

# Metabolism of Dihydrotestosterone in Human Liver: Importance of 3 $\alpha$ - and 3 $\beta$ -Hydroxysteroid Dehydrogenase\*

EDYTA C. PIROG AND DELWOOD C. COLLINS

Department of Pathology, Cornell University School of Medicine and Veterans Administration Medical Center, and the Department of Obstetrics and Gynecology, University of Kentucky College of Medicine, Lexington, Kentucky 40536

## ABSTRACT

This study compared the enzyme activity of 3 $\alpha$ -hydroxysteroid dehydrogenase (3 $\alpha$ HSD) and 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ HSD) in the human liver. 3 $\alpha$ HSD was found in both microsomal and cytosolic liver fractions. Contrary to that in rat liver, microsomal 3 $\alpha$ HSD activity was 12-fold higher than cytosolic 3 $\alpha$ HSD activity, and 3 $\alpha$ HSD was not inhibited by indomethacin (10  $\mu$ mol/L). The rate of 5 $\alpha$ -dihydrotestosterone (DHT) reduction to 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol (3 $\alpha$ DIOL) by 3 $\alpha$ HSD was 2 times higher than the rate of 3 $\alpha$ DIOL oxidation to DHT. 3 $\beta$ HSD was present primarily in the microsomal fraction of the human liver, and the rate of DHT reduction to 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol (3 $\beta$ DIOL) by 3 $\beta$ HSD was 3 times higher than

the rate of 3 $\beta$ HSD oxidation to DHT. When 3 $\alpha$ HSD and 3 $\beta$ HSD activities were compared, the rate of DHT reduction by 3 $\beta$ HSD was 3-fold lower than the rate of DHT reduction by 3 $\alpha$ HSD. No sex or age differences were found in either 3 $\alpha$ HSD or 3 $\beta$ HSD activity. As the activity of DHT-metabolizing enzymes is not sex dependent, the sex differences in plasma levels of 3 $\alpha$ DIOL glucuronide probably reflect differences in DHT production rather than in DHT metabolism. Comparison of the activities of 3 $\alpha$ HSD, 3 $\beta$ HSD, and androgen UDP-glucuronyl transferase suggests that the major pathway of DHT metabolism in human liver involves 3 $\alpha$ HSD reduction in the liver, followed by subsequent glucuronidation and clearance via the kidney. (*J Clin Endocrinol Metab* 84: 3217–3221, 1999)

THE SYNTHESIS and metabolism of an active androgen, 5 $\alpha$ -dihydrotestosterone (DHT), takes place in extragonadal, androgen target tissues. For this reason, DHT is considered to be a paracrine or an autocrine hormone. DHT is synthesized from testosterone in an irreversible reaction catalyzed by the microsomal enzyme, 5 $\alpha$ -reductase. Modification of DHT at the 17 $\beta$ -hydroxy or 3-keto position renders it inactive (Fig. 1). The reactions carried out by DHT-metabolizing enzymes are reversible, and the relative activities of these enzymes determines tissue exposure to the active androgen, DHT. One of the enzymes involved in DHT metabolism is 3 $\alpha$ -hydroxysteroid dehydrogenase (3 $\alpha$ HSD), which reduces DHT to 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol (3 $\alpha$ DIOL). Interestingly, 3 $\alpha$ HSD activity favors net production of DHT in certain tissues, such as the prostate (1). Inadequate metabolism of DHT by 3 $\alpha$ HSD may contribute to the development of prostate hyperplasia (1). Skin fibroblasts show different patterns of 3 $\alpha$ HSD activity in different skin areas (2). In nongenital skin, DHT formation from 3 $\alpha$ DIOL was approximately equal to DHT reduction to 3 $\alpha$ DIOL (2). However, in genital skin, the rate of DHT formation from 3 $\alpha$ DIOL was twice as high as DHT reduction (2). Little information is available about the regulation of 3 $\alpha$ HSD activity in other tissues. It is tempting to speculate that abnormal DHT metabolism may contribute not only to disorders such as pros-

tate hyperplasia, but also to other androgen-related disorders such as acne and hirsutism.

Other enzymes involved in DHT metabolism are 3 $\beta$ HSD, which reduces DHT to 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol (3 $\beta$ DIOL) and microsomal UDP-glucuronyl transferase (UDPGT), which conjugates DHT to glucuronic acid. This reaction can be reversed by another enzyme,  $\beta$ -glucuronidase. The relative contributions of the above-mentioned enzymes to the overall metabolism of DHT are poorly understood.

The equilibrium of reductive and oxidative activities of the hydroxysteroid dehydrogenases may be important in the regulation of intracellular levels of DHT. This study was designed to determine the activity and kinetic properties of 3 $\alpha$ HSD and 3 $\beta$ HSD in the human liver. In addition, the activities of these enzymes were measured in liver homogenates from five females and five males to determine whether there are sex- or age-specific differences in the expression of these enzymes in the liver.

## Subjects and Methods

### Subjects

Liver samples from female (34–60 yr) and male (33–75 yr) organ donors were obtained from the National Disease Research Interchange (Philadelphia, PA). Samples were procured postmortem (within 5 h), snap-frozen upon removal, and stored at  $-70^{\circ}\text{C}$  until used.

### Chemicals

The radioactive androgens, [1,2- $^3\text{H}$ ]3 $\alpha$ -DIOL (SA, 30.5 Ci/mmol) and [1,2,4,5,6,7- $^3\text{H}$ ]DHT (SA, 119.6 Ci/mmol) were purchased from Amersham Pharmacia Biotech (Arlington Heights, IL). The nonradioactive steroids were obtained from Sigma Chemical Co. (St. Louis, MO) or Steraloids, Inc. (Wilton, NH). The purity of each androgen was con-

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Address all correspondence and requests for reprints to: Dr. D. C. Collins, 204 Health Sciences Research Building, University of Kentucky Medical Center, Lexington, Kentucky 40536-0305.

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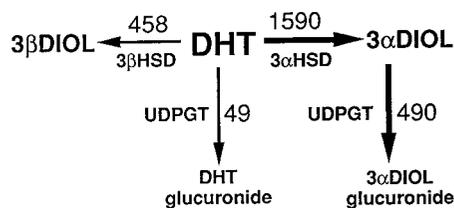


FIG. 1. Major pathways for DHT metabolism leading to glucuronyl metabolites. Minor pathways leading to sulfonated metabolites are not shown.

firmed by high pressure liquid chromatography. The protein assay reagents were obtained from Bio-Rad Laboratories, Inc. (Richmond, CA). All other chemicals were analytical grade.

### Tissue fractionation

Liver samples were homogenized with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY) in 4 vol ice-cold 50 mmol/L phosphate buffer, pH 7.4, containing 250 mmol/L sucrose and 0.5 mmol/L dithiothreitol. Aliquots of the liver homogenates were taken for analysis of  $3\alpha\text{HSD}$  and  $3\beta\text{HSD}$  activities. The homogenates were centrifuged for 15 min at  $12,000 \times g$ , followed by ultracentrifugation of the supernatant at  $100,000 \times g$  for 60 min. The  $100,000 \times g$  pellets were rinsed once with 250 mmol/L sucrose and 50 mmol/L phosphate buffer, pH 7.4, and re-homogenized in 250 mmol/L sucrose and 50 mmol/L phosphate buffer, pH 7.4, with 20% glycerol. The microsomal and cytosolic fractions were stored at  $-80^\circ\text{C}$  for up to 2 months with no significant change in enzyme activity. At the time of analysis, the viability of the microsomal fractions was confirmed by determining the activity of the microsomal marker enzyme, glucose-6-phosphatase, as described by Aronson and Touster (3), using 100  $\mu\text{mol}$  glucose-6-phosphate as a substrate.

### Enzyme kinetics

The enzyme activity studies were performed using liver homogenates or subcellular fractions. The  $K_m$  and  $V_{\max}$  values were determined in the microsomal and cytosol fractions using the Michaelis-Menten equation with a nonlinear regression data analysis software program (Enzfitter 1.05, Biosoft, 1987).  $3\alpha\text{HSD}$  activity was determined using tritiated substrates (DHT or  $3\alpha\text{DIOL}$ ). The incubation mixture (600  $\mu\text{L}$ ) contained 150,000 dpm of either [ $^3\text{H}$ ]DHT or [ $^3\text{H}$ ]3 $\alpha\text{DIOL}$  and cold androgens (final concentration, 0.1–20  $\mu\text{mol/L}$ ), an aliquot of the cell fraction (100  $\mu\text{g}$  total homogenate protein, 150  $\mu\text{g}$  cytosol protein, or 50  $\mu\text{g}$  microsomal protein), 200  $\mu\text{mol/L}$  NADP or NADPH, and 0.05% Tween-80 (vol/vol) in 25 mmol/L phosphate buffer, pH 7.4. The androgens were added to the tubes in ethanol solution before the assay, and the ethanol was evaporated before the other components of the incubation mixture were added. Control incubations were carried out without adding the cofactors, NADP or NADPH. Samples were incubated at  $37^\circ\text{C}$  in a shaking water bath for 0.5–5 min. DHT or  $3\alpha\text{DIOL}$  was used as substrate at a concentration of 50 nmol/L (100 times the physiological concentration). The conditions of the assay used both cofactor (NADPH or NADP) and substrate at excess concentrations to ensure that product formation reflected maximal  $3\alpha\text{HSD}$  activity and was linear with time and protein concentration.

The reaction products were extracted from the incubation mixture using reverse phase  $C_{18}$  SPC columns. Aliquots of the incubation mixture (150  $\mu\text{L}$ ) were applied to the columns and eluted with 1.5 mL water, 0.5 mL hexane, and finally, 1.5 mL ethanol. All of the radioactivity was recovered in the ethanol eluate. The ethanol fractions were evaporated using a Speed-Vac evaporator (Savant Instruments, Farmingdale, NY), reconstituted in ethanol containing cold tracer (100  $\mu\text{g}$  each of DHT,  $3\alpha\text{DIOL}$ , and  $3\beta\text{DIOL}$ ), and separated by high pressure liquid chromatography (Waters, Millipore Corp., Millford, MA) using a  $C_{18}$  column (Waters, Millipore Corp.) and a mobile phase of acetonitrile-water (45:55) at a flow rate of 1 mL/min. The retention times for the androgens were:  $3\alpha\text{DIOL}$ , 9.46;  $3\beta\text{DIOL}$ , 7.32; and DHT, 10.95. The radioactivity in the eluted samples was quantitated using a Radio-Chromatography Detector (Radiomatic FLO-ONE Beta, Series A-500, Radiomatic Instru-

TABLE 1. Michaelis-Menten constants ( $K_m$ ), maximal velocities ( $V_{\max}$ ), and  $V_{\max}/K_m$  ratios of  $3\alpha\text{HSD}$  and  $3\beta\text{HSD}$  in liver microsomes and cytosol of a 65-yr-old Caucasian male

Enzyme	Substrate	$K_m$ ( $\mu\text{mol/L}$ )	$V_{\max}$ (nmol/min-mg)	$V_{\max}/K_m$ ratio
<b><math>3\alpha\text{HSD}</math></b>				
Microsomes				
Reduction	DHT	2.2	2.50	1.136
Oxidation	$3\alpha\text{DIOL}$	2.4	1.57	0.654
Cytosol				
Reduction	DHT	2.2	0.20	0.091
Oxidation	$3\alpha\text{DIOL}$	2.5	0.21	0.084
<b><math>3\beta\text{HSD}</math></b>				
Microsomes				
Reductive	DHT	1.13	0.37	0.327
Oxidative	$3\beta\text{DIOL}$	3.35	0.39	0.116
Cytosol				
Reduction	DHT	ND	ND	ND
Oxidation	$3\beta\text{DIOL}$	ND	ND	ND

DHT was added in excess as the substrate for reduction, and  $3\alpha\text{DIOL}$  or  $3\beta\text{DIOL}$  was added in excess as the substrate for oxidation. ND, Not detectable.

ments & Chemical Co., Inc., Meriden, CT) and analyzed with the Radiomatic FLO-ONE Software program (Radiomatic Instruments & Chemical Co., Inc.).

$3\beta\text{HSD}$  activity was determined as described above, except that  $3\beta\text{DIOL}$  was the substrate for the oxidative reaction, and 150  $\mu\text{g}$  microsomal protein, 450  $\mu\text{g}$  cytosolic protein, or 100  $\mu\text{g}$  total homogenate protein were used in the incubation.

### Protein determination

Protein concentrations of the homogenates and cellular fractions were determined as described by Bradford (4), using BSA as a standard.

## Results

### Kinetic characteristics of $3\alpha\text{HSD}$ and $3\beta\text{HSD}$ in liver

The  $K_m$  and maximum velocity ( $V_{\max}$ ) values of  $3\alpha\text{HSD}$  and  $3\beta\text{HSD}$  for both the reduction of DHT and the oxidation of  $3\alpha\text{DIOL}$  and  $3\beta\text{DIOL}$  in the microsomes and cytosol are shown in Table 1 for a 65-yr-old Caucasian male. A similar pattern of  $K_m$  constants and reductive and oxidative activities was noted in the other samples that were fractionated.  $3\alpha\text{HSD}$  activity was expressed in both the microsomal and cytosolic fractions. The  $V_{\max}$  value was 12-fold higher in the microsomes than in the cytosol for both the oxidative and reductive reactions. The  $K_m$  values for DHT and  $3\alpha\text{DIOL}$  were similar in both cellular fractions. The  $V_{\max}/K_m$  ratio represents the relative reaction velocity (see Table 1), assuming that the initial concentrations of the substrates, DHT and  $3\alpha\text{DIOL}$ , are equal (see Table 1). This is, in fact, the physiological situation, as plasma levels of DHT and  $3\alpha\text{DIOL}$  are approximately equal (5). The relative rate of DHT reduction was 2 times the relative rate of  $3\alpha\text{DIOL}$  oxidation in the microsomes. In cytosol, the  $V_{\max}/K_m$  ratios for reduction and oxidation were similar. Neither microsomal nor cytosolic  $3\alpha\text{HSD}$  activity was inhibited by indomethacin at a concentration as high as 10  $\mu\text{mol/L}$  (data not shown).

$3\beta\text{HSD}$  activity was present mainly in the microsomal fraction of the liver. The  $K_m$  and  $V_{\max}$  values of microsomal  $3\beta\text{HSD}$  for reduction of DHT to  $3\beta\text{DIOL}$  and oxidation of  $3\beta\text{DIOL}$  to DHT are shown in Table 1.  $3\beta\text{HSD}$  activity in the cytosol was below the assay detection limit. DHT reduction

to 3 $\beta$ DIOL was 3 times faster than DHT formation from 3 $\beta$ DIOL. In the microsomes, the relative rate of DHT reduction by 3 $\beta$ HSD was 3-fold lower than the relative rate of DHT reduction by 3 $\alpha$ HSD. This suggests that the major product to DHT metabolism in the human liver is 3 $\alpha$ DIOL.

#### Activity of 3 $\alpha$ HSD and 3 $\beta$ HSD in liver homogenates from males and females

3 $\alpha$ HSD activity in liver homogenates from five male and five female subjects is shown in Fig. 2. In all subjects, the velocity of DHT reduction to 3 $\alpha$ DIOL was 2 times higher than the velocity of 3 $\alpha$ DIOL oxidation to DHT. There was no significant difference between the mean rate  $\pm$  SE for 3 $\alpha$ HSD reduction in men ( $38.5 \pm 16.9$  pmol/min·mg protein) and women ( $38.3 \pm 10.6$ ; Table 2). 3 $\alpha$ HSD activity (DHT reduction) was 9.4–103.7 pmol/min·mg for men and 10.0–73.1 pmol/min·mg for women. Furthermore, no significant correlation was found between 3 $\alpha$ HSD activity and the age of the subjects.

As suggested by the results in Table 1, the enzyme activity of 3 $\beta$ HSD was about 10-fold lower than that of 3 $\alpha$ HSD and was near the sensitivity of the assay for the oxidation of 3 $\beta$ DIOL to DHT. The mean 3 $\beta$ HSD activity in liver homogenates for DHT reduction in men ( $8.71 \pm 2.3$  pmol/min·mg protein; range, 6.4–12.6) was not significantly different from the mean activity in women ( $6.78 \pm 1.7$  pmol/min·mg protein; range, 4.4–8.9; Table 2). In addition, there was no significant correlation between the age of the subjects and 3 $\beta$ HSD activity.

### Discussion

The need to understand the pathways for metabolism of DHT in human tissues was brought about by the long and unsuccessful search for a reliable biochemical test of androgen excess. The concentration of DHT in plasma does not correlate with the clinical symptoms of hyperandrogenism (6). Horton *et al.* (7) proposed that plasma levels of 3 $\alpha$ DIOL glucuronide reflected the formation and metabolism of DHT

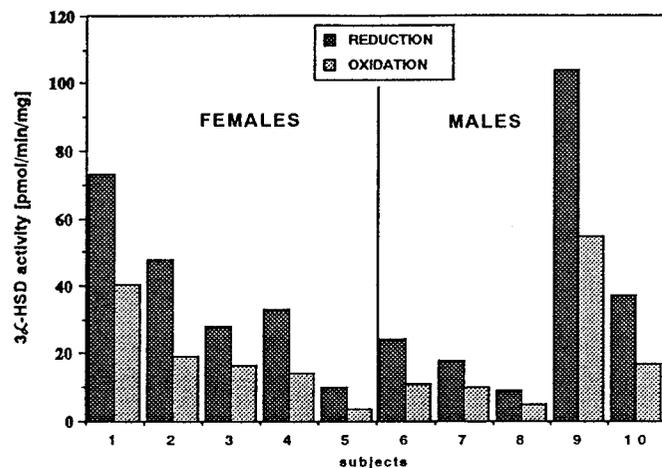


FIG. 2. 3 $\alpha$ HSD activity (picomoles per min/mg protein) in liver homogenates from five male and five female subjects. DHT (50 nmol/L) was the substrate for the reductive reaction (DHT $\rightarrow$ 3 $\alpha$ DIOL). 3 $\alpha$ DIOL (50 nmol/L) was the substrate for the oxidative reaction (3 $\alpha$ DIOL $\rightarrow$ DHT).

**TABLE 2.** Mean activity (picomoles per min/mg  $\pm$  SE) of 3 $\alpha$ HSD and 3 $\beta$ HSD in liver homogenates from five male and five female subjects

Enzyme	Activity	Substrate	Mean $\pm$ SE	
			Males	Females
3 $\alpha$ HSD	Reduction	DHT	$38.5 \pm 16.9$	$38.5 \pm 10.6$
3 $\alpha$ HSD	Oxidation	3 $\alpha$ DIOL	$19.5 \pm 9.0$	$18.9 \pm 6.0$
3 $\beta$ HSD	Reduction	DHT	$8.7 \pm 2.3$	$6.7 \pm 1.7$

DHT (50 nmol/L) or 50 nM 3 $\alpha$ DIOL was used as the substrate. Values are expressed as the mean  $\pm$  SE for three determinations performed in duplicate.

in extrahepatic, androgen target tissues, such as skin and prostate (7). However, only limited studies of the enzymes involved in DHT metabolism in human tissues other than skin and prostate have been performed, and the relative contributions of these tissues to plasma levels of androgen conjugates were not clearly established. In a recent study of human subjects, Duffy *et al.* (8) demonstrated that percutaneously applied DHT is more readily converted to 3 $\alpha$ DIOL than DHT infused iv, suggesting significant activity of 3 $\alpha$ HSD in skin. On the other hand, conversion of 3 $\alpha$ DIOL to 3 $\alpha$ DIOL glucuronide was more efficient after iv infusion of DHT than with percutaneous application. Giagulli *et al.* (9) reported that the plasma level of 3 $\alpha$ DIOL glucuronide was an order of magnitude higher after oral administration of testosterone compared to transdermal application. These observations suggested the importance of the splanchnic organs for androgen glucuronidation and support *in vitro* data obtained from several studies. Recent studies of *in vitro* androgen UDPGT activity revealed that androgen glucuronidation occurs almost exclusively in the liver (10–13). These results from several different laboratories raise serious concerns about the hypothesis that plasma levels of 3 $\alpha$ DIOL glucuronide reflect exclusively androgen action and metabolism in extrahepatic tissues (8–13).

The level of DHT in androgen target tissues is regulated mainly by 5 $\alpha$ -reductase and 3 $\alpha$ HSD. 3 $\alpha$ HSD was initially purified from rat liver cytosol, and subsequently, the gene was cloned from human liver and prostate (14–16). Although 3 $\alpha$ HSD activity from rat liver and rat and human prostate has been extensively characterized (17–20), no comparative studies of the enzyme oxidative and reductive activities in human liver have been conducted.

We present here a comparative study of the kinetic properties of 3 $\alpha$ HSD and 3 $\beta$ HSD involved in DHT metabolism in the human liver. 3 $\alpha$ HSD, the most active DHT-metabolizing enzyme, was present in both the microsomal and cytosol fractions. The  $V_{max}$  of microsomal 3 $\alpha$ HSD was 12-fold higher than that of cytosolic 3 $\alpha$ HSD. This is in contrast to rat liver, where cytosolic 3 $\alpha$ HSD was 10 times more active than microsomal 3 $\alpha$ HSD (18). Another distinctive feature was that 3 $\alpha$ HSD in the human was not sensitive to indomethacin, a strong inhibitor of 3 $\alpha$ HSD in the rat (18, 21). Our laboratory and others (18, 21) have shown that rat 3 $\alpha$ HSD possesses high affinity binding and sensitivity to inhibition by nonsteroidal antiinflammatory drugs. If human 3 $\alpha$ HSD was inhibited by nonsteroidal antiinflammatory drugs, significant clinical consequences would be expected. However, we

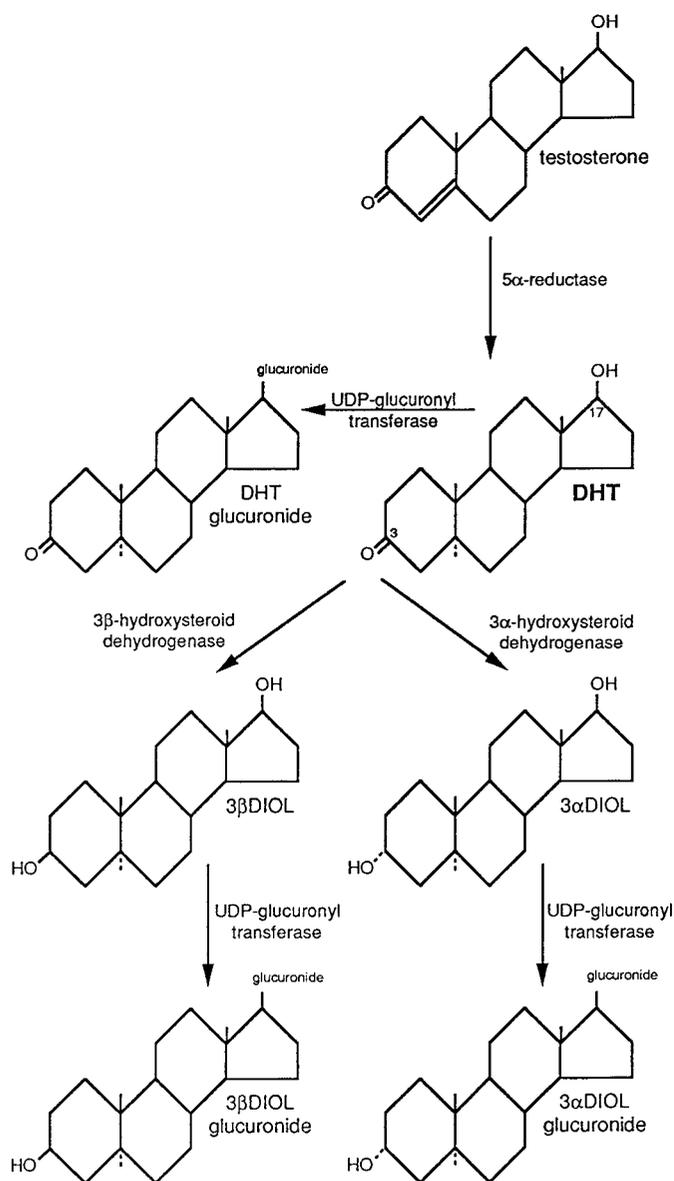


FIG. 3. Proposed scheme for DHT metabolism in human liver. The values are the enzyme velocities at a physiological concentration ( $V_{\max}/K_m \times [S]$  in femtomoles per min/mg protein).

found no inhibition of this enzyme activity even with a high concentration of indomethacin (10  $\mu\text{mol/L}$ ).

The reductive activity of 3 $\alpha$ HSD (DHT $\rightarrow$ 3 $\alpha$ DIOL) was 2 times higher than the oxidative activity (3 $\alpha$ DIOL $\rightarrow$ DHT). This indicates that 3 $\alpha$ HSD in the human liver favors reduction of DHT to 3 $\alpha$ DIOL. Liver samples from both male and female subjects showed a wide range of values in 3 $\alpha$ HSD activity. However, the mean activity in men and women was not statistically different. This suggests that human 3 $\alpha$ HSD is not regulated by sex steroids. In contrast, rat liver 3 $\alpha$ HSD exhibits sexual dimorphic expression. The enzyme activity is elevated in female rat liver and appears to be under the control of estrogens (22). In addition, no change in 3 $\alpha$ HSD activity was found to be related to age in our samples.

3 $\beta$ HSD activity was found primarily in the human liver

microsomes and was 3-fold lower than the activity of microsomal 3 $\alpha$ HSD. DHT reduction to 3 $\beta$ DIOL was 3 times higher than the oxidation of 3 $\beta$ DIOL to DHT. This indicates that 3 $\beta$ HSD in liver contributes to the clearance of DHT, but to a lesser extent than 3 $\alpha$ HSD. These *in vitro* observations support *in vivo* studies of the metabolic clearance and origin of DHT in humans (23). Mahoudeau *et al.* (23) also found that the ratio for *in vivo* conversion of DHT to 3 $\alpha$ DIOL was greater than that to 3 $\beta$ DIOL, as we report here in the liver *in vitro*. In contrast, Dijkstra *et al.* (24) found that 3 $\beta$ DIOL was the major product of DHT metabolism in the sebaceous glands in skin. This result is particularly interesting because plasma levels of 3 $\beta$ DIOL have been reported to be 3-fold higher than plasma levels of 3 $\alpha$ DIOL in both men and women (5). There are at least two potential explanations for this observation. First, a tissue other than the liver may be the primary source for plasma 3 $\beta$ DIOL (*e.g.* skin). Second, the clearance of 3 $\beta$ DIOL may be much slower than the clearance of 3 $\alpha$ DIOL, perhaps due to the low affinity of UDP-glucuronyl transferase for 3 $\beta$ DIOL. This is supported by the observation that plasma levels of 3 $\beta$ DIOL glucuronide are lower than those of 3 $\alpha$ DIOL glucuronide (5). No sex or age differences in 3 $\beta$ HSD activity were found in the human liver.

The scheme for DHT metabolism leading to formation of glucuronated metabolites in the human liver is shown in Fig. 3. The numbers represent calculated enzyme velocities (velocity =  $V_{\max}/K_m \times [S]$  with a physiological substrate concentration for males). The values for androgen UDPGT velocity were calculated from previous studies from our laboratory (13). The scheme was created using results from *in vitro* kinetic studies of enzyme activity in subcellular fractions. The values represent relative enzyme velocities *in vitro* and may not represent the *in vivo* state. A well controlled perfusion study would be required to determine *in vivo* dynamics.

In summary, the results of our *in vitro* kinetic studies suggest that the main pathway of DHT metabolism leading to glucuronated metabolites in human liver is 3 $\alpha$ -reduction, followed by subsequent conjugation to glucuronic acid. Furthermore, no sex or age differences were observed in either 3 $\alpha$ HSD or 3 $\beta$ HSD activity in human liver.

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