05 for all), and DHEA, E₁, and E₂ correlated negatively with age in postmenopausal women (DHEA, *r*=−0.786; E₁, *r*=−0.786; E₂, *r*=−0.786; *P*<0.05 for all). Serum DHEA was positively related to serum DHEAS in premenopausal (*r*=0.734, *P*=0.001, *n*=18) and in postmenopausal women (*r*=0.893, *P*=0.007, *n*=7) as well as in all women analyzed together (*r*=0.843, *P*<0.001, *n*=25). Furthermore, serum E₂ correlated with serum E₁ in premenopausal (*r*=0.796, *P*<0.001), postmenopausal (*r*=1.0, *P*<0.001), and all women (*r*=0.898, *P*<0.001).

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Table 1 Clinical characteristics of the patients. The data are expressed as median (range).

	Premenopausal (n=18)	Postmenopausal (n=7)	P value*
Age, years	43.9 (29.3–51.9)	56.0 (52.3–74.8)	<0.001
BMI, kg/m ²	28 (21–35)	25 (22–34)	0.615
Waist to hip ratio	0.85 (0.73–0.99)	0.90 (0.82–1.05)	0.130
Serum FSH, IU/l	7 (1–18)	70 (26–188)	<0.001
Serum SHBG, nmol/l	61 (31–96)	60 (24–97)	0.929
Serum DHEAS, μ mol/l	3.7 (1.2–7.1)	1.5 (0.2–4.2)	0.025
Serum DHEA, nmol/l	14.1 (1.9–58.0)	4.7 (0.2–20.5)	0.012
Serum estrone, pmol/l	228 (122–970)	94 (15–216)	0.001
Serum estradiol, pmol/l	339 (33–1342)	42 (9–358)	0.005

Statistically significant *P* values are bold.

*Comparison premenopausal vs postmenopausal (independent-samples Mann–Whitney *U* test).

Steroid sulfatase activity in adipose tissue

Radiolabeled DHEAS was used as the substrate to investigate activity of adipose tissue steroid sulfatase. The hydrolysis of ³H-DHEAS during the incubation with pooled subcutaneous and visceral adipose tissue homogenate increased as a function of time up to 24 h (Fig. 1). Based on this enzyme assay validation, we chose the 3-h incubation time for further experiments. To observe steroid sulfatase activity in subcutaneous and visceral adipose tissue samples of pre- and postmenopausal women, we incubated ³H-DHEAS with adipose tissue homogenates and determined the conversion of ³H-DHEAS to ³H-DHEA. Steroid sulfatase activity was higher in postmenopausal compared to premenopausal women both in subcutaneous (median 379 vs 257 pmol/kg adipose tissue per hour; *P*=0.006) and visceral (545 vs 360 pmol/kg per hour; *P*=0.004) adipose tissue (Fig. 2). Visceral adipose tissue showed higher steroid sulfatase activity compared to that observed in subcutaneous adipose tissue (Fig. 2). This difference was significant in premenopausal women (*P*=0.035) and in all women analyzed together (*P*=0.010), but not in postmenopausal women (*P*=0.128). Steroid sulfatase activity in subcutaneous adipose tissue correlated positively with the activity in visceral adipose tissue in premenopausal (*r*=0.472, *P*=0.048) and all women (*r*=0.571, *P*=0.003), but not in postmenopausal women (*r*=0.250, *P*=0.589). Serum concentrations of DHEA or DHEAS did not correlate with the steroid sulfatase activity in any of the study groups. Steroid sulfatase activity in both adipose tissue depots correlated positively with age (subcutaneous, *r*=0.604, *P*=0.001; visceral, *r*=0.416, *P*=0.039) when all women were analyzed together. However, there was no positive correlation between sulfatase activity and age, when premenopausal and postmenopausal women were analyzed separately (premenopausal: subcutaneous,

r=0.360, *P*=0.142; visceral, *r*=−0.040, *P*=0.874; postmenopausal: subcutaneous, *r*=−0.750, *P*=0.052; visceral, *r*=0.000, *P*=1.0).

Gene expression of steroid-converting enzymes

STS gene displayed similar expression in premenopausal compared to postmenopausal women (Fig. 3). The relative mRNA expression level of *STS* was, however, higher in subcutaneous than in visceral adipose tissue in premenopausal women (*P*=0.001) and in all women (*P*<0.001). In postmenopausal women, the difference was non-significant (*P*=0.080) (Fig. 3).

Figure 1

The hydrolysis of DHEAS in adipose tissue as a function of time. The hydrolysis is expressed as a percentage of ³H-DHEAS converted to ³H-DHEA. ³H-DHEAS was incubated with (filled circle) and without (open circle, control) homogenized, pooled, combined subcutaneous and visceral adipose tissue samples obtained from premenopausal women, followed by extraction and purification of ³H-DHEA on Sephadex LH-20 chromatography.

Figure 2

Steroid sulfatase activity measured as the conversion of ^3H -DHEAS to ^3H -DHEA in subcutaneous (Sc) and visceral (Visc) adipose tissue. The hydrolysis is expressed as the amount of liberated ^3H -DHEA (pmol)/adipose tissue mass (kg) per incubation time (h). ^3H -DHEAS was incubated in the presence of adipose tissue homogenates for 3 h at 37 °C, followed by extraction, purification, and quantification of ^3H -DHEA. The data are expressed as median (interquartile range). Pre, premenopausal women ($n=18$); Post, postmenopausal women ($n=7$); All, all women combined ($n=25$). *, §, $P<0.01$, postmenopausal compared to premenopausal (Mann–Whitney U test). # $P<0.05$, subcutaneous compared to visceral adipose tissue (Wilcoxon signed rank test).

In addition to *STS* gene, we analyzed expression levels of some key genes regulating steroid metabolism in adipose tissue. In subcutaneous adipose tissue, the relative mRNA expression levels of *CYP19A1* and *HSD17B12* were higher in postmenopausal compared to premenopausal women. Similarly, the expression of *LIPE* was higher in postmenopausal than in premenopausal women in visceral fat (Fig. 3). *LIPE* and *CYP19A1* displayed higher expression in subcutaneous compared to visceral adipose tissue in premenopausal women (Fig. 3) and in all women (*LIPE*, $P=0.004$; *CYP19A1*, $P=0.001$). The expression of *HSD17B7* was, however, higher in visceral compared to subcutaneous fat in premenopausal women (Fig. 3) and in all women ($P<0.001$). We found no measurable mRNA expression of estrogen sulfotransferase gene (*SULT1E1*) in adipose tissue.

Serum DHEA and DHEAS concentrations did not correlate with the mRNA expression levels of any of the

genes studied. In postmenopausal women, serum E_1 and E_2 concentrations displayed strong positive correlations with the *STS* mRNA expression in both subcutaneous and visceral adipose tissue ($P=0.005$) (Table 2). The expression of the other genes studied did not correlate with serum E_1 and E_2 . *CYP19A1* expression in visceral fat correlated positively with BMI ($r=0.886$, $P=0.019$) and WHR ($r=0.829$, $P=0.042$) in postmenopausal women, and the expression of the same gene in subcutaneous fat showed a positive correlation with WHR in premenopausal women ($r=0.574$, $P=0.016$) and all women ($r=0.541$, $P=0.008$).

In subcutaneous adipose tissue, the DHEA sulfatase activity correlated positively with the mRNA expression levels of *STS* ($r=0.412$, $P=0.045$), *CYP19A1* ($r=0.694$, $P<0.001$), and *HSD17B12* ($r=0.536$, $P=0.007$) in all women analyzed together. In visceral fat, the sulfatase activity correlated with the expression of *CYP19A1* in all women ($r=0.414$, $P=0.050$).

Figure 3

The relative mRNA expression of genes related to steroid hormone biosynthesis in abdominal subcutaneous (Sc) and visceral (Visc) adipose tissue in premenopausal (Pre) and postmenopausal (Post) women. *STS*, steroid sulfatase; *LIPE*, hormone-sensitive lipase; *CYP19A1*, aromatase; *HSD17B1*, *HSD17B7*, *HSD17B12*, 17 β -hydroxysteroid dehydrogenase types 1, 7, and 12. The data are expressed as median (interquartile range). * $P\leq 0.01$, postmenopausal compared to premenopausal (Mann–Whitney U test). # $P<0.01$, subcutaneous compared to visceral adipose tissue (Wilcoxon signed rank Test).

Table 2 Correlations between serum hormone concentrations and mRNA expression of steroid sulfatase (*STS*) in adipose tissue in postmenopausal women.

	Subcutaneous <i>STS</i>		Visceral <i>STS</i>	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
DHEAS	0.371	0.468	0.714	0.111
DHEA	0.657	0.156	0.714	0.111
Estrone	0.943	0.005	0.943	0.005
Estradiol	0.943	0.005	0.943	0.005

Statistically significant correlations are bold. $n=6$.
r, correlation coefficient (Spearman's correlation).

Discussion

Our study demonstrates that the steroid sulfatase activity, when determined as the conversion of DHEAS to DHEA, in subcutaneous and visceral adipose tissue is significantly higher in postmenopausal compared to premenopausal women. This finding suggests that DHEAS hydrolysis may play a more important role after menopause in steroid biosynthesis in adipose tissue. To the best of our knowledge, this is the first report comparing steroid sulfatase activity in adipose tissue derived from pre- and postmenopausal women. After the arrest of ovarian estrogen secretion at menopause, estrogens and androgens are synthesized in peripheral tissues through the enzymatic conversion from steroid precursors (1). Our results suggest that DHEAS, circulating at micromolar concentrations, could be more efficiently targeted to and utilized by postmenopausal than premenopausal adipose tissue.

We found that the DHEA sulfatase activity was higher in visceral compared to subcutaneous adipose tissue as analyzed in premenopausal women and in all women. This difference has not been previously reported. Our finding suggests that body fat distribution may have relevance in modulating hormone metabolism. Accumulation of visceral adipose tissue is associated with obesity-related metabolic derangements that increase the risk for metabolic syndrome, type 2 diabetes, and ultimately cardiovascular disease (20, 21). Body fat distribution is different between men and women suggesting that sex hormones are important regulators of body fat distribution patterns (22). Furthermore, reduced estrogen levels after menopause have been associated with increased accumulation of visceral adipose tissue (23, 24).

While DHEAS was more efficiently converted to DHEA in postmenopausal compared to premenopausal adipose tissue, the mRNA expression levels of the *STS* gene did not

differ between these groups. Moreover, in premenopausal and all women, the DHEA sulfatase activity was higher in visceral fat, but the *STS* mRNA expression was lower. These differences may be due to the possibility that steroid sulfatase can be controlled via posttranscriptional or posttranslational modifications (7) such as glycosylation (25). For example, in breast cancer cells, it has been reported that cytokines interleukin 6 (IL6) and tumor necrosis factor α (TNF α) enhance steroid sulfatase activity without increase in the *STS* gene expression (26). Furthermore, in hepatocytes, inflammatory factors induce expression and activity of steroid sulfatase, thus enhancing estrogen activity, which subsequently attenuates the inflammatory response (27). It has been reported that postmenopausal women have an increased inflammatory response in abdominal subcutaneous adipose tissue (28) as well as in breast adipose tissue (29). Increased adipose tissue inflammation in postmenopause could, in theory, explain our finding of higher steroid sulfatase activity but similar *STS* gene expression in postmenopausal compared to premenopausal women.

We observed that the mRNA expression levels of 17 β -HSD type 12, an enzyme converting E_1 to E_2 (30, 31), and aromatase (*CYP19A1* gene), an enzyme converting androstenedione to E_1 and testosterone to E_2 (32, 33), were higher in postmenopausal compared to premenopausal subcutaneous adipose tissue. Furthermore, the mRNA expression of hormone-sensitive lipase (*LIPE* gene), an enzyme hydrolyzing adipose triacylglycerols as well as fatty acyl esters of DHEA and E_2 (18), was higher in postmenopausal compared to premenopausal women in visceral fat. Since all these three enzymes participate in the metabolic pathway leading to the formation of biologically active estrogens our data support the possibility that the local production of estrogens is enhanced after menopause.

Subcutaneous adipose tissue had a higher mRNA expression of *STS*, aromatase, and hormone-sensitive lipase compared to visceral adipose tissue, in line with previous studies (8, 11, 34). In contrast to previous studies (11, 35), we observed a higher mRNA expression of 17 β -HSD type 7, an enzyme converting E_1 to E_2 (36), in visceral compared to subcutaneous fat. Interestingly, in subcutaneous adipose tissue, all women analyzed together, steroid sulfatase activity correlated positively with the mRNA expression of *STS*, aromatase, and 17 β -HSD type 12. Based on this, we can hypothesize that the increased hydrolysis of DHEAS could be related to the enhanced gene expression of aromatase and 17 β -HSD

type 12, which could further drive this pathway towards the formation of active E_2 .

Serum concentrations of DHEAS and DHEA did not correlate with mRNA expression of any of the genes studied. As the adrenals secrete large amounts of these prohormones into the circulation, it is rather expected that the expression of steroid-converting enzymes in adipose tissue is not reflected in serum prohormone levels. Serum E_1 and E_2 , however, correlated positively with *STS* mRNA expression in subcutaneous and visceral adipose tissue of postmenopausal women. In premenopausal women, no such correlation was found, as expected due to the high variation of estrogen levels in different phases of the menstrual cycle. After the arrest of ovarian estrogen production at menopause, local estrogen synthesis in peripheral tissues becomes more important. Our finding that, in postmenopausal women, *STS* mRNA expression in adipose tissue correlated with serum E_1 and E_2 concentrations raises the possibility that steroid sulfatase in adipose tissue may contribute to serum estrogen levels. Steroid sulfatase activity did not correlate with serum hormone levels. However, as we used DHEAS as a substrate it is worth noting that DHEAS is only one of the substrates for steroid sulfatase and the hydrolysis of others, such as E_1S , was not investigated in the present study.

In conclusion, the DHEA sulfatase activity in adipose tissue was higher in postmenopausal compared to premenopausal women suggesting that DHEAS, derived from the circulation, could be more efficiently hydrolyzed in postmenopausal adipose tissue, and utilized for the formation of biologically active androgens and estrogens. Visceral adipose tissue showed a higher DHEA sulfatase activity compared to subcutaneous adipose tissue suggesting that body fat distribution may influence hormone metabolism. Further studies are needed to characterize differences in steroid-metabolizing enzyme expression and activity between various adipose tissue depots.

Declaration of interest

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

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