

Concentration of sex steroids in adipose tissue after menopause

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Adipose tissue is a site of uptake, storage, action, and metabolism of sex steroids. After menopause aromatization of androgens to estrogens in adipose tissue is one of the most important sources of estrogen in the circulation and for peripheral tissues. The aim of this study was to estimate local sex steroid concentrations in breast and abdominal subcutaneous (s.c.) adipose tissue, to compare them with plasma concentrations and to investigate possible correlations with body mass index (BMI). The patients were postmenopausal women undergoing surgery for non-oncological reasons (Group A; n = 35) and breast cancer patients (group B; n = 19). The concentrations of estrone, 17 β -estradiol, estrone sulfate, 17 β -estradiol sulfate, androstenedione, androstenediol (androst-5-ene-3 β ,17 β -diol), testosterone and dehydroepiandrosterone were measured. The method was based on frozen tissue homogenization, extraction with ethanol : acetone, delipidation, extraction of estrogens with ether, and of androgens with iso-octane in toluene, followed by RIA. The mean levels of steroids were higher in fat than in plasma, apart from testosterone. Levels of sulfates of estrogens and androstenediol were higher in breast than abdominal adipose tissue, and levels of estradiol lower. Positive correlations were found between BMI and tissue and plasma concentration of both estrone and androstenedione. (Steroids 63:319–321, 1998) © 1998 by Elsevier Science Inc.

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Introduction

Adipose tissue plays an important role in the metabolism of sex hormones.^{1,2} Obesity is associated with an increased incidence of cancer of breast, endometrium, prostate and colon. Despite decreased gonadal estrogen production and plasma levels after menopause, the incidence of breast cancer increases with age. Adipose tissue is the major site of peripheral aromatization of androgens to estrogens after menopause.^{3,4} The aim of our work was to compare sex steroid levels in plasma and adipose tissue and to seek correlations between body mass index and steroid concentrations. To determine whether tissue levels of sex hormones varies with different body sites, their concentration was measured in adipose tissue from breast cancer in postmenopausal women and in s.c. adipose tissue taken from the abdomen.

Experimental

Patients and samples

Two groups of postmenopausal Polish women were studied: Group A consisted of 17 women undergoing abdominal surgery

for non-oncological reasons. The mean age was 61.2 ± 5.2 years, and BMI was 27.6 ± 6.4 [kg/m²]. Group B consisted of 35 breast cancer patients who underwent mastectomy. The mean age in this group was 61.6 ± 6.1 years, and BMI 26.5 ± 4.5 [kg/m²].

Patients had no previous hormonal or chemotherapeutic medication, nor had had preoperative radiotherapy. Blood samples were collected before operation, and plasma samples kept at -20°C until processing. Adipose tissue (1 g) was isolated from mastectomy specimens immediately after tumor resection, and abdominal s.c. adipose tissue before abdominal wound closure in the other group of patients. All tissues were frozen immediately and stored at -80°C until assay for estrone (E₁), 17 β -estradiol (E₂), estrone sulfate (E₁S), 17 β -estradiol sulfate (E₂S), androstenedione (Adion), androstenediol (androst-5-ene-3 β ,17 β -diol; Adiol), testosterone (T), and dehydroepiandrosterone (DHEA).

Assay of steroids

Determination of estrogen and androgen levels in tissue samples was performed by methods previously described⁵ with some slight modifications.

Briefly, frozen tissue was pulverized in a dismembrator, suspended in phosphate buffer pH 7.4, and the suspension extracted twice with ethanol/acetone 1:1 (v/v). The extract was concentrated and delipidated with 70% methanol at -20°C , the methanol evaporated, and the residue dissolved in buffer. Free steroids were extracted into ether by a two step procedure, and those remaining in the buffer were hydrolyzed enzymatically (Helix Pomatia) be-

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Table 1 Comparison of Adipose Tissue and Plasma Sex Steroid Concentrations^a

	Breast adipose tissue	Tissue versus plasma	Plasma	Abdominal adipose tissue	Tissue versus plasma	Plasma
	Mean ± SE	<i>p</i>	Mean ± SE	Mean ± SE	<i>p</i>	Mean ± SE
E ₁ (fmol)	670 ± 61	<0.0001	208 ± 16	685 ± 68	<0.0001	219 ± 30
E ₂ (fmol)	203 ± 25	<0.0001	56 ± 9	334 ± 67	<0.001	58 ± 11
T (pmol)	0.62 ± 0.08	<0.0001	1.3 ± 0.1	0.79 ± 0.2	NS	1.1 ± 0.1
Adion (pmol)	16 ± 1.8	<0.0001	4.6 ± 1.1	18 ± 1.9	<0.0001	5.1 ± 0.91
Adiol (pmol)	3.8 ± 0.6	<0.01	1.8 ± 0.2	2.0 ± 0.3	NS	1.5 ± 0.3
DHEA (nmol)	99 ± 13	<0.0001	11 ± 1.3	69 ± 15	<0.001	8.9 ± 2.3

^a Values are expressed in mol/g tissue or mol/mL plasma.

fore ether extraction. The extract was evaporated to dryness and the residue dissolved in 2.2 mL absolute ethanol.

Androgens were estimated by specific and sensitive radioimmunoassay in 1.0 mL of this solution, following Celite chromatography. Columns were eluted with a discontinuous gradient of 2.5% to 20% iso-octane in toluene (v/v). The radioimmunoassays were standardized against plasma pools containing different concentrations of androgens.

Estrogens in plasma and tissue extracts were separated by LH-20 chromatography with toluene/ethanol 92: 8 (v/v) and assayed by radioimmunoassay.

Statistical procedures

Results are expressed as mean ± SE, and statistical comparisons made by Student's *t*-test. Pearson's correlation test was used to determine the relationship between variables; *p* values less than 0.05 were regarded as significant.

Results

Mean concentrations of steroids examined were higher in breast and abdominal s.c. fatty tissue than in plasma, with the exception of testosterone; androstenediol concentrations were higher in breast adipose tissue than in plasma, but not in abdominal adipose tissue. These results are summarized in Table 1.

Adipose tissue and plasma hormone concentrations were positively correlated for estrone ($r = 0.75$) and testosterone

($r = 0.57$) in women with breast cancer, and for estrone ($r = 0.79$) and Adiol ($n = 0.63$) in the other group. No differences in plasma hormone concentration were detected between the two groups.

Tissue hormone concentrations were slightly different in the two groups, with levels of estrogen sulfates and Adiol higher in breast than abdominal adipose tissue, but levels of estradiol lower, as shown in Table 2.

In terms of considering the relationship between obesity and hormones, we sought possible differences in tissue and plasma hormone concentrations between obese (BMI ≥ 26 kg/m²) and non-obese (BMI < 26 kg/m²) women, which were significant only in the case of estrone in group B. In obese breast cancer patients ($n = 16$) estrone concentrations in adipose tissue (812 ± 115 fmol/g) were higher than in non-obese women (557 ± 52 fmol/g; $p = 0.037$). Similarly, plasma estrone concentrations were higher in obese women (261 ± 23 fmol/ml) than in non-obese (160 ± 6 fmol/ml; $p < 0.001$; $n = 19$). We observed a positive correlation between body mass index (BMI) and concentration of E₁S ($r = 0.54$), testosterone ($r = 0.42$) and Adiol ($r = 0.51$) only in breast adipose tissue ($p < 0.05$). There was no correlation between E₂ tissue concentrations and BMI. Plasma hormone concentrations were positively correlated with BMI for estrone and androstenedione considered separately for each group, and for both groups together. In obese (BMI ≥ 26) women this correlation was also significant for E₂ in plasma ($r = 0.41$; $n = 24$). Individual between-hormone correlations are shown in Table 3

Table 2 Comparison of Estrogen and Androgen Concentrations in Subcutaneous Abdominal Adipose Tissue (Group A) and in Breast Adipose Tissue (Group B)

	Group A	Group A versus Group B	Group B
	Mean ± SE	<i>p</i>	Mean ± SE
E ₂ (fmol/g)	334 ± 67 <i>n</i> = 17	0.034	203 ± 25 <i>n</i> = 30
E ₁ S (fmol/g)	52 ± 8 <i>n</i> = 17	0.034	142 ± 29 <i>n</i> = 33
E ₂ S (fmol/g)	32 ± 4 <i>n</i> = 17	0.002	59 ± 6 <i>n</i> = 32
Adiol (pmol/g)	2.0 ± 0.3 <i>n</i> = 17	0.02	3.8 ± 0.6 <i>n</i> = 25

Table 3 Pearson's Correlation Coefficients for Concentrations of Pairs of Hormones in Adipose Tissue and Plasma

Correlated hormones	Breast adipose tissue	Abdominal adipose tissue	Plasma
	Group B (<i>n</i> = 25)	Group A (<i>n</i> = 17)	Group B and A (<i>n</i> = 40)
Adion and E ₁	0.44	0.57	0.59
Adion and E ₂	0.46	0.53	0.42
DHEA and Adiol	0.86	0.32 (NS)	0.60

Discussion

Adipose tissue comprises about 20% of the normal human body mass, and in obese people this can reach 50%. The concentrations of estrone, estradiol, androstenedione and dehydroepiandrosterone in adipose tissue are several fold higher than in plasma, results comparable with those of our previous studies.⁶

In both groups, estrone concentrations in tissue and plasma were positively correlated. In terms of potential connections between amounts of fatty tissue (represented indirectly by BMI) and hormone concentration in plasma and fatty tissue, the positive correlation between BMI and Adiol and estrone is of interest; in addition we found higher E₁ concentration in obese than non-obese breast cancer patients, both in adipose tissue and plasma.

Adipose tissue in postmenopausal women is quantitatively the most important site of aromatization of androgens to estrogens and thus the main source of extraglandular estrogen synthesis.^{3,4} The correlations Adion-E₁ and Adion-E₂ in fatty tissue and plasma (as shown in Table 3) confirm the importance of Adion as a source of estrogens. Similarly, dehydroepiandrosterone and Adiol concentrations are positively correlated in fat and plasma, which most likely reflects their *in vivo* interconversion.

The lack of correlation between DHEA and Adion concentrations in adipose tissue is intriguing. Androstenedione may be the principal hormone stored in fat over long period, and thus is minimally affected by changes in DHEA levels. *In vitro* 96% of medium androstenedione is absorbed by adipocytes within 1 minute, where it appears to remain for a long time.⁷ The data in the literature on testosterone concentrations in tissues generally show testosterone levels comparable with those in plasma.^{1,8}

The differences in hormone concentrations between breast and abdominal adipose tissue observed in our work suggest that local factors must be involved in the hormone uptake, storage, act and metabolism.^{4,9} The higher concentrations of estrogen sulfates in breast adipose tissue than that from abdomen suggest that sulfates are stored in breast fat, and may thus be used as a source for free estrogens,^{10,11}

especially since E₁S levels in breast fatty tissue, despite its polar nature, were positively correlated with BMI.

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