

# Pregnancy-Induced Perturbation of Urinary Androgenic Steroid Disposition

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**Objective:** To investigate the excretion and conjugation profile of testosterone (T), Epitestosterone (EpiT), and other androgen metabolites in different phases of pregnancy and postpregnancy as a reflection of the “androgenic exposure.”

**Design:** Consecutive recruitment of pregnant women.

**Setting:** Maternity outpatient low-risk pregnancy clinic.

**Patients:** Seventy-seven pregnant women.

**Interventions:** Collection of urine for analyses of sulfate (S) and glucuronide (G) conjugates and metabolic ratios of androgens and androgen metabolites using liquid chromatography-tandem mass spectrometry.

**Main Outcome Measures:** Excretion profiles and metabolic ratios of G and S conjugates of T, EpiT, dehydroepiandrosterone (DHEA), androsterone (A), etiocholanolone (Etio), and dihydrotestosterone in relation to trimester and postpartum, body mass index, fetal sex, and ethnicity.

**Results:** T-S excretion increased significantly between the second and third trimester, whereas excretion of T-G did not change. In contrast, both conjugates of EpiT increased markedly, more so for the S- (17-fold) than the G-conjugate (1.6-fold). The preference for S over G conjugation was conspicuous for EpiT and DHEA (S/G ratio 2.1 and 4.7, respectively, in the third trimester), whereas the reverse was true for T, A, and Etio (S/G 0.6, 0.13, and 0.11, respectively).

**Conclusions:** Pregnancy influences the androgen excretion profile, with the most profound change being an increase in EpiT excretion throughout the trimesters. EpiT may modulate the effect of T, but its exact role during pregnancy is not known. There were marked differences in the S/G conjugate ratios between androgens upstream and downstream from T in the metabolic network. These results are interesting to compare with the androgen disposition in women with endocrine disorders or abuse of steroids.

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**Freeform/Key Words:** androgens, epitestosterone, glucuronidation, pregnancy, sulfoconjugation, testosterone

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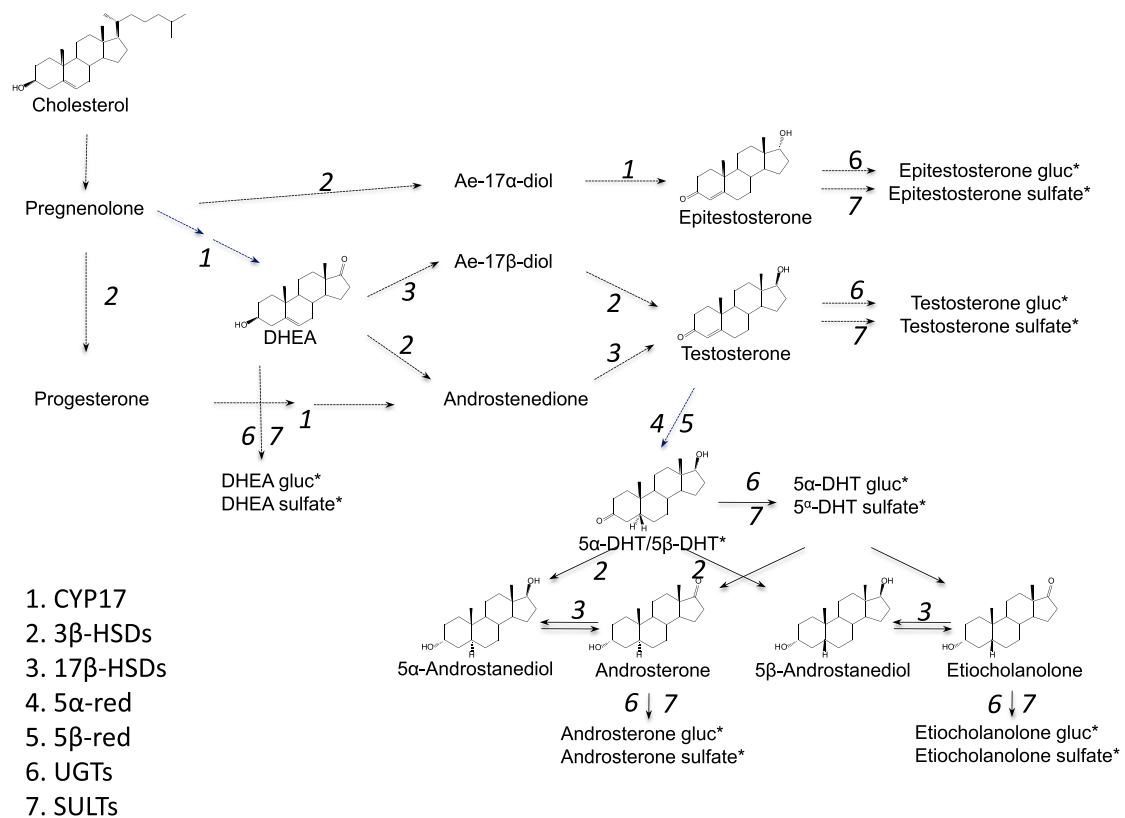
Abbreviations: A, androsterone; BMI, body mass index; CYP, cytochrome P450; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; EpiT, epitestosterone; Etio, etiocholanolone; G, glucuronide; NO, nitric oxide; pp, postpartum; S, sulfate; T, testosterone.

There is substantial knowledge about estrogens, progesterone, FSH, and LH in women, mostly related to fertility and pregnancy. By comparison, the information about androgens is sparse, in particular, androgen disposition in pregnancy. Androgens are as important in women as in men. Testosterone (T) has an important role in many physiological processes, such as protein synthesis, muscle growth, and maturation of the reproductive organs. It has a fat-reducing effect in obese women and a negative effect on the serum lipid profile. It may also increase insulin resistance, coagulation activity, *etc.* T also promotes sexual activity and sexual desire in women. Thus, it is a hormone of great interest in relation to several disorders, such as the metabolic syndrome (type 2 diabetes, obesity, arteriosclerosis), breast cancer, sexual dysfunction, and general physical well being [1, 2].

Epitestosterone (EpiT) is a 17 $\alpha$ -epimer of T that is present in the human circulation. Its physiological role is largely unknown. The serum concentrations of EpiT are lower than T (T/EpiT ratio in serum ~10–30) and higher in men than in women (average 2.5 vs 1.2 nM in serum) [3, 4].

Androgens are predominantly excreted as glucuronides (G) and to a lesser extent, as sulfates (S; Fig. 1). As was previously shown, the T-G excretion varies 100-fold among adult individuals [5]. There are also marked differences among ethnic groups, which were shown by us to be caused by a gene-deletion polymorphism in the major glucuronidating enzyme [5]. No physiological implications of this variation are known to date [6], but the polymorphism may be a confounder in doping tests [7].

The weak dehydroepiandrosterone (DHEA) and DHEA-S precursors from the adrenal cortex are converted in peripheral tissues to contribute substantially to the circulating T [8–10]. Thus, the direct contribution of the ovaries and the adrenals to the circulating T and its downstream products is only marginal (Fig. 1) in pregnancy.



**Figure 1.** Formation and metabolism of key androgens that were investigated. The metabolites denoted with an asterisk (\*) are the metabolites analyzed in this study. Enzymes involved are indicated with numbers and include the following: 1, cytochrome P450 (CYP)17; 2, 3 $\beta$ -hydroxysteroid dehydrogenases (HSDs); 3, 17 $\beta$ -HSDs; 4, 5 $\alpha$ -reductase (red); 5, 5 $\beta$ -red; 6, uridine diphosphoglucuronosyl transferases (UGTs); and 7, sulfotransferases (SULTs). DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone.

In women, serum levels of T are suggested to reflect only part of the androgen load, as the major adrenal androgen precursor DHEA exerts its effects “on site” (sometimes denoted as an intracrine mechanism of action) in peripheral tissues after biotransformation to various androgens [11]. Therefore, serum concentrations of major T metabolites, in particular, androsterone A-G, which accounts for >90% of the total androgen G in serum, have been suggested as a more accurate analyte of choice for estimation of total androgen exposure in women [12]. As a corollary, the urinary excretion of androgen metabolites is of interest, as it is supposed to reflect the endocrine androgen exposure.

Given the background described previously, we thought it of interest to measure the urinary excretion of major androgens in different phases of pregnancy to assess the total “androgenic load,” assuming that the major bulk of androgens and androgen metabolites is excreted in urine. The aim was to describe the urinary excretion profile of S and G conjugates of T, EpiT, DHEA, as well as other androgen metabolites in different phases of pregnancy and after parturition.

## 1. Materials and Methods

### A. Study Population

We recruited 77 women who visited the maternity unit at St Michael’s Hospital, Toronto University (ON, Canada), in 2014 and 2015. They were pregnant in their first or second trimester of pregnancy and visited the low-risk pregnancy clinics of the hospital. The average age of the participants was 31.4 years, and the average body mass index (BMI) was 24.6. The ethnic origins were as follows: 43 white, 10 were from Southeast Asia (Indian, Bangladesian), seven were black, 15 were Asian (Korean, Japanese, Chinese, Vietnamese, Phillipino), and two were mixed Asian/Caucasian. Eight of the women were excluded because three dropped out of the study, and five did not show up at the clinic or did not leave urine samples. All women were included only after informed consent was obtained. The study was approved by the Toronto Academic Health Sciences Network Human Subjects Research Ethics Committee.

For 30 participants, this pregnancy was the first gestation; for 25 participants, it was the second gestation; and for 13 participants, this pregnancy was the third or more gestation. None of the women in the study suffered from any androgen-related disorder, such as polycystic ovarian syndrome, before pregnancy. Fifty-seven of the women did not take any medication chronically, whereas 15 women took chronic medication [five took Diclectin (pyridoxine/doxylamine), one woman took Feramax (iron supplement), one woman took Zopiclone (generic name), five women took Synthroid (levothyroxine sodium)/Thyroxine/Eltroxin (levothyroxine), one woman took Paxil (paroxetine) and Symbicort (budesonide/formoterol fumarate dehydrate), one woman took progesterone and estrogen, one woman took Cipralext (escitalopram), one woman also took Ventolin (albuterol sulfate) and aspirin (acetylsalicylic acid), and one woman took Ursolit (ursodeoxycholic acid)]. The mean gestational age at delivery was 39.5 weeks. The average weight of the neonate at birth was 3.364 g. Regarding sex, 30 girls and 32 boys were born. Forty-three of the women had a vaginal delivery, whereas 13 had a Caesarean section and six had a mechanical delivery.

Urine samples were collected from the participants, with a maximum of one per woman per trimester: 27 urine samples in the first trimester, 73 in the second trimester, 64 in the third trimester, and 33 in the postpartum (pp) period, 3 to 6 weeks after parturition. All samples were collected between 8 AM and 2 PM.

### B. Urinary Analyses

The androgen metabolites were analyzed using liquid chromatography-tandem mass spectrometry as described [13]. Sample preparation for analyses of conjugated endogenous steroids (S and G) was made using a 96-well solid-phase extraction plate, Oasis hydrophilic-lipophilic-balanced extraction plate (Waters, Taunton, MA) for extraction and preconcentration of the

analytes. Separation was performed by an Ultimate 3000 ultra HPLC system (Thermo Scientific, Dionex Products, Sunnyvale, CA) on a YMC-UltraHT Hydrosphere C18 column with a precolumn YMC-Hydrosphere C18 (YMC Co. Ltd, Kyoto, Japan). The analytes were detected using a high-resolution/high-accuracy mass spectrometer by a Q Exactive, with an HESI-II probe electrospray interface [13]. Urinary data were normalized for a specific gravity of 1.020, measured using a Digital Urine SG Refractometer (UG-1; Atago, Tokyo, Japan). Specific gravity was used instead of creatinine, as it was presumed to be less influenced than creatinine by pregnancy-specific physiology. The concentrations are expressed in molarity to permit direct comparison of different androgens and conjugates.

### C. Data Analysis

Most of the urinary metabolites did not show normal distribution, and therefore, non-parametric tests were consistently used throughout the study. All data are reported as medians with 25 and 75 percentiles or as ratios of the means, if nothing else is stated. Differences were considered significant at the level of  $P < 0.05$  two-sided tests. When comparing the intra-individual variation among different trimesters, paired analysis (Wilcoxon) was used. To study if there were any differences at a group level among the different trimesters ANOVA, Kruskal-Wallis analysis was done. Mann-Whitney was used for comparison of steroid concentration and sex of the fetuses. For correlation studies, Spearman rank test was used.

## 2. Results

### A. The Disposition of Androgen Phase 2 Metabolites in the Different Trimesters

There were moderate, although important, changes in excretion of the T conjugates in the different trimesters (ANOVA,  $P = 0.007$ ). T-S increased moderately from the second [median 8.35 nM (4.90 to 14.18)] to the third [median 12.41 nM (4.34 to 20.96);  $n = 35$ ,  $P = 0.016$ ] trimester and declined to lower values pp [median 3.1 nM (1.45 to 5.18);  $n = 6$ ,  $P = 0.031$ ; Fig. 2]. The excretion of T-G did not change significantly among the trimesters (Fig. 2).

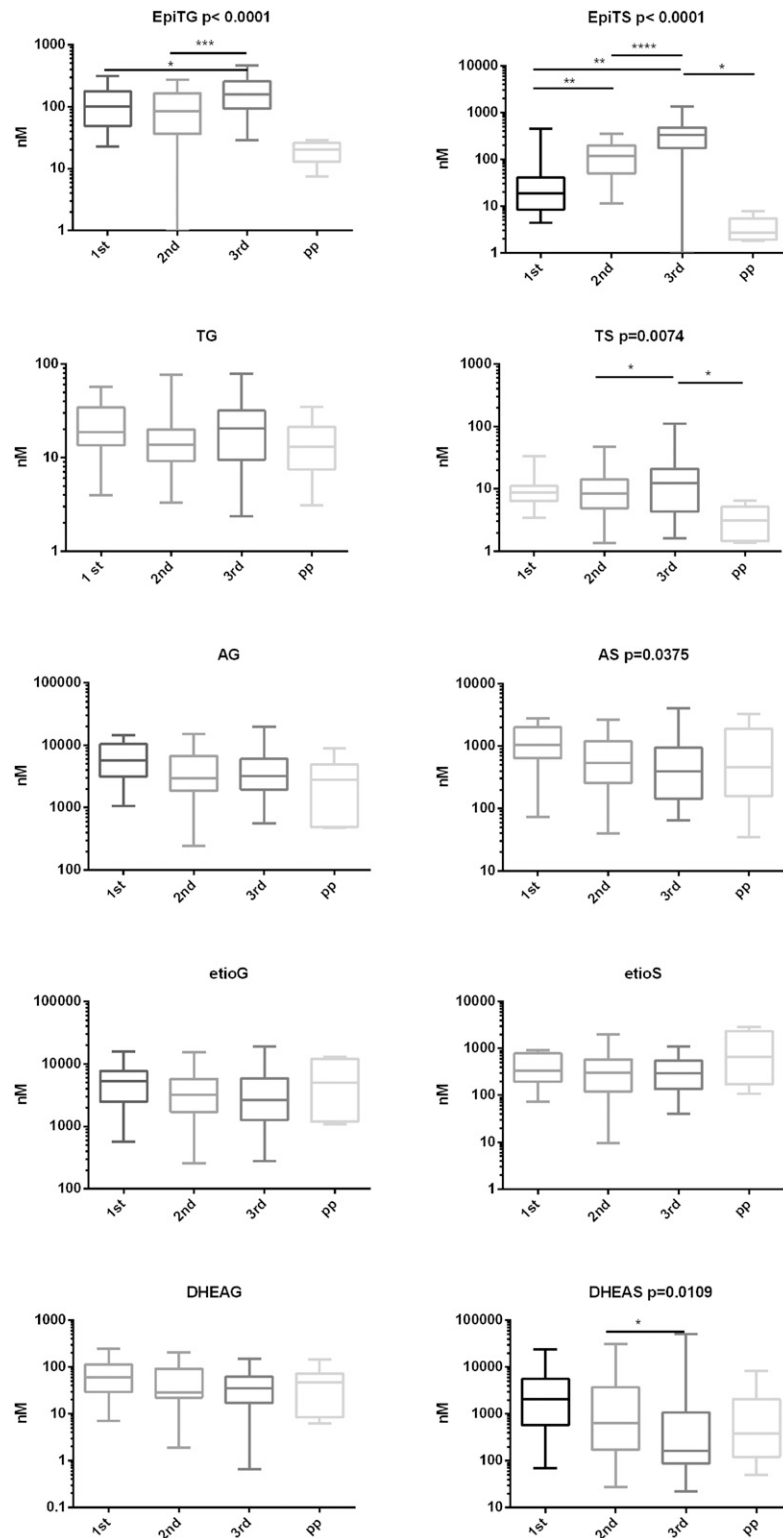
In contrast to T, both the conjugates of EpiT increased markedly in pregnancy. Most significant changes were observed for the EpiT-S (ANOVA,  $P < 0.0001$ ). The urinary concentration of EpiT-S was highest in the third trimester [median 330.5 nM (172.2 to 484.2)], followed by the second [median 117 nM (49.71 to 200.5)] and first [median 19.0 nM (8.54 to 40.49)] trimesters. After delivery, the excretion declined markedly to a very low concentration [median 2.72 nM (1.91 to 5.48)]. Paired analysis revealed significant differences between first and second ( $n = 15$ ,  $P = 0.008$ ), first and third ( $n = 8$ ,  $P = 0.008$ ), and second and third ( $n = 35$ ,  $P < 0.0001$ ) trimesters, as well as between third trimester and pp ( $n = 6$ ,  $P = 0.031$ ; Fig. 2).

The excretion of EpiT-G also changed significantly in the different trimesters (ANOVA,  $P < 0.0001$ ), peaking in the third trimester [median 158.9 nM (92.91 to 257.8)], with a marked decline after delivery [median 20.25 nM (13.04 to 26.17)]. When paired analyses were performed, differences between first and third ( $n = 9$ ,  $P = 0.027$ ), as well as second and third ( $n = 35$ ,  $P = 0.0002$ ) trimesters were observed (Fig. 2).

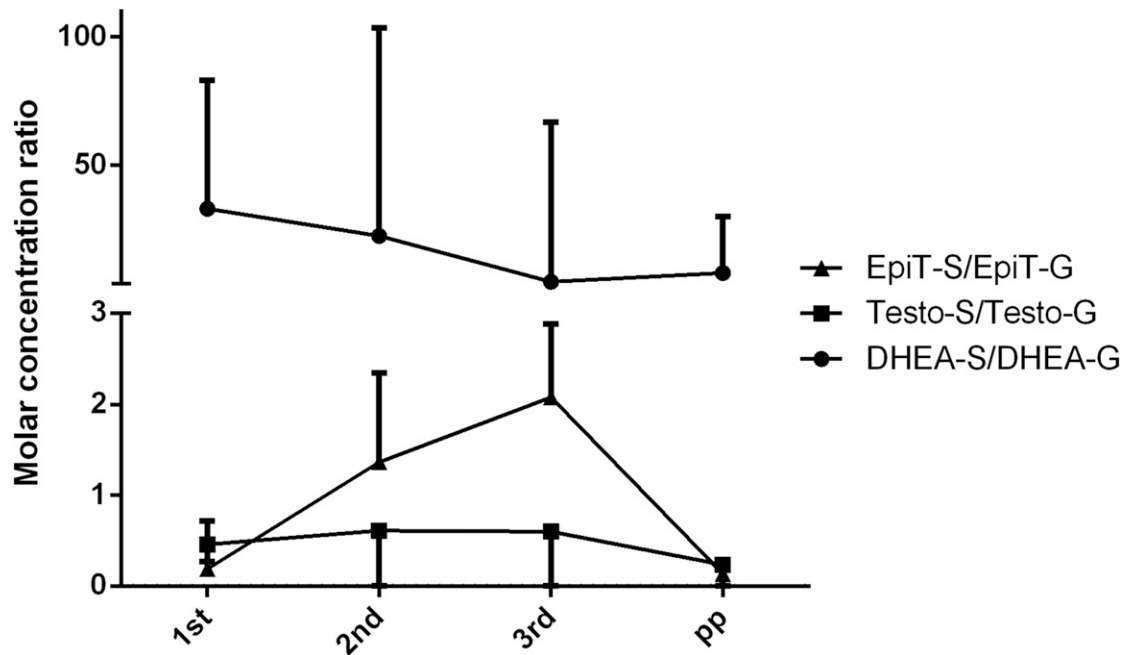
The urinary concentration of A-S decreased significantly throughout pregnancy (ANOVA,  $P = 0.037$ ; Fig. 2). However, there were no intraindividual changes in A-G when paired analysis was performed. Etiocholanolone (Etio) excretion was not affected by pregnancy.

DHEA-S decreased significantly throughout pregnancy (ANOVA,  $P = 0.01$ ) and was highest in the first trimester [median 2035 nM (574.2 to 5647); Fig. 2]. In contrast, there was no change in DHEA-G excretion during pregnancy.

The S/G urinary ratios reflect the preference of the conjugating enzymes for the different androgen substrates. This is depicted in Fig. 3 in which the medians of the ratios for EpiT (line with ▲), T (line with ■), and DHEA (line with ●) are compared in different trimesters and pp. The S/G ratio for DHEA was ~33 in the first trimester. The sulfation preference over glucuronidation was, by far, highest with DHEA as substrate (Fig. 3).



**Figure 2.** Urinary concentrations of androgens in the three trimesters and after delivery (pp). Note differences in scales of the y-axes. Left panels include G conjugates; right panels include S conjugates. Paired panels from top include EpiT, T, androsterone (A), etiocholanolone (Etio), and DHEA. The data are median values in box plots. \*\*\*\* $P < 0.0001$ , \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ .



**Figure 3.** Urinary molar ratios for S/G conjugates for EpiT, T (Testo), and DHEA in different trimesters and pp. Note the different scales on the y-axes. The data points indicate means, and the bars indicate SDs.

Interestingly, the abundance of S over G conjugate for EpiT increased more than two times in the third trimester, and it was three times higher than the S/G ratio for T in the second ( $P < 0.05$ ) and third ( $P < 0.0001$ ) trimesters, respectively (Fig. 3).

The urinary dihydrotestosterone (DHT)-G and DHT-S concentrations were very low and below the detection limit in 25% and 50% of the samples, respectively, in the first trimester. The corresponding numbers in the second and third trimesters were 32% and 23%, and 26% and 14%, respectively, and 57% pp. In patients with detectable concentrations, there was no variation in DHT-G or DHT-S throughout pregnancy. There was virtually no sulfation of DHT, as the average S/G ratio was only 0.058.

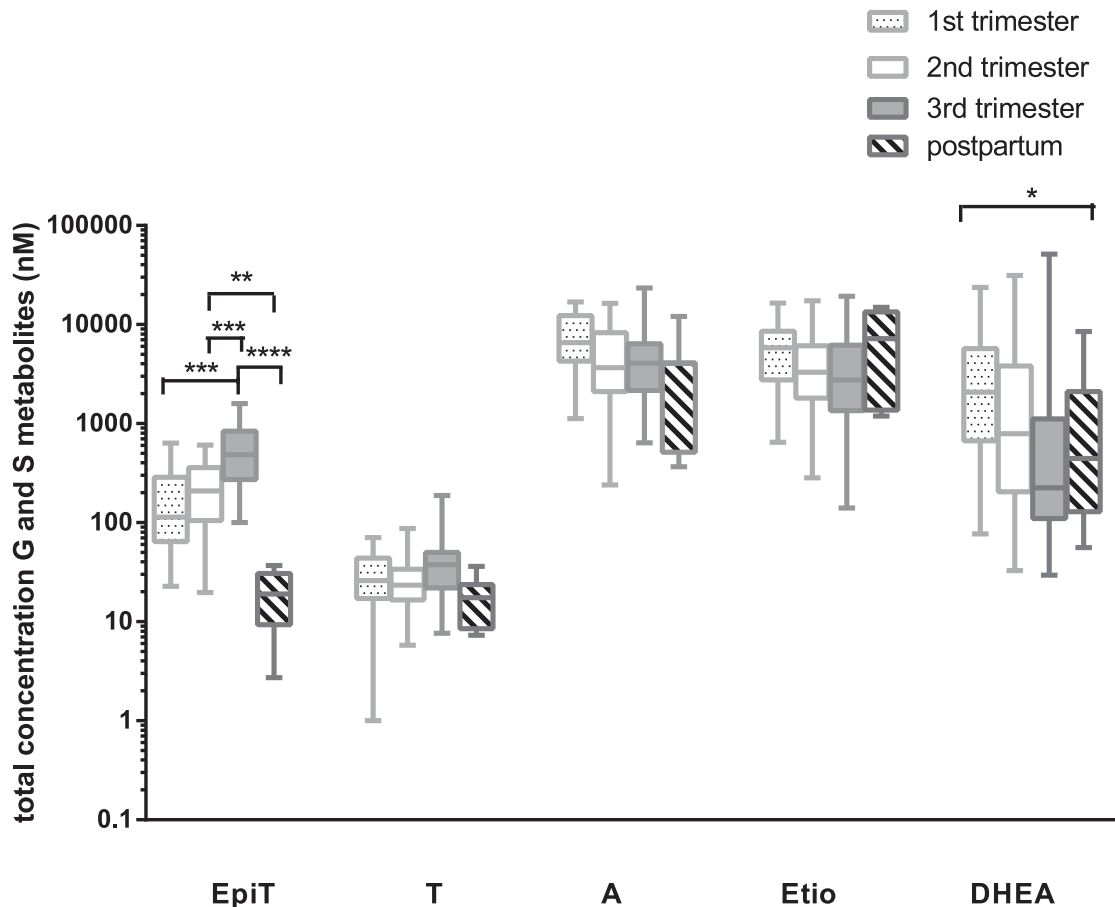
Total concentrations in different trimesters: the differences in the total urinary concentrations in different trimesters are depicted in Fig. 4. There is a conspicuous difference between EpiT and all other analytes, as EpiT is the only metabolite with a marked increase from the first to the third trimester. The excretion of all other androgens analyzed here decreased in pregnancy. Large differences between total concentrations (S and glucuronic acid conjugates combined) were observed, e.g., on one hand, the low concentrations of T and EpiT and on the other hand, the high concentrations of A, Etio, and DHEA.

### B. Urinary Excretion Profile in Relation to Clinical Parameters

Strong correlations between pregnancy BMI and excretion rates of all G were found in the first trimester (EpiT-G  $r = 0.82$ ,  $P = 0.0006$ ; T-G  $r = 0.84$ ,  $P = 0.0004$ ; DHT-G  $r = 0.81$ ,  $P = 0.007$ ; A-G  $r = 0.66$ ,  $P = 0.012$ ; DHEA-G  $r = 0.79$ ,  $P = 0.0013$ ; Etio-G  $r = 0.63$ ,  $P = 0.018$ ). For the S metabolites, only Etio-S correlated significantly with BMI ( $r = 0.65$ ,  $P = 0.01$ ). In the second trimester, no such correlations were found between the pregnancy BMI and urinary excretion of any metabolite.

### C. Urinary Excretion Profile in Relation to Ethnicity, Gestational Age, Birth Weight, and Sex

Attempts to relate ethnicity to the androgen-excretion profile were only partially possible, probably because of the small groups that were identified with certainty, namely 14 (out of 43) women of white and nine (out of 15) women of Asian origin.



**Figure 4.** Comparison of total molar urinary concentrations of androgens (S and G combined) in different trimesters and pp. Kruskal-Wallis, followed by Dunn's multiple comparisons test was performed. \*\*\*\* $P < 0.0001$ , \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ .

EpiT-G concentrations were lower in white [median 50.7 nM (25.07 to 103.2)] than in Asian women [median 102.0 nM (80.40 to 193.2);  $P = 0.02$ ; Fig. 5]. The other metabolites did not differ between the ethnic groups.

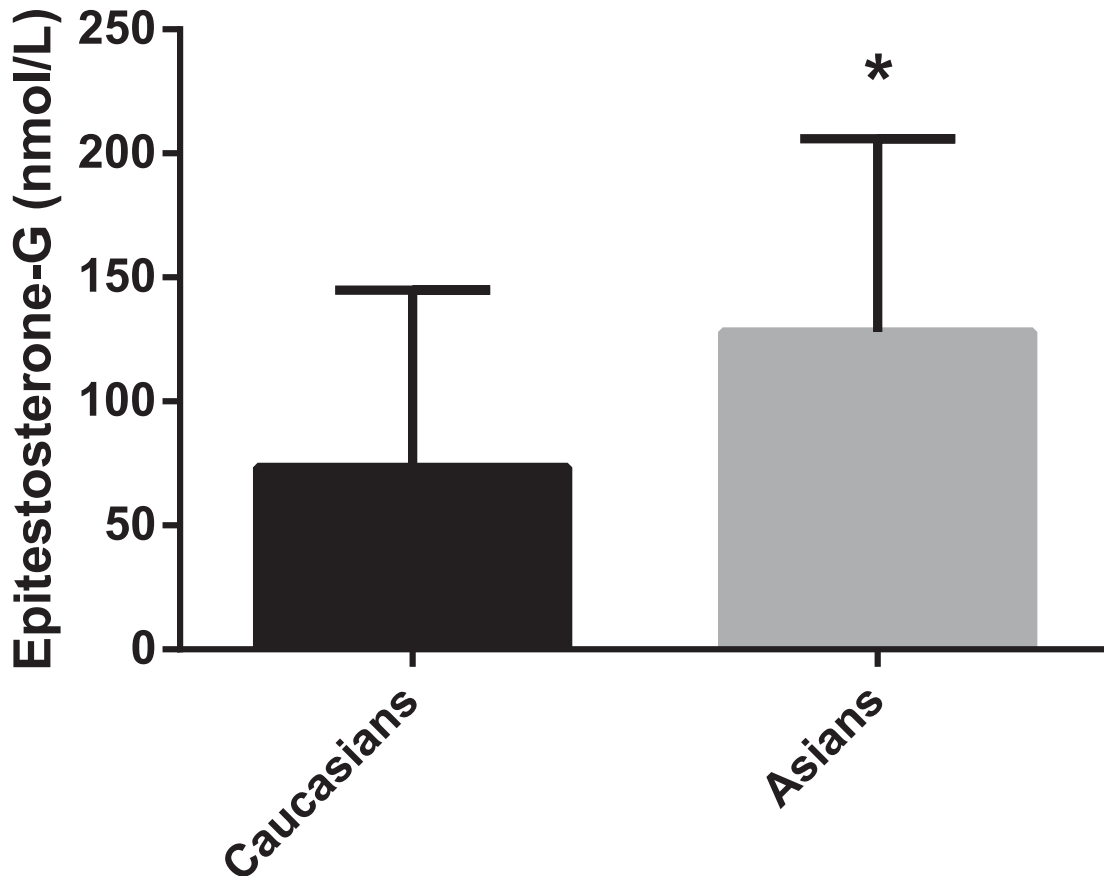
The sex of the fetus was not correlated to the urinary levels of the phase II androgen metabolites, except for EpiT-S in the second trimester, which was excreted at higher concentrations in the urine of women carrying a male fetus (median  $67.7 \pm 36.5$ ) compared with female fetuses (median  $38.4 \pm 40.8$ ;  $P = 0.02$ ).

There was no correlation between urinary concentrations of any metabolite and birth weight, or week of delivery (data not shown).

### 3. Discussion

The role and importance of androgens in pregnancy are less known than those of estrogens or progestogens, and only little interest to this research area has been devoted. Here, we have studied the urinary excretion profile of T, its epimeric congener EpiT, some precursors, and several metabolites of T in pregnancy and pp (Fig. 1).

The most interesting finding was a conspicuous and marked increase of EpiT from the first to second and from the second to third trimester. This excretion pattern was not seen for any other androgens measured. The physiological role of this steroid is virtually unknown, but on the basis of animal experiments, it has been suggested to have a putative antiandrogenic effect, thus being able to moderate the androgenic effects mediated via the androgen receptor



**Figure 5.** EpiT urinary concentrations in women of Caucasian, white (median 40.8 nM; range 8.14 to 183.7 nM) or Asian (median 102.0 nM; range 9.1 to 272.3 nM;  $P = 0.008$ ) origin. \* $P < 0.05$ .

[4]. EpiT may have a physiological role, or it may simply be a marker or a byproduct in the formation of other endogenous steroids. Its close similarity to T, with the 17-OH isomerism as the only difference, makes the atypical excretion profiles in pregnancy an interesting question.

The mechanism for the EpiT rise in pregnancy is unclear. The dissimilarity in excretion profiles lends further support to the assumption that these steroids use different formation pathways despite their structural similarity. Various pieces of information point to a key role of cytochrome P450 (CYP)17 for the synthesis of EpiT. Earlier findings in our group [14] showed that a promoter polymorphism in the *CYP17* gene is related to the excretion of EpiT and to androstene- $3\beta$ 17 $\alpha$ -diol, which is a putative precursor of EpiT. This is consistent with earlier supposition and experimental evidence of a separate formation pathway for EpiT [15]. Furthermore, Weusten *et al.* [16] showed that androstene- $3\beta$ 17 $\alpha$ -diol is synthesized from pregnenolone in a single step, most probably by direct action of CYP17, and as androstene- $3\beta$ 17 $\alpha$ -diol is the precursor of EpiT, it is conceivable that the unique excretion profile of EpiT is dependent on a separate regulatory mechanism of the CYP17 pathway. Given this background, it seems logical that the pregnancy-induced early rapid rise in progesterone would result in similar changes in EpiT, as EpiT is a metabolite formed by CYP17 [14] via 17 $\alpha$ -hydroxylation of pregnenolone. This possible metabolic explanation of the systemic increase in EpiT is in line with the marked elevation in progesterone and estrogen concentrations through increased synthesis, preferentially by the placenta (progesterone) but also by the fetus (estradiol). Progesterone and estrogens are also elevated in the luteal phase of the menstrual cycle in parallel with elevated EpiT concentrations [13].

Differences in metabolism of EpiT and T may also contribute to the unique behavior of EpiT in pregnancy. Early studies of the role of the position of the OH group for transacylation



and sulfatase hydrolysis demonstrated that a  $17\alpha$  position makes EpiT a less favorable substrate than steroids with  $3\beta$ -,  $17\beta$ -, or phenolic OH groups [17]. Although these results were generated with microbial steroid esterase, a similar mechanism may exist in humans, particularly in pregnancy, yielding an accumulation of EpiT-S in the circulation and an increased urinary excretion.

Our results have demonstrated stable excretion rates of T-S and T-G (total rate) throughout pregnancy, although at a higher level than postpregnancy. A previous case report of three pregnant women also demonstrated a rise of T-G, but no information on S conjugates was included [18]. The report included only three women, but one of them had spuriously low, close-to-unmeasurable concentrations. In the residual two women, the concentrations in all trimesters were in the same range (5.8 to 13 ng/ml) as in our 43 women (total S + G fractions: 1 to 36 ng/ml).

The large variation in urinary androgen metabolite excretion is an interesting result in itself, as such interindividual variation is unusual among biochemical physiological parameters in healthy individuals. However, we have previously described similar variation in many androgens in healthy men [5], largely as a result of genetic polymorphisms in androgen-metabolizing enzymes [19]. Interestingly, women with initially high excretion seemed, in most cases, to maintain this position among all women throughout pregnancy and *vice versa*.

The two major conjugation pathways catalyzed by uridine diphosphate glucuronosyl transferase(s) and sulfotransferase have different preferences for T and EpiT as substrates. The sulfation pathway was much more important for EpiT than the glucuronidation during pregnancy (Fig. 2). In nonpregnant women, 4% to 52% of EpiT is excreted as S conjugates [13]. The relations (quotients) between different metabolites give some information about pregnancy physiology. As seen in Figure 3, the S/G ratio for EpiT increased continuously in the trimesters, showing that EpiT is a preferred substrate of the sulfotransferase enzyme. The preference for sulfation of DHEA was even more pronounced, with a +30-fold difference in the first trimester (Fig. 3). This is in contrast to T, which was excreted, preferably as G, without any significant change among the trimesters. The T S/G ratio was only  $\sim 0.75$ .

As predicted, DHEA-S decreased continuously in pregnancy (Fig. 4). This may partly be ascribed to the intense synthesis of estrogens by the placenta, with one-half of the precursors from the mother and one-half from the fetus, altogether leading to a marked rise in estrogen concentrations from  $\sim 0.4$  nM to 50 to 100 nM in the pregnant state.

The predominance of sulfation conjugation of many androgens in the pregnant state is believed to have a metabolic explanation rather than a difference in renal excretory mechanisms for S and G conjugates. This phenomenon has an interesting clinical correlate in the newborn baby in which the degree of sulfation of several drugs is higher than in adult life. This has been demonstrated in newborns for morphine [20], ritodrine [21], paracetamol [22], and other drugs that switch postnatally from sulfation to glucuronidation. Moreover, the S-over-G conjugation preference was also demonstrated *in vitro* in human fetal liver in our laboratory. We were able to demonstrate sulfation, but not glucuronidation, with acetaminophen as substrate [23]. Thus, the fetomaternal endocrine interior seems to include major metabolic alterations both for endobiotics and xenobiotics.

Strong correlations between the G metabolites and BMI were noted in the first trimester. The reason that the G metabolites, but not the S metabolites, are associated with BMI may be that androgen G metabolites better reflect the androgen activity in women [12]. Our group [24] and other investigators [25] have also found that the activities of uridine diphosphoglucuronosyl transferases are associated with body composition in men. It is believed that the glucuronidated androgens reflect the circulatory levels to a higher degree than the S metabolites [3].

The EpiT excretion was associated with ethnicity, as the excretion rate of EpiT-G was higher in the Asian women than in white women. In our original study [5], comparing men with Asian and Caucasian origin, no difference in EpiT concentration was noted. To our knowledge, such data have not been published for women. It is possible that this ethnic association is only seen in female populations and/or among pregnant women.

There is more information on T concentrations in serum than in urine from pregnant women. An early study demonstrated only a 1.7-fold increase in serum total T concentration

from week 5 to the end of pregnancy [26]. In a study from 2012, Järvelä *et al.* [27] found no change in serum T concentration in the first 12 weeks of pregnancy. Therefore, it seems that this important androgen has marginal dynamic change in relation to the maintenance of pregnancy in the different phases, and not to the extent of estrogens and progesterone.

The nonsexual-related role of T in pregnancy is still fairly unexplored. However, recent research has demonstrated an androgen-mediated endothelial regulation of vascular tonus in pregnant rats [28] leading to an increase of the arterial pressure and blunting of the endothelial-dependent vascular relaxation induced by acetylcholine or by nitric oxide (NO). These findings are in concert with our studies in healthy individuals [29] showing that a single dose of T significantly decreased the urinary NO and inhibited the gene expression of endothelial NO synthase in human vascular endothelial cells.

The role of androgens in pathological conditions, such as polycystic ovarian syndrome, has been subject to much attention [30]. Women with polycystic ovary syndrome or hyperandrogenism were, however, excluded from participation in the study, as the aim was to establish data on androgens levels in normal pregnancy in healthy women. It would, however, be interesting to study the concentrations of T and EpiT and the ratios thereof in pregnancy disorders supposed to be associated with the endocrinology of androgens.

There are also reasons to believe that androgens are related to preeclampsia, although their role in regulating endometrial function and vascular function in pregnancy is poorly understood [31]. Androgen receptors are expressed in the endometrium, and androgens could negatively regulate placental oxygenation and may oppose the progesterone/estrogen-mediated decrease of the resistance in the uterine arteries [32]. In view of this, the previously mentioned androgen-mediated endothelial regulation of vascular tonus [28, 29] is highly interesting. Given this background, the rise of EpiT in pregnancy may serve to moderate the effects of androgens on the vascular resistance at the androgen receptor.

In summary, androgen endocrinology in pregnancy has not been extensively explored. This work demonstrates a conspicuous excretion pattern of EpiT that is an epimer of T with an unknown physiological role. Its marked rise in the second to third trimesters and its unique preference for sulfation rather than glucuronidation are in contrast to any other androgen or androgen metabolite studied. Whether EpiT is a parallel byproduct of other key steroids, or its secretion and excretion pattern is an independent, unique phenomenon remains to be studied. Its putative role in the vascular regulation in the feto-maternal unit warrants further investigations.

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**Disclosure Summary:** The authors have nothing to disclose.

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