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ANDROGEN METABOLISM IN ADIPOSE TISSUE: CONVERSION OF 5α-DIHYDROTESTOSTERONE TO 3α-ANDROSTANEDIOL BY HAMSTER TISSUE

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SUMMARY

Metabolism of androgens was investigated in tissue piece preparations of adipose tissue from several sites of the golden Syrian hamster. Androgens (testosterone, 5α-dihydrotestosterone and 5α-androstane-3α,17β-diol) were [1,2-^{3}H] labeled and used at 5×10^{-8} M. Virtually no 5α-reduction or 17β-hydroxy dehydrogenation of testosterone occurred. The predominant reaction was formation of 5α-androstane-3α,17β-diol (3α-androstanediol) from 5α-DHT. The reverse reaction occurred to a much lesser (1/30) extent. Activity of the 3α-hydroxysteroid oxidoreductase of adipose tissue (5α-DHT → 3α-ADIOL) per μg of DNA was between those of striated muscle and prostate. The enzymatic profile of adipose tissue (low 5α-reductase, high 3α-hydroxysteroid oxidoreductase) is similar to that previously reported for striated muscle and kidney, two androgen-responsive, non-reproductive organs. It is suggested that such organs and tissues may be disposed to prevent access of 5α-DHT to cellular receptors while permitting access of other androgens, e.g. testosterone. The enzymatic data are regarded as adding to the evidence that adipose tissue is directly androgen-responsive.

INTRODUCTION

Metabolism and interconversion of sex steroids by adipose tissue are of interest from several standpoints. One concerns the mechanism of the normal sex difference in anatomical distribution of fat [1], another the endocrinopathies associated with extreme obesity [2] and yet another, the increase in adiposity associated with ageing [3]. Thus metabolic modification of androgens and estrogens by adipose tissue could affect this tissue itself as a possible endocrine-responsive organ, or could affect levels of endocrine-active steroids in the general circulation, and hence the responses of remote tissues.

Human adipose tissue can effect steroid A-ring aromatization, e.g. conversion of androstenedione and testosterone to estrone and estradiol, respectively [4, 5, 6] and 17β-hydroxysteroid-17-oxidoreductase has been demonstrated in adipose tissue of man [2, 8, 9].

So far as we are aware, no reports exist on the presence or absence in adipose tissue of the enzymes currently believed [10, 11] to be involved in target tissue activation of circulating androgens, i.e. 4-ene-3-ketosteroid 5α-oxidoreductase (5α-reductase) and the 3α- and 3β-hydroxysteroid oxidoreductases. We now report the presence of 3α-hydroxysteroid oxidoreductase in hamster adipose tissue; corresponding evidence for the existence of 5α-reductase and 3β-ketosteroid oxidoreductase was not obtained.

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MATERIALS AND METHODS

Animals. Male Golden Syrian hamsters of the ELA-Engle strain, weighing 110–120 gm were obtained from Engle Laboratory Animals, Inc., Farmersburg, Indiana.

Labeled substrates. [1,2-^{3}H]-testosterone, [1,2-^{3}H]-5α-dihydrotestosterone and [1,2-^{3}H]-5α-androstane-3α-17β-diol, all of specific activity 40 Ci/mmol, were obtained from New England Nuclear Corp. and further purified by chromatography in t.l.c. System A (below).

Unlabeled steroids. Testosterone and 5α-dihydrotestosterone (5α-DHT) were obtained from Schwarz/Mann, Orangeburg, NY; 5α-androstane-3α, 17β-diol (3α-androstanediol), 5α-androstane-3β,17β-diol (3β-androstanediol) and androst-4-ene-3,17-dione from Sigma Chemical Co., St. Louis, MO.; 5α-androstane-3,17-dione from G. D. Searle & Co., Chicago, IL. All were reasonably pure by t.l.c. in System A (below) and were used without further purification.

Tissue preparation and incubation. Animals were sacrificed by cervical dislocation. Adipose tissue was removed as quickly as possible and extraneous tissue and visible blood vessels trimmed away under a dissecting microscope. In the case of epididymal fat pad, samples were cut transversely through the greatest width of the flattened tissue. Adjacent tissue was used for DNA analysis, when desired.

In the experiments with adipose tissue from different sites (Tables 1 and 2) weights of samples varied between 4.8 and 14.6 mg. In the experiment exploring

reversibility of the 3α-hydroxysteroid oxidoreductase reaction (Table 4) samples weighed 32–37 mg, and in the experiment comparing adipose tissue to muscle and prostate, adipose samples weighed 25–29 mg. Tissue was rinsed with saline and used prompty and was never cooled below room temperature (25°C). Diaphragms were trimmed free of connective tissue; one hemi-diaphragm was cut into pieces for incubation (approx. 10 mg tissue) and the other used for DNA analysis. Prostates were dissected out, minced, and 8–9 mg taken for incubation; remainder was used for DNA determination.

Incubations were in Krebs-Ringer phosphate, pH 7.4, containing 5 mM glucose, at 37°C in a Dubnoff shaking incubator, in capped tubes flushed with 95% O_2–5% CO_2. Total vol. per tube was 0.5 ml. Labeled steroid substrates were added in 10 μl ethanol–buffer (1:1) to a final concentration of 5×10^{-8} M, 1–2 μCi per tube, at zero time, and the incubations continued for 60 minutes. Blank tubes containing substrate, but no tissue, were carried through the entire procedure in all experiments.

Extraction and measurement of metabolites. The reaction was stopped by addition of 20 volumes of chloroform–methanol (2:1, v/v) to each tube. 0.5 mg each of the following carrier steroids were added in a single 0.5 ml $CHCl_3$:MeOH: 5α-androstane-3α,17β-diol (3α-androstanediol), testosterone, 5α-dihydrotestosterone, androst-4-ene-3,17-dione, 5α-androstane-3,17-dione. The tube contents were transferred to a Dounce homogenizer and the tissue homogenized in the solvent. The extract was filtered and an aliquot washed with water, followed by "solvent upper phase" [12]. The chloroform layer was evaporated under N_2, the residue dissolved in 100 μl of chloroform, and an aliquot streaked on a silica gel G t.l.c. sheet (Polygram®, Machery-Nagel Co., 0.25 mm thickness, without $CaSO_4$ binder). Sheets were developed twice in $CHCl_3$–MeOH (147:2.8, v/v) (System A) and air-dried. Spots corresponding to carrier steroids were located with I_2 vapor, marked, cut out with scissors and added to scintillation vials containing scintillation fluid (100 ml Biosolve BBS-3®, Beckman Instruments, Palo Alto, CA., 4 gm PPO and 50 mg POPOP per liter of toluene). Tritium activity was counted in a Beckman LS-330 liquid scintillation spectrometer. Recoveries of DHT and androstanediol through the entire procedure were 91 and 94%, respectively.

Identification of 3α-androstanediol as a metabolite. t.l.c. System A (above) does not separate 3α- and 3β-androstanediols, so the following t.l.c. system (System B) was used for that purpose [13]. Alumina t.l.c. sheets (aluminum Oxide F_{254} neutral, Type E plastic sheets, E. Merck, Darmstadt) were activated at 100° for 2 h, then stored in a vacuum desiccator briefly before use. Developing solvent was benzene–ethanol (96:4, v/v). Double development completely separated the 3α- and 3β-diols, which, along with 5α-dihydrotestosterone (5α-DHT) were used as carriers. As described under Results, the androstanediol radioactivity was associated almost completely with the 3α-isomer. In additional chromatograms, the 3α-androstanediol spots were scraped off the plastic backing and eluted with $CHCl_3$–MeOH (2:1) 40 mg of 3α-androstanediol was added to the residue from this extract, and the whole recrystallized to constant specific activity successively from methanol and acetone–water.

Determination of DNA. Tissues, which had been stored frozen, were homogenized with over 20 vols of cold acetone. Fat pads and prostates were homogenized with a Potter–Elvehjem type tissue grinder; diaphragms were ground with a Tekmar Tissuemizer. After centrifugation and washing once with acetone, the dried powders were analyzed for DNA by the method of Burton[14] using calf thymus DNA (Type I, Sigma Chemical, St. Louis, MO) as a standard.

RESULTS

Metabolism of testosterone by adipose tissue

Results of incubation of [1,2-^{3}H]-testosterone with adipose tissue from various anatomical locations are presented in Table 1. Conversion to 5α-reduced metabolites was uniformly low, consistent with the low

Table 1. Metabolism of [1, 2-^{3}H]-testosterone by adipose tissue from various sites

Anatomical site	Sample No.	Conversion to: 5α-Reduced Metabolites (ADIOL + DHT) (%)	(pmol/gm)	Androstenedione (%)	(pmol/gm)
Subcutaneous	1	0.125	2.14	0.094	1.61
(below flank organ)	2	0.150	2.98	0.145	2.88
Subcutaneous	1	0.189	4.63	0.110	2.70
(cervical)	2	0.004	0.09	0.087	1.94
Epididymal	1	0.119	2.04	0.043	0.73
	2	0.197	3.52	0.072	1.28
Perirenal	1	0.055	1.05	0.005	0.096
	2	0.210	4.24	0	0

ADIOL = androstanediol, DHT = 5α-dihydrotestosterone.

Table 2. Metabolism of [1, 2-^{3}H]-DHT by adipose tissue from various sites

Anatomical site	Sample No.	Conversion to: Androstanediol (% of Added DHT)	Androstanediol (pmol per gm tissue)	3,17-Androstanedione (%)	3,17-Androstanedione (pmol/gm)
Subcutaneous (under flank organ)	1	0.856	44.6	0.04	2.1
	2	0.897	33.0	0	0
Subcutaneous (cervical)	1	1.08	56.3	0.023	1.2
	2	1.01	43.7	0.025	1.1
Epididymal	1	4.56	154	0.056	1.9
	2	4.03	96.9	0.035	0.8
Perirenal	1	2.99	98.2	0.084	2.8
	2	2.61	54.4	0.036	0.8

levels of 5α-reductase in tissues which are not generally identified as androgen target organs. Conversion of testosterone to androstenedione was no greater, although the presence of 17β-dehydrogenase in adipose tissue of man has been reported [2, 8, 9]. Other areas of the t.l.c. sheets contained less radioactivity than those reported in Table 1.

Metabolism of 5α-dihydrotestosterone

Corresponding data for conversion of DHT are presented in Table 2. In this case a major metabolite appeared in the thin-layer chromatographic area corresponding to the androstanediols. Again, 17β-dehydrogenase activity was low on the basis of radioactivity recovered in the androstanedione area, and other metabolites were not evident.

Androstanediol formation occurred in adipose samples from all anatomical sites examined. Possible variation between sites was not explored further, and subsequent incubations were carried out with epididymal fat pad tissue. Perfusion of fat pads with saline to remove blood did not reduce androstanediol formation.

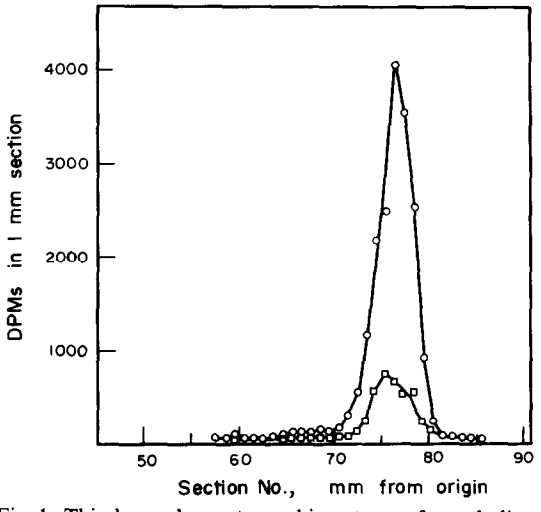

Fig. 1. Thin layer chromatographic pattern of metabolites derived from [1,2-^{3}H]-DHT in the androstanediol area, t.l.c. System B. Carrier 3β-androstanediol occurred in sections 60–69; 3α-androstanediol in sections 71–82; solvent front at section 150. Circles, epididymal fat; squares, subcutaneous fat.

Identity of DHT metabolite

Unused portions of the chloroform–methanol extracts from the previous experiment (Table 2) were used for chromatography in t.l.c. system B, which separates the isomeric 3α- and 3β-androstanediols [13]. Areas corresponding to these carrier steroids were marked, and the area encompassing both cut out and cut into 1 mm strips with scissors. Strips were added directly to scintillation vials and counted. Results are shown in Fig. 1. For both epididymal and subcutaneous fat, radioactivity fell almost entirely within the 3α-androstanediol area.

The 3α-androstanediol area from a second epididymal fat sample was cut out and eluted with chloroform–methanol (2:1, v/v). The evaporated eluate was dissolved in methanol, 40 mg of 5α-androstane-3α, 17β-diol was added and recrystallized. A second recrystallization was carried out from acetone–water. Specific activities of the two sets of crystals, as well as the calculated specific activity after addition of carrier, are presented in Table 3. Results indicate that greater than 90% activity isolated as the major DHT metabolite is 3α-androstanediol.

Reversibility of 3α-androstanediol formation

The question of whether adipose tissue can catalyze the reverse reaction, i.e. conversion of 3α-androstanediol to DHT, was explored by comparing conversions of each to the other under identical conditions of incubation (see methods). Formation of 5α-androstane-3,17-dione from each substrate was

Table 3. Identity of 3α-androstanediol by recrystallization

Solvent	S.A. of Crystals (d.p.m./mg)
	467 (initial, calc.)
Methanol	436
Acetone–H_2O	432

Table 4. Metabolism of 1,2-[^{3}H]-DHT and 1,2-[^{3}H]-3α-androstanediol by hamster epididymal adipose tissue

Substrate	Sample No.	Conversion to: 3α-Androstanediol (%)	3α-Androstanediol (pmol/gm)	5α-DHT (%)	5α-DHT (pmol/gm)
DHT	5	10.63 ± 1.44†	78.4 ± 8.8		
Androstanediol	5			0.35 ± 0.08	2.6 ± 0.6

† Values given as means ± S.E.

also measured. Results are presented in Table 4. Conversion of 3α-androstanediol to DHT was only 3.3% of that in the reverse direction. Androstanedione formation was too low to be measured from either substrate. In another experiment, uptakes of 3α-androstanediol, DHT and testosterone by adipose tissue were shown to be very similar (data not shown).

Comparison to other tissues

The enzyme which catalyzes conversion of DHT to 3α-androstanediol, 3α-hydroxysteroid oxidoreductase, has been studied chiefly in prostate [13, 15, 16], liver [17, 18] and kidney [19, 20]. Prostate is a typical androgen target organ, while liver and kidney are organs having detoxication and excretion functions, although they are also capable of responding to hormonal stimulation. Recently, Massa and Martini [21] have shown that organs such as pancreas and skeletal muscle, which do not fit the classical concept of androgen target organ, and which do not have obvious deactivation functions, have high levels of 3α-hydroxysteroid oxidoreductase activity. It seemed desirable, therefore, to compare the activity of adipose tissue to those of skeletal muscle and prostate. As before, single pieces of epididymal fat pad were used; prostate tissue was minced with scissors, and hemidiaphragms were cut into pieces. Conversions of DHT to 3α-androstanediol by these preparations are shown in Table 5. Because of the great differences between these tissues in number of cells per unit weight, DNA determinations were carried out, and the data expressed in the last column as activity per μg of DNA. On this basis the order of activity is: Muscle > adipose > prostate, with all 3 being within an order of magnitude. On a unit weight basis the order becomes muscle > prostate > adipose.

DISCUSSION

Since the original reports of Bruchovsky and Wilson[22], and Anderson and Liao[23], it has become generally accepted [10, 11] that androgen target organs convert the circulating androgen, testosterone, to 5α-dihydrotestosterone (DHT), which is firmly bound in the cell nucleus, and which in receptor-bound form, constitutes the androgenic stimulus to these organs. The T-to-DHT conversion requires the enzyme, 4-ene-3-ketosteroid 5α-reductase (5α-reductase), found in target organs, which also contain enzymes (3α- and 3β-hydroxysteroid oxidoreductases) which convert DHT to 3α- and β-androstanediols, respectively. In rat prostate, the 3α-enzyme predominates [13].

The physiological significance of the formation of androstanediols from DHT remains unclear, and is difficult to discover due to the ability of target organs to reverse the reaction to produce the known effective androgen, DHT, under most experimental conditions. There are several reports [24–26], however, of greater androgenic potency of 3α-androstanediol than of DHT, as well as a report of a qualitative difference in ability to stimulate prostate growth in the dog [27], 3α-androstanediol being the effective androgen. There is thus a definite possibility that androstanediols may mediate some aspects of androgen action even in target tissues, even though they are not bound to nuclear receptors which bind DHT in these tissues.

Several organs and tissues, not usually classified as androgen targets, nevertheless exhibit unmistakable responses to androgen administration. Among such responsive entities are skeletal muscle, kidney and bone marrow.

In these tissues testosterone is bound preferentially over DHT [29, 30], and Massa and Martini have

Table 5. Conversion of 1,2-[^{3}H]-DHT to 3α-androstanediol by epididymal adipose tissue, diaphragm muscle and prostate

Tissue	Conversion (%)	Conversion (pmol/gm tissue)	Conversion (pmol/μg DNA)
Adipose (6)	7.90 ± 0.81	71.4 ± 7.1	0.518 ± 0.050
Muscle (6)	21.8 ± 1.6	552 ± 22	0.961 ± 0.076
Prostate (6)	8.26 ± 1.45	233 ± 42	0.161 ± 0.045 (5)

Numbers in parentheses indicate number of tissue samples. Means ± S.E.

reported [21] that the enzymatic profile of several such tissues, including skeletal muscle and kidney, is one of low 5α-reductase activity and high 3α-hydroxysteroid oxidoreductase activity. Such an enzymatic posture can be considered defensive against accumulation of DHT, but permissive of testosterone accumulation.

The present work shows that hamster adipose tissue exhibits the same enzymatic profile as these non-reproductive androgen-responsive tissues. In addition, it was shown that conversion of 3α-androstanediol to DHT is much lower than the reverse reaction in this tissue, which we construe as additional evidence for an integrated enzymatic defense against DHT accumulation.

If the enzymatic profile of adipose tissue is the same as that of somatic androgen-responsive tissues, is there other evidence that adipose tissue itself is androgen-responsive? It is, of course, an ancient observation that castration of men and animals increases adiposity and changes the anatomical distribution of fat. Vague and co-workers [1] have documented the differences between males and females in adipose tissue distribution, and Vague *et al.*[28] have reported that chronic testosterone administration to eunuchoid men decreased total adipose tissue mass and caused reciprocal changes in the number and volume of adipose cells in the deltoid and trochanter areas. It has been reported recently [31] that administration of dehydroepiandrosterone to an obese strain of mouse prevented development of obesity without a corresponding decrease of food consumption. Finally, Esanu *et al.*[7] have reported that testosterone, at a concentration of 2×10^{-6} M, inhibits incorporation of thymidine into DNA of adipose tissue *in vitro*, a result consistent with an inhibitory effect on adipose tissue growth.

All together, this evidence, combined with the enzymatic profile, suggests that adipose tissue belongs in the category of androgen-responsive tissues outside the reproductive system. In contrast to the male accessory sex organs, such tissues seem to be enzymatically disposed toward prevention of accumulation of the potent androgen, DHT, and toward accumulation of testosterone and possibly 3α-androstanediol. Obviously, much additional work, including demonstration of specific androgen receptors, will be required before an androgen-responsive status of adipose tissue can be regarded as established.

Much of the literature on androgen-adipose tissue relationships is concerned with the activity of steroid 17β-dehydrogenase in this tissue [2, 8, 9]. To our surprise activity of this enzyme, with either testosterone or DHT as substrate, was very low in adipose tissue of the hamster, at least relative to 3α-oxidoreductase activity.

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