The Metabolic Clearance Rate and Origin of Plasma Dihydrotestosterone in Man and Its Conversion to the 5α-Androstanediols

JACQUES A. MAHOUDEAU, C. WAYNE BARDIN, and MORTIMER B. LIPSETT

From the National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT Dihydrotestosterone metabolism was studied with a constant infusion technique in three men, three women, five hirsute women, and four estrogen-treated hirsute women. The mean dihydrotestosterone metabolic clearance rate was higher in men (336 liters/24 hr per m^2 [range, 239–448]) than in women (153 liters/24 hr per m^2 [range, 108–184]). The metabolic clearance rates in hirsute patients were intermediate between those men and women and were decreased by estrogen treatment. These observations demonstrate similarities in the metabolic rates of testosterone and dihydrotestosterone.

The conversion of plasma testosterone and androstenedione to dihydrotestosterone was studied in men and hirsute women. Approximately 4 and 2% of plasma testosterone and androstenedione, respectively, were converted to plasma dihydrotestosterone in both groups. From these observations it was determined that a major fraction of plasma dihydrotestosterone was derived from these plasma precursors rather than from glandular secretion.

Both 5α-androstan-3α,17β-diol (3α-diol) and 5α-androstan-3β,17β-diol (3β-diol) were identified in plasma during dihydrotestosterone and testosterone infusions. The conversion ratio of dihydrotestosterone to 3α-diol ($C_{BB}^{DHT-3\alpha}$) was greater than the conversion ratio to the 3β-isomer ($C_{BB}^{DTH-3\beta}$) in all the patients studied. Both $C_{BB}^{DHT-3\alpha}$ and $C_{BB}^{DHT-3\beta}$ were higher in men (mean values of 0.151 [range, 0.110–0.222] and 0..031 [range, 0.022–0.042]) than in women (means of 0.044 [range, 0.037–0.048] and 0.012 [range 0.010–0.013]). A smaller fraction of testosterone was converted to 3α-diol and 3β-diol.

INTRODUCTION

Interest in dihydrotestosterone (1) has been stimulated by recent suggestions that his potent androgen is an intracellular effector of testosterone action (1, 2). Testosterone and androstenedione are metabolized to dihydrotestosterone (DHT) [1] in many tissues (3–7). The entry of this steroid into the plasma from these sources would contribute to total blood androgens. It was pertinent, therefore, to examine the conversion of plasma testosterone and androstenedione to plasma dihydrotestosterone in order to estimate the importance of secretion of DHT as compared with its peripheral production.

Patients. The following subjects were studied: three normal men and one normal woman (subjects 1–4, age 21–23). Patient 5 (age 26) had been successfully treated for choriocarcinoma 12 months previously, and patient 6 (age 23) was receiving replacement doses of cortisol and 9α-fluorohydrocortisone after bilateral adrenalectomy for adrenal hyperplasia. These three women had regular menses and no hirsutism. Five women with hirsutism and oligomenorrhea were studied: Patients 7–9 (age 25–28) had "idiopathic hirsutism" and patients 10 and 11 (age 24 and 28), had polycystic ovaries. Patient 11 was studied both before and after ovarian

Dr. Mahoudeau's present address is 91, Rue Jouffroy, 91, Paris (XVII[E]), France.

Dr. Bardin's present address is the Department of Medicine, The Milton S. Hershey Medical Center, The Pennsylvania State University, College of Medicine, Hershey, Pa. 17033.

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[1] *Abbreviations used in this paper:* A, androst-4-ene-3,17-dione; C, conversion ratios; 3α-diol, 5α-androstan-3α,17β-diol; 3β-diol, 5α-androstan-3β,17β-diol; 5α-diols, 5α-androstan-3α,17β-diol and 5α-androstan-3β,17β-diol; DHT, dihydrotestosterone (17β-hydroxy-5α-androstan-3-one); MCR, metabolic clearance rate; MCR^T, testosterone metabolic clearance rate; ρ, transfer rates; TeBG, testosterone-estradiol binding globulin; TLC, thin-layer chromatography.

wedge resection. Four hirsute patients were studied during estrogen administration: patients 7, 8, and 13 (age 30, 14 months posttreatment for choriocarcinoma) had been treated with estrogen and a gestagen cyclically for 6–14 months and each received 50 μg of ethinyl estradiol for 7–10 days before the study. Patient 12, a woman with polycystic ovaries, had received ethinyl estradiol, 150 μg/day for 4 yr, and medroxyprogesterone, 20 mg daily for 5 days every 2 months.

METHODS

Solvents. All solvents were glass distilled.

Chromatography. Thin-layer chromatography (TLC) was performed on 20 × 20 cm glass plates coated with Silica Gel GF-250 by Brinkmann Instruments Inc., Westbury, N. Y., and by Analtech, Inc., Wilmington, Del. Paper chromatography was performed on Whatman No. 1 paper in sytem Bush A_2 (ligroine: methanol: water, 10:7:3).

Reactions. Acetylation and saponification were accomplished as previously described (8). O-methyloximes were made according to Fales and Luukkainen (9). Steroid reductions were performed with freshly prepared 2% potassium borohydride in water: 20 sec were required to convert androstenedione to testosterone, and 60 sec to reduce dihydrotestosterone-17β-acetate to 3β-hydroxy-5α-androstan-17β-acetate, in over 90% yield.

Radioactive steroids. The following radioactive steroids were purchased from New England Nuclear Corp., Boston, Mass. and were repurified by TLC: testosterone-1,2-^{3}H (57 Ci/mmole), dihydrotestosterone-1,2-^{3}H (50 Ci/mmole), androstenedione-1,2-^{3}H (50 Ci/mmole), testosterone-4-^{14}C (50 mCi/mmole), and androstenedione-4-^{14}C (50 mCi/mmole).

Other ^{14}C steroids were synthesized by incubating 300 mg of minced rat preputial glands with 5 μCi of testosterone-4-^{14}C in 5 ml of Dulbecco's buffer containing 6.0 mg glucose, 5 U glucose-6-phosphate dehydrogenase, 24 μmoles glucose-6-phosphate, and 6.2 μmoles NADP (triphosphopyridine nucleotide) under 0_2: CO_2 (95:5) at 37°C for 5 hr. The content of the incubation flask was homogenized and extracted with 8 volumes of methylene chloride. The extract was dried, spotted on thin-layer plate, and developed in system I (see Table I). The areas corresponding to DHT and the 5α-diols (5α-androstan-3α,17β- and 3β,17β-diol) were eluted and the steroids acetylated and chromatographed in system III. After saponification, TLC was performed in system II. The 3α- and 3β-diols were then separated by paper chromatography in system IV. The radiochemical purity of the isolated DHT and 5α-diols was established by crystallizing a portion of each sample with authentic standard.

Infusion of labeled steroids. Tritium-labeled DHT, testosterone, and androstenedione were infused as described previously (8). A priming dose of 7 μCi was given intravenously and 30 min later 15–25 μCi was infused for 150 min. Each infusion was monitored to assure a constant rate. 40 ml-heparinized blood samples were obtained at 90, 120, and 150 min of the infusion. Plasma was separated from red cells and stored at −16°C. When possible, the same patient was also infused with testosterone or androstenedione.

Plasma samples. Before extraction, 100 μg of the steroids to be isolated was added to each sample, except for the 5α-diols of which only 20 μg was added. Approximately 500 cpm of the infused steroid-^{14}C and 100–150 cpm of the product steroid-^{14}C were also added to correct for procedural losses. After addition of 1.0 ml 1 N NaOH, plasma samples were extracted twice with 5 volumes ether. The extracts were washed, dried, and processed according to Fig. 1.

Radioactivity was measured in portions of each sample as indicated (Fig. 1) in a Packard Tri-Carb liquid scintillation spectrometer, model 4322 (Packard Instrument Co., Downers Groves, Ill.) with efficiencies of 25 and 54% for ^{3}H and ^{14}C, respectively; 19% of ^{14}C was counted in the tritium channel and less than 0.04% of ^{3}H was counted in the ^{14}C channel. Sufficient counts were accumulated to give counting errors of less than 5%.

The metabolic clearance rates (MCR), conversion ratios (C), and transfer constants ([ρ]) were calculated as previously described (10). In all studies of MCR's the ^{3}H/^{14}C ratios of the last two derivatives were equal. No systematic deviation from equilibrium conditions was noted in 31 of 34 studies. In three studies, there was a significant trend, the third point being 15, 25, and 30% higher than the first. These studies have been included using the mean level.

The ^{3}H/^{14}C ratios of the conversion products were measured in two successive derivatives. In 56% of the cases they were the same. In 15%, too few counts were present in one derivative. In the remainder, the ^{3}H/^{14}C ratios differed by 10–30% and the ^{3}H/^{14}C ratio of the final derivative was used.

Body surface area was obtained according to Du Bois and Du Bois (11).

RESULTS

Dihydrotestosterone. For consistency, all MCRs here and in the discussion will be given as liter/24 hr per m^2. The mean MCR^{DHT} was 336 in the three normal men (Table II); the mean MCR^{T} measured in these three subjects was 545 (Table III). The mean MCR^{DHT} in the normal women was lower, 153; but we were unable to compare this with the MCR^{T}. MCR^{DHT} of 277 (mean) of the hirsute women was between the means for men and normal women, whereas estrogen treatmena reduced the mean MCR^{DHT} to 125, a value below that of normal women. There was no overlap between the MCR^{DHT} values of hirsute women and hirsute women treated with estrogen. It should be appreciated that hirsute women do not represent a homogeneous population and that variations in MCR and conversion ratios (Table II) are not unexpected. The MCR^{DHT} in patient 11 decreased 33% after ovarian wedge resection and MCR^{T} decreased by 27%.

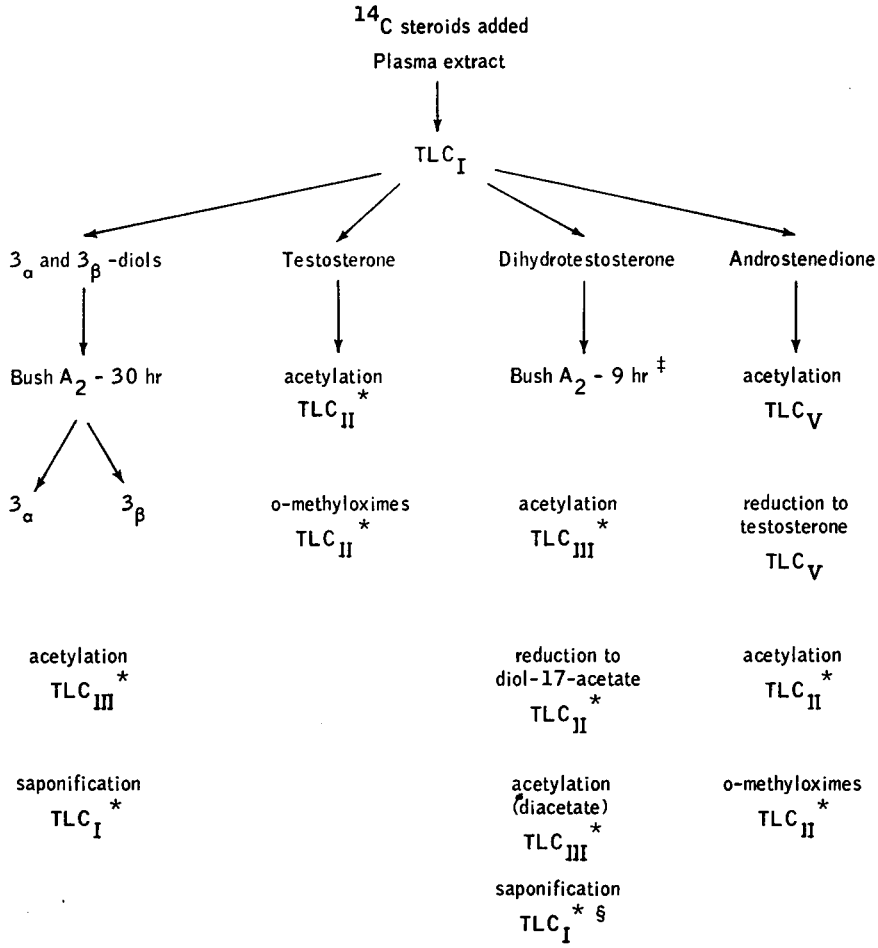
TABLE I
Chromatography Systems

System	Support	Solvents
I	Silica gel	Chloroform: methanol (98:2)
II	Silica gel	Benzene: ethyl acetate (8:2)
III	Silica gel	Benzene: ethyl acetate (9:1)
IV	Paper	Ligroine: methanol:water (10:7:3)
V	Silica gel	Benzene: ethyl acetate (6:4)

The conversion ratio of DHT to 5α-androstan-3α,17β-diol ($C_{BB}^{DHT-3\alpha}$) was greater than the conversion ratio to the 3β-isomer ($C_{BB}^{DHT-3\beta}$) in all the patients studied (Table II). Both $C_{BB}^{DHT-3\alpha}$ and $C_{BB}^{DHT-3\beta}$ were higher in men and virilized women than in normal women and hirsute women given estrogens.

Testosterone infusions. The testosterone metabolic clearance rate (MCR^T) and the conversion of testosterone to DHT and the 5α-diols are given in Table III. The MCR^T values in men and hirsute women are in our previously reported range for these groups (8). Only two studies of conversion of testosterone to DHT in men were obtained. The C_{BB}^{T-DHT} for normal men and hirsute women was similar, about 4% of plasma testosterone being converted to plasma DHT. An even smaller fraction of testosterone was converted to the 5α-diols. Because of the few data, these estimates have wide confidence limits.

Androstenedione infusions. The androstenedione metabolic clearance rates, conversion ratios, and transfer constants are summarized in Table IV. The MCR^A, C_{BB}^{A-T}, and $[\rho]_{BB}^{A-T}$ were similar to those previously



* Fraction counted for $^3H/^{14}C$

‡ This paper chromatography was necessary in the Testosterone and Androstenedione infusions to separate Androsterone from Dihydrotestosterone, but in the Dihydrotestosterone infusions, the separation by the two following steps was sufficient and the paper step was omitted.

§ The $^3H/^{14}C$ ratios as Dihydrotestosterone were counted at the first 2* in the Testosterone and Androstenedione infusions, at the 2 last * in the Dihydrotestosterone infusions.

FIGURE 1 Flow chart for purification of plasma steroids.

TABLE II

^{3}H-Dihydrotestosterone Infusions

Subjects	^{3}H-DHT		MCR^{DHT}		Conversion ratios*	
	Infusion rate	Plasma level			DHT-3α-diol	DHT-3β-diol
	dpm/hr × 10^7	*dpm/liter* × 10^5	*Liter/24 hr*	*Liter/24 hr per m²*		
Normal men						
1	1.057	5.608	452	239	0.110	0.022
2	2.571	9.978	618	322	0.128	0.031
3	2.025	7.789	832	448	0.222	0.042
Mean			634	336	0.153	0.031
±SD			±190	±105	±0.059	±0.010
±SE			±109	± 60	±0.033	±0.005
Normal women						
4	2.740	21.183	310	184	0.048	0.013
5	1.267	16.988	179	108	0.047	0.010
6	3.163	31.320	242	168	0.037	0.013
Mean			243	153	0.044	0.012
±SD			±65	±40	±0.005	±0.002
±SE			±37	±23	±0.003	±0.001
Hirsute women						
7	0.217	0.630	827	411	0.244	0.032
8	2.913	23.578	296	148	0.029	0.009
9	2.490	23.357	255	157	0.043	0.014
10	2.543	12.240	498	278	0.045	0.018
11	1.250	5.613	712	394		
Mean			517	277	0.090	0.018
±SD			±250	±125	±0.102	±0.009
±SE			±112	± 55	±0.051	±0.005
Estrogen-treated women						
7	2.266	22.968	236	110	0.034	0.009
8	2.761	28.440	233	116	0.019	0.005
12	2.501	19.535	307	141	0.037	0.007
13	2.988	27.112	264	134	0.046	0.012
Mean			260	125	0.034	0.008
±SD			±34	±14	±0.011	±0.002
±SE			±17	± 7	±0.005	+0.001

* The average counting rates of tritium in the final derviatives, less background and ^{14}C run over, were: 3α-diol, 105 cpm; 3β-diol, 35 cpm.

reported from this and other laboratories (8, 10). Approximately 2% of androstenedione was converted to DHT in both men and hirsute women. In one nonhirsute woman $[\rho]_{BB}^{A\text{-}DHT}$ was also 2%. In each case $[\rho]_{BB}^{A\text{-}T}$ was greater than $[\rho]_{BB}^{A\text{-}DHT}$.

DISCUSSION

Dihydrotestosterone, MCR, and sources. In several bio-assay systems, DHT is a more potent androgen than testosterone (12, 13). Both androgens have high binding affinities for the same plasma transport protein, testosterone-estradiol binding globulin (TeBG) (14). Further similarities in the metabolism of these two steroids have been demonstrated in these studies.

The MCR^{DHT} measured by us was the same as that reported by some authors (15), but lower than that given by Saez, Morera, and Bertrand (16). In view of the higher affinity of DHT, than of testosterone, for TeBG (14), it is surprising that the latter authors (16) found MCR^{DHT} equal to MCR^{T}. The MCR^{DHT} is higher in hirsute women than in normal women and is decreased by estrogens. The comparison with testosterone is also valid here; MCR^{T} was higher in hirsute women than in normal women (8), and was decreased by estrogen (17–19).

The difference between the MCR^{DHT} of men and women is probably related in part to the higher TeBG binding capacity in women. With increasing androgen production as seen in most hirsute women (8), binding capacity decreased (20) and MCR^{DHT} and MCR^{T} (8) increased. However, the binding capacity of TeBG is not the sole factor determining the MCR as shown by the studies of Southern, Gordon, and Tochimoto (21) and Vermeulen, Verdonck, Van der Straeten, and Orie (20) who noted that chronic administration of testosterone increased MCR^{T} above that which was observed after saturation of TeBG.

In normal men, the DHT plasma concentrations have been reported as 55 ng/100 ml (15, 22), and the production rate as determined by the metabolic clearance technique was 280 μg/day. We found that approximately 4 and 2% of plasma testosterone and androstenedione, respectively, are converted to plasma DHT. Plasma testosterone is thus the precursor of about 280 μg/day (7000 × 0.04), and androstenedione of 40 μg/day (2000 × 0.02) of plasma DHT. Even though these estimates are based on a limited number of studies, they nevertheless suggest that a major fraction of DHT production can result from peripheral conversion of plasma precursors. Knowledge of the exact quantity of secreted DHT must await further study.

Similarly, in normal women DHT may be derived largely from plasma precursors. The DHT production rate is 75 μg/day and 10 μg of this is derived from plasma testosterone (15). If $[\rho]_{BB}^{A-DHT}$ for all normal women is 2% as it is in men and hirsute women (and in one woman with normal ovarian function No. 6), then 60 μg/day (3000 × 0.02) of DHT would be derived from plasma androstenedione. Unfortunately, we were unable to study more normal women. However, it is unlikely that the transfer factor would be much lower. We would therefore conclude tentatively that little DHT is secreted in women provided our assumption that $[\rho]_{BB}^{A-DHT}$ is not greatly different from that observed in our other subjects.

In normal men, DHT contributes very little to the total blood androgens, as its production rate was only 1/26 that of testosterone (15, 22). By contrast in women, DHT can be considered an important androgen since its production rate is 1/3 that of testosterone. Since a major portion of DHT is synthesized in peripheral tissues from plasma precursors, increased androstenedione production rates will result in increased DHT

TABLE III
^3H-Testosterone Infusions

Subjects	^{3}H-testosterone		MCR^T		Conversion T-DHT		Conversion T-diols	
	Infusion rate	Plasma level			C_{BB}^{T-DHT}*	$[\rho]_{BB}^{T-DHT}$	$C_{BB}^{T-3\alpha\text{-diol}}$*	$C_{BB}^{T-3\beta\text{-diol}}$*
	dpm/hr × 10^7	*dpm/liter* × 10^5	*Liter/24 hr*	*Liter/24 hr per m²*				
Normal men								
1	1.438	3.732	924	488				
2	1.726	3.478	1191	621	0.056	0.029	0.009	0.009
3	2.483	6.106	976	526	0.077	0.066	0.017	0.008
Mean			1030	545	0.066	0.047	0.013	0.0085
±SD			±141	±68	±0.014	±0.026	±0.005	±0.0007
±SE			± 81	±39	±0.010	±0.018	±0.004	±0.0005
Hirsute women								
7	1.486	3.603	990	492	0.074	0.062		
9	2.370	9.612	591	365	0.047	0.020	0.004	0.005
10	1.741	4.740	885	495	0.075	0.042		
11	2.966	5.893	1208	669				
Mean			918	505	0.065	0.041		
±SD			±256	±124	±0.015	±0.021		
±SE			±128	± 62	±0.009	±0.012		
Hirsute estrogen-treated woman								
7	4.203	13.929	724	360	0.030	0.010	0.003	0.004

* The average counting rates of tritium in the final derivatives, less background and ^{14}C runover, were: DHT, 42 cpm; 3α-diol, 9 cpm; 3β-diol, 6 cpm.

TABLE IV

^{3}H-Androstenedione Infusions

Subjects	^{3}H-androstenedione		MCR$_A$		Conversion A-T		Conversion A-DHT	
	Infusion rate	Plasma level			C_{BB}^{A-T}*	$[\rho]_{BB}^{A-T}$	C_{BB}^{A-DHT}*	$[\rho]_{BB}^{A-DHT}$
	dpm/hr × 10^7	*dpm/liter* × 10^5	*Liter/24 hr*	*Liter/24 hr per m²*				
Normal men								
1	1.753	2.073	2029	1073	0.114	0.052	0.088	0.020
2	2.603	2.838	2201	1149	0.124	0.067	0.074	0.021
3	3.140	3.786	1990	1072	0.088	0.043	0.089	0.037
Mean			2073	1098	0.108	0.054	0.083	0.026
±SD			±112	± 44	±0.018	±0.012	±0.008	±0.009
±SE			± 64	± 25	±0.010	±0.007	±0.004	±0.005
Hirsute women								
7	1.536	2.000	1842	916	0.097	0.052	0.062	0.028
9	3.796	4.726	1928	1190	0.074	0.023	0.111	0.015
10	2.903	3.818	1825	1022	0.067	0.032	0.085	0.023
11	2.083	2.572	1944	1077				
Mean			1884	1051	0.079	0.035	0.086	0.022
±SD			±259	±114	±0.015	±0.014	±0.024	±0.006
±SE			±129	± 57	±0.009	±0.008	±0.014	±0.003

* The average counting rates of tritium in the final derivatives, less background and ^{14}C run over, were: testosterone, 42 cpm; DHT, 49 cpm.

A, androstenedione.

levels. However, DHT production will not exceed the production rate of testosterone, as $[\rho]_{BB}^{A-T} > [\rho]_{BB}^{A-DHT}$, unless a significant fraction of blood DHT is secreted or derived from a precursor other than androstenedione and testosterone.

The role of androstenedione as a precursor of plasma DHT can assume considerable importance in hirsutism. In a short communication, it was suggested that DHT was secreted (23). However, in hirsutism, since androstenedione production rates average 6 mg/day (24) and the $[\rho]_{BB}^{A-DHT}$ is 0.02, 120 μg of plasma DHT would be produced daily from this precursor. Thus, in hirsutism, androstenedione is an even more important source of plasma DHT.

5α-diols production. We have shown that the conversion of DHT and testosterone to the 3α-diol is higher in men than in women. This confirms the study of Mauvais-Jarvis, and associates (25, 26) who reported similar findings using urinary metabolites. If the MCRs of the 5α-diols are in the same range as MCR^{DHT} which is a reasonable assumption since their binding affinities are not greatly different (14), then the conversion ratios $C_B^{DHT-3\alpha}{}_B$ and $C_B^{DHT-3\beta}{}_B$ will be equal to the transfer constants $[\rho]_{BB}^{DHT-3\alpha}$ and $[\rho]_{BB}^{DHT-3\beta}$, respectively (Table II). We can similarly estimate transfer constants $[\rho]_{BB}^{T-3\alpha}$ as 0.008 and $[\rho]_{BB}^{T-3\beta}$ as 0.005 in the males (Table II). Thus, at least 73 μg of plasma 3α-diol would be produced in men daily, 17 μg coming from DHT, 56 μg from testosterone [(280 × 0.059) + (7000 × 0.008)]. About 40 μg of 3β-diol would be similarly produced, mainly from plasma testosterone. In women, only 4 μg of 3α-diol and 1 μg of 3β-diol can be estimated to be produced by these two precursors.

Always assuming $MCR^{DHT} = MCR^{3\alpha\text{-diol}} = MCR^{3\beta\text{-diol}}$, the product $[\rho]_{BB}^{T-DHT} \times [\rho]_{BB}^{DHT\text{-diols}}$ in males is 0.0071 and 0.0014 for the 3α- and 3β-diol, respectively. These values are lower than the $[\rho]_{BB}^{T\text{-diols}}$; 0.013, (T-3α) and 0.0085 (T-3β). This means that the conversion of testosterone to both 3α- and 3β-diols either involves intermediates other than plasma DHT (possibly androstenedione-androstanedione-androsterone and isoandrosterone-androstanediols) or that part of the DHT formed from T in tissues is further metabolized to diols before entering the blood. Both mechanisms may be correct.

Plasma DHT is converted to a greater extent to the 3α- than to the 3β-diol. The 3α-diol has a very high biological activity on rat prostate growth and the 3β-diol has no action in this system (13). But in prostate culture, the 3β-diol has been shown to maintain both height and secretion of the cells, while the 3α-diol has no effect (27). Further studies are needed to evaluate the biological significance of the diols in human plasma.

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