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THE INTERCONVERSION AND AROMATIZATION OF ANDROGENS BY HUMAN ADIPOSE TISSUE

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SUMMARY

Incubations have been carried out to study the interconversion and aromatization of androstenedione and testosterone in human adipose tissue. In abdominal and omental fat using androstenedione at a variety of substrate concentrations, testosterone was isolated from all incubations, estrone from 8 out of 12 incubations and estradiol from 3 out of 12 incubations. Using breast adipose tissue from male and female subjects, testosterone, estrone and estradiol were isolated from all incubations. No consistent difference in the capacity of adipose tissue to aromatize androstenedione was noted in adipose tissue from different sources. In studies on isolated fat cells and the fibrovascular stroma of adipose tissue the interconversion of testosterone and androstenedione was demonstrated in both tissues. Human adipocyte precursors isolated from the stromal-vascular fraction of human omental adipose tissue converted androstenedione to testosterone and estrone. These studies confirm the aromatization of androstenedione in adipose tissue from a variety of sources. They also demonstrate the interconversion of androgens in adipose tissue and emphasize that the net effect of a gonadal steroid acting on a peripheral tissue may depend on the nature of the metabolites formed in that tissue.

INTRODUCTION

The demonstration that adipose tissue is capable of metabolizing androgens has stimulated interest in the role of adipose tissue in modulating the biological effects of gonadal steroids. The studies of Bleau *et al.* [1] and Owens *et al.* [2] have clearly demonstrated that androgens and estrogens are taken up by adipose tissue. The studies of Schindler *et al.* [3] demonstrated the conversion of androstenedione (A) to estrone (E) and suggested that fat from patients with endometrial carcinoma was more effective in this conversion than fat from control subjects. Bolt and Gobel[4] presented evidence for the conversion of dehydroepiandrosterone and testosterone (T) as well as A to E_1 and E_2 in homogenates of human adipose tissue. Schindler and Aymar[5] were unable to detect the conversion of dehydroepiandrosterone to E_1 and E_2. More extensive studies by Nimrod and Ryan[6] have compared the conversion of A and T to E_1 and E_2 in abdominal, breast and axillary fat. They found that breast and abdominal fat had similar aromatizing activity and axillary fat was more active in the conversion of T and A to estrogens.

A series of *in vivo* studies by Siiteri, MacDonald and co-workers have demonstrated the importance of peripheral conversion of A to E_1. Their studies have shown that the major circulating estrogen in the post menopausal female is E_1 and that this is formed primarily from peripheral aromatization of A [7]. They have also shown that the conversion of A increases with increasing degrees of adiposity [8] and with increasing age [9]. Longcope *et al.* [10] using a forearm perfusion technique demonstrated the interconversion of A and T and the aromatization of A and T in muscle and adipose tissue. The present studies were undertaken to compare androgen interconversion and aromatization in adipose tissue from different sites and in isolated adipocytes and adipocyte precursors.

MATERIALS AND METHODS

Chemicals; reference steroids were obtained from Ikapharm, Ramat-Gan, Israel and the Sigma Chemical Company, St. Louis, Missouri and were recrystallized prior to use. The radioactive steroids [7α-^{3}H]-A [S.A. 11.4 Ci/mmol]; [7α-^{3}H]-T [S.A. 11.4 Ci/mmol]; [1,2 ^{3}H]-A [S.A. 40 Ci/mmol]; [1,2 ^{3}H]-T [S.A. 40 Ci/mmol]; [1,2,6,7 ^{3}H]-A [S.A. 86.8 Ci/mmol]; [4 ^{14}C]-E_1 [S.A. 50 mCi/mmol]; [4 ^{14}C]-E_2 [S.A. 50 mCi/mmol]; [4 ^{14}C]-T [S.A. 50 mCi/mmol]; and [4 ^{14}C]-A [S.A. 50 mCi/mmol] were obtained from New England Nuclear Corporation, Boston, Massachusetts. The radioactive steroids were purified by paper chromatography prior to use. In order to get a desired substrate concentration, unlabelled steroid was added to the radioactive compound for each incubation.

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INCUBATION PROCEDURE

Adipose tissue obtained from surgery was washed in buffer, blotted dry, weighed and homogenized in cold Krebs–Ringer bicarbonate buffer (pH 7.4), using an all glass homogenizer. The amount of buffer used for homogenization varied depending on the texture of the adipose tissue. The homogenate was centrifuged at 4°C and 2000 rev./min for 20 min and the fat layer was discarded. The supernatant was filtered through glass wool and a 1 ml aliquot was taken for protein determination. The remaining supernatant was incubated with [^{3}H]-A or [^{3}H]-T in the presence of the cofactors ATP (5×10^{-3} M) and NADPH (2×10^{-3} M) at 37°C for 90 min in an atmosphere of 95% O_2 and 5% CO_2.

Isolated adipose cells

Isolated mature fat cells were prepared by collagenase digestion according to the method of Rodbell[12]. The number of free fat cells was determined using a Coulter counter. Each incubation with 1×10^6 fat cells and varying amounts of ^{3}H substrate was carried out in 5 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 0.9% glucose. Incubations proceeded for 90 min in a shaking water bath at 37°C under an atmosphere of 95% O_2 and 5% CO_2.

Adipose tissue stroma

The residue (stroma) from the adipose tissue remaining after the fat cell liberation was homogenized and incubated under the same conditions as described for the adipose tissue homogenate.

Human adipocyte precursors

Human adipocyte precursors were isolated by the method of Van *et al.* [13] and harvested after 3 weeks in culture. Incubations containing 1×10^6 cells in 5 ml buffer were performed as described for the isolated fat cells.

Homogenate of adipocyte precursors

1×10^6 human adipocyte precursors were homogenized in a glass homogenizer and incubated under the same conditions as described for the adipose tissue homogenate.

Isolation procedure

The incubations were stopped by the addition of five volumes of ethyl acetate. ^{14}C labelled E_1, E_2, T and A were added for recovery correction and 100 μg of each steroid was added to facilitate TLC detection. The reaction mixture was kept overnight at −4°C, extracted three times with ethyl acetate, dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in 5 ml methanol and a 0.1 ml aliquot was taken for counting.

The extract was subjected to phenolic partition as follows: the dried sample was dissolved in 5 ml of 1 N NaOH and the neutral steroids were extracted three times with 5 ml of benzene–hexane (1:1 V/V). The phenolic steroids were extracted from NaOH with three times 5 ml of ethyl acetate. The phenolic and neutral steroids were purified separately by thin layer chromatography in the system cyclohexane–ethyl acetate (1:1, V/V). After elution with methylene chloride–methanol (9:1, V/V), the eluates were dried and acetylated. The acetates were further chromatographed using the systems cyclohexane–ethyl acetate (1:1, V/V), hexane–ethyl acetate (1:1, V/V) and benzene–methanol (95:5, V/V).

Evidence for radiochemical homogeneity of the isolated products was established by recrystallization with authentic steroids to constant specific activity of crystals and mother liquors. When it had been demonstrated that purity was achieved by repeated thin layer chromatography to a constant ^{3}H:^{14}C ratio, the crystallization step was omitted. The total percent conversion was calculated from the fraction of ^{14}C steroid remaining after purification and multiplying this factor by the amount of ^{3}H isolated in each compound.

Protein concentration was determined in each incubation and the final results were expressed as ng of steroid formed per 100 mg of tissue protein [11]. In control incubations, boiled tissue was used.

Determination of radioactivity

Radioactivity was determined in a Philips liquid scintillation analyzer in vials containing 10 ml toluene with 4% Permafluor (Packard Instrument Co.) and 2% methanol. The efficiency of counting, determined by use of an external standard was approx. 25% for ^{3}H and 38% for ^{14}C when both isotopes were counted simultaneously.

RESULTS

The conversion of A to T, E_1 and E_2 in abdominal fat from a series of patients is shown (Table 1). The concentration of androstenedione used per 100 mg of protein in these studies varied from 12 to 506 ng. The protein concentration per gram of fat was extremely variable from patient to patient with no obvious relationship to the origin of the tissue. Testosterone was isolated from all incubations, E_1 was isolated from most incubations, and E_2 was isolated from three incubations in this series. The failure to recover E_1 and E_2 in some of these incubations was due in part to the low conversions and in part to the low recovery of E_1 and E_2 encountered in some of our earlier studies.

Table 2 shows the conversion of A to T, E_1 and E_2 in breast adipose tissue. The protein concentration per gram of fat was somewhat less variable in breast fat than in abdominal and omental fat, but there was still a wide variation from patient to patient. The formation of T, E_1 and E_2 did not show any consistent differences in tissue from patients with different breast disorders. The formation of T was roughly propor-

Table 1. Conversion of androstenedione to testosterone, estrone and estradiol by abdominal and omental adipose tissue

Source	Sex	Diagnosis	Wt. of A* (ng/100 mg protein)	g fat/ incub.	mg protein/ gm fat	ml/incub.	T (ng/100 mg protein)	E_1 (pg/100 mg protein)	E_2
Abdominal fat	Female	Adrenal carcinoma	74	8.5	10.1	20	1.0	—	—
	Female	Cholecystectomy	84	10	7.6	20	2.5	—	—
	Female	Hysterectomy	104	5	5.2	10	10.3	38.5	40
	Female	Cholecystectomy	114	3.5	16	12	4.3	46	6.8
	Female	Hysterectomy	442	10	5.5	16	3.1	88	—
	Male	Laparotomy	506	9	10.7	21	27	300	—
Omental fat	Male	Adrenal carcinoma	12	4	25	22	0.12	14	—
	Female	Cushing's Syndrome	84	5	3.4	13	2.94	25.3	2.4
	Female	Renal carcinoma	91	10	7.0	11.5	0.86	—	—
	Female	Renal carcinoma	105	6	6.1	18	3.8	50	—
	Female	Adrenal carcinoma	213	8	3.8	15	2.6	—	—
	Male	Cholecystectomy	245	5	6.5	6	9.4	119	—

* Exogenous Substrate.

Table 2. Conversion of androstenedione to testosterone, estrone and estradiol by breast adipose tissue

Diagnosis	Age	Wt. of A (ng/100 mg protein)	g fat/incub.	mg protein/gm fat	ml/incub.	T (ng/100 mg protein)	E_1 (pg/100 mg protein)	E_2
				Female				
Hypertrophy	49	83	7	10.3	16	3	33	10
	24	102	4.5	5.4	5.5	3.8	29	4
	24	118	4	5.3	4.5	3.4	19	5
	55	187	5	6.4	6.8	15	47	9
Carcinoma	73	50	1	10.7	16	3.5	140	15
	71	196	4.5	5.7	11	11.5	90	44
	76	311	5	6.4	8	14.7	37	—
	73	408	1	10.7	16	26	260	82
	64	602	5	3.3	5.5	41	84	—
				Male				
Gynecomastia	24	420	2.5	4.8	8.5	12.5	57	12
Carcinoma	47	541	2.5	7.4	4.5	24.5	108	22

Table 3. Conversion of testosterone to androstenedione by adipose tissue

Source	Sex	Diagnosis	Wt. of T* (ng/100 mg protein)	g fat/ incub.	mg protein/ gm fat	vol/incub.	A (ng/100 mg protein)
Omental fat	Male	Adrenal carcinoma	6.1	4	25	22	0.1
	Female	Renal carcinoma	63	10	11.5	11.5	0.8
	Female	Renal carcinoma	72	6	10.2	18	0.5
Abdominal fat	Male	Laparotomy	236	9	10.7	21	1.7
	Female	Adrenal carcinoma	51	8.5	10.1	20	0.02

* Exogenous Substrate.

Table 4. Interconversion of testosterone and androstenedione by isolated human adipocytes and residual stroma

Source	Substrate	Wt. of substrate (ng)	Number of cells	Total protein (mg)	% Conv.	Product (ng per 1×10^6 cells)	Product (ng/100 mg protein)
ABD fat* cells	A	243	1×10^6		0.57	1.38	
ABD fat cells	A	243	1×10^6		0.52	1.26	
ABD fat cells	A	267	1×10^6		0.34	0.9	
ABD fat cells	A	486	1×10^6		0.39	1.9	
ABD fat cells	T	227	1×10^6		0.33	0.75	
Stroma	A	243		12.4	2.68		52.5
Stroma	A	243		43	0.12		0.67
Stroma	A	486		83	3.3		19.3
	T	227		83	2.2		6.18

* Fat cells isolated from subcutaneous abdominal fat.

Table 5. Conversion of androstenedione to testosterone and estrone by human adipocyte precursors

	Source	Wt. of A (ng)	Number of cells	T (% conv.)	T (ng/100 mg protein)	E_1 (% conv.)	E_1 (pg/100 mg protein)
Cells	Human Adipocyte Precursors	11.5	1×10^6	0.5	0.05	0.022	2.5
Homogenate	Human Adipocyte Precursors	11.5	1×10^6	0.47	0.05	0.024	2.8

tional to the concentration of A substrate, while the formation of E_1 and E_2 was more variable from patient to patient.

The formation of A from T in omental and abdominal fat is shown in Table 3. Under these conditions, the formation of A from T was less than the formation of T from A at similar substrate concentrations. Table 4 shows the interconversion of T and A by isolated human adipocytes and stromal tissue. In each incubation the cells isolated by collagenase digestion were counted, and an estimated 1×10^6 cells were added to each incubation. The stromal tissue remaining after liberation of fat cells was also incubated to determine if the steroid interconversion was due to fat cells or stroma. Both cells and stroma were capable of the interconversion of A and T. A comparison of the activity of cells and stroma was not possible because the amounts of tissue incubated were not comparable.

Human adipocyte precursors isolated by Dr. D. A. Roncari and R. L. Van were subcultured until sufficient cells were available for incubation. These adipocyte precursors were derived from the stromal-vascular fraction of adult omental adipose tissue and have been shown to accumulate lipid in culture [13]. Incubation of 1×10^6 cells and a homogenate of a similar number of cells with A resulted in the formation of T with a percent conversion similar to that seen with mature fat cells. These cells were also shown to aromatize A to E_1 (Table 5).

DISCUSSION

Previous *in vitro* studies on the metabolism of T and A in adipose tissue have focused on the aromatization of these compounds to estrogens. The results of the present study emphasize the potential for the formation of both androgens and estrogens in adipose tissue suggesting that the net effect of a substrate such as A on a target tissue will depend on the ratio of products formed.

In these incubations, A was converted to E_1 in subcutaneous, omental, and breast fat and E_2 was consistently isolated from incubations with breast fat and in some incubations with subcutaneous and omental fat. The interconversion of T and A was demonstrated in adipose tissue from all sources and the formation of T from A was greater than the formation of A from T at similar substrate concentrations. In other incubations, we have demonstrated the conversion of A to T, E_1 and E_2 in muscle tissue (unpublished data). These *in vitro* studies are in agreement with the *in vivo* studies of Longcope *et al.* [10] using a forearm perfusion technique. These authors demonstrated that adipose tissue as well as muscle was active in the interconversion of A and T and that A and T could be aromatized to estrogens. They also found that the conversion of A to T was greater than the conversion of T to A in their system.

Schweikert and Wilson[15] have shown that fibroblasts in culture can aromatize T to E_2. Since adipose

tissue includes mature adipocytes and a fibrovascular stroma, incubation studies with both of these fractions were carried out. Both fractions were found to be capable of the interconversion of T and A. Since the adipocyte precursors are derived from the stromal fraction no conclusions can be drawn regarding the type of cell responsible for the androgen interconversion in the stromal fraction.

Using the adipocyte precursors a comparison was made between an incubation of whole cells and a homogenate of a similar number of cells. It was felt that the large amount of lipid present in intact cells could sequester some of the steroid entering the cell and prevent access to the enzymes which metabolize the steroids. The data in Table 5 reveals that there was no difference in these two incubations both with respect to T and E_1 formation.

Roncari and Van[16] have demonstrated that estrogens stimulate the growth of adipocyte precursors in culture. This observation along with results in the present study indicating that adipocyte precursors can form estrogen locally from circulating androgens suggests a possible mechanism for the effects of gonadal steroids on the formation of adipose tissue and their effect on body habitus.

These studies emphasize that the local formation of androgens and estrogens may be an important factor in determining the effect of gonadal steroids on target tissue. This local hormone formation may not be reflected in the measurement of circulating hormone levels, but the total effect of gonadal steroids on these tissues may be a function of both the circulating hormone levels and local hormone formation and interconversion.

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