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MATERNAL TESTOSTERONE AND FETAL SEX

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Summary—To investigate the influence of fetal sex on maternal testosterone levels throughout pregnancy, blood was sampled from 37 healthy pregnant women from week 14 until term and at 6 weeks postpartum. Testosterone concentrations were measured with a highly specific RIA after chromatographic purification. Mean (±SD) testosterone at the end of gestation was significantly higher compared to non-pregnant values (3.10 ± 2.38 nM/l, $n = 32$ vs 1.14 ± 1.06 nM/l, $n = 35$). It appeared that in women carrying a male fetus testosterone levels gradually increased during pregnancy up to 3.99 ± 2.72 nM/l. In women carrying a female fetus the levels decreased after the first trimester from 2.44 nM/l to 1.80 nM/l. A statistically significant difference ($P < 0.01$) existed in maternal testosterone concentrations between both groups during the second half of pregnancy.

INTRODUCTION

In the human fetus sex differentiation starts with the evolution of the testosterone synthesizing enzyme system at week 6–8 of pregnancy, reaching a maximum activity at week 12–14, coinciding with the differentiation of the urogenital organs [1, 2]. Fetal production of testosterone is for the greater part limited to the testis with negligible synthesis occurring in adrenals and ovaria [3–6].

The fetal testis remains productive until term, with differing activities of testosterone synthesizing enzyme systems in the course of prenatal life [3, 5, 7–9]. Sex-related differences in androgen concentrations have been reported to exist in amniotic fluid, the umbilical cord and neonatal blood [10–13].

Several attempts have been made to predict fetal sex from maternal testosterone levels indicating the supposition of an influence of fetal testosterone levels on maternal testosterone levels. In 1978 Klinga *et al.* [14] could demonstrate higher maternal testosterone in women carrying a male fetus compared with women carrying a female fetus in the first half of pregnancy. In the present study maternal testosterone was measured with highly specific assay from week 14 until term to determine any effect of fetal sex on maternal testosterone concentrations during gestation.

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MATERIALS AND METHODS

Subjects and sampling

The study group consisted of 37 healthy pregnant women, attending the gynecologic clinic of the St Jozef Hospital in Doetinchem. All participants gave informed consent. Blood was routinely sampled 4 times during gestation between week 14 until term and at 6 weeks postpartum. The sampling scheme was: week 14–19 = period 1, $n = 37$; week 20–26 = period 2, $n = 36$; week 27–34 = period 3, $n = 36$; week 35–40 = period 4, $n = 32$; 6 weeks postpartum = period 5, $n = 35$. Blood was drawn in heparinized tubes, centrifuged (5 min at 1500 g) and the plasma was stored at −20°C until analysis.

Methods

Extraction and chromatography. To enhance the specificity of the testosterone assay plasma samples were extracted and chromatographically purified prior to RIA. To 0.2 ml of plasma was added approx. 10,000 dpm [1,2,6,7-^{3}H]-testosterone (102 Ci/mmol, New England Nuclear Corp., Boston, MA) as a recovery tracer. After equilibration for at least 2 h the plasma was extracted with 15 ml diethylether. The extracts were applied to Whatman No. 1 paper lanes and chromatographed for about 2.5 h in a descending Bush B3 solvent system (petroleum ether 80–10°–toluene–methanol–water 133:167:400:100, by vol.). All

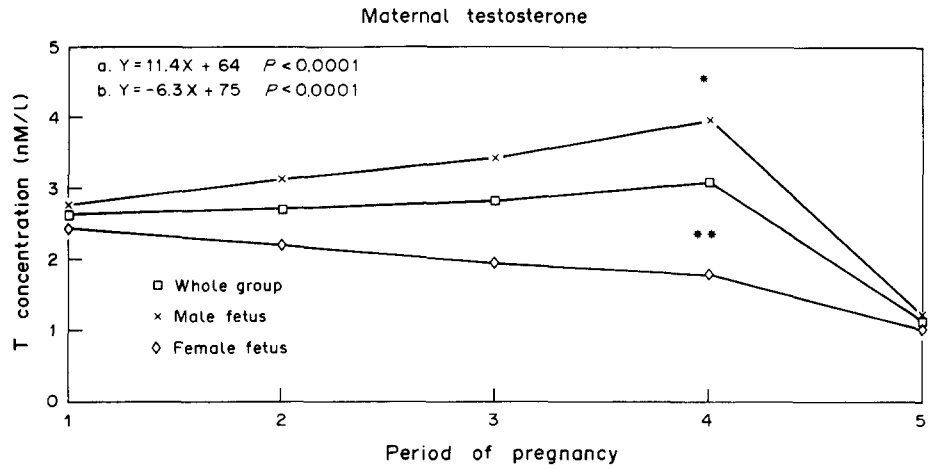

Fig. 1. Mean values (nM/l) of maternal testosterone concentrations per period of pregnancy. Period 1 = week 14–19, period 2 = week 20–26, period 3 = week 27–34, period 4 = week 35–40, period 5 = 6 weeks postpartum. Regression equation: a, male fetus; b, female fetus. *Mean concentration significantly different from period 1 ($P < 0.0002$, t-test). **Mean concentration significantly different from period 1 ($P < 0.001$, t-test).

chromatography solvents were of "Baker-Analyzed" grade (J. T. Baker Chemical Co., Phillipsburg, NJ). After chromatography and location of the steroid with radioactivity scanning the appropriate areas were isolated and eluted in 1.5 ml 0.2% ethyleneglycol in water. The eluates were used for recovery counting and RIA.

RIA. Standards of testosterone (300 μl in duplicate), ranging from 2–500 pg/test tube, and eluates (300 μl in triplicate) were incubated with 100 μl [^{3}H]testosterone ($\approx$8000 dpm) and 100 μl antiserum, both in assay buffer (0.1 M borate buffer, pH 8.0, containing 0.5% BSA). The antiserum (titer 1:99,000) was raised against testosterone–7α-carboxyethyl-thioether–BSA, kindly provided by Dr Pratt (Groningen, The Netherlands). After overnight incubation at 4°C the bound and free fractions were separated with dextran-coated charcoal. The detection limit of the assay was 1 pg/tube. Intra- and inter-assay coefficients of variation were 4.5 and 7.2%, respectively. Concentrations calculated were corrected for procedural losses.

Statistics. Results are presented as mean $\pm$ SD. Data were evaluated by Student's t-test, Anova Bonferoni t-test and regression analysis using the SAS statistical software (SAS Institute Inc., Cary, NC).

RESULTS

The results of the testosterone determinations in maternal plasma are depicted in Fig. 1. In the course of pregnancy the mean concentrations are significantly raised above those of the postpartum period ($P < 0.05$). In the group of women carrying a male fetus (M-group) testosterone significantly increased from a mean ($\pm$SD) level of 2.77 ± 1.12 nM/l in period 1 to 3.99 ± 2.72 nM/l in period 4, the regression equation being $Y = 11.4\ X + 64$ ($P < 0.0001$). The difference between the mean concentrations in the first and the fourth period was statistically significant ($P < 0.0002$, t-test). In the group of women carrying a female fetus (F-group) the mean testosterone level declined significantly from 2.44 ± 1.46 nM/l to 1.80 ± 0.71 nM/l, the regression equation being $Y = -6.3X + 75$ ($P < 0.0001$). The difference between the mean concentration in the first and the fourth period was statistically significant ($P < 0.001$, t-test).

Comparison of the mean testosterone concentrations per period between the M- and the

Table 1. Values of maternal testosterone (nM/l, mean $\pm$ SD) during pregnancy

Time of pregnancy	Male fetus	n	Female fetus	n	P-value[a]
Period 1 (W14–19)	2.77 ± 1.12	21	2.44 ± 1.46	16	NS[b]
Period 2 (W20–26)	3.14 ± 1.16	20	2.20 ± 0.51	16	<0.01
Period 3 (W27–34)	3.45 ± 1.37	21	1.95 ± 0.49	15	<0.005
Period 4 (W35–40)	3.99 ± 2.72	19	1.80 ± 0.71	13	<0.01
Period 5 (W6 post partum)	1.22 ± 1.25	21	1.02 ± 0.73	14	NS

[a](Students t-test) signify the difference between both groups.
[b]Not significant.

F-group revealed a statistically significant difference (Student's t-test) in period 2, 3 and 4 ($P < 0.01$, $P < 0.005$ and $P < 0.01$, respectively). Throughout gestation the mean testosterone concentration in the M-group was higher than in the F-group.

DISCUSSION

The rise in testosterone levels in maternal plasma during the course of pregnancy is in agreement with earlier data obtained by others [15–17]. From the first sampling period (week 14) until term testosterone concentrations were significantly higher than at 6 weeks postpartum ($P < 0.05$, Bonferoni).

To investigate any influence of fetal sex on maternal testosterone concentrations, the study group was divided into an M-group and an F-group, indicating women carrying a male or a female fetus, respectively. It appeared that in the M-group the mean level of testosterone steadily and significantly ($P < 0.0001$) increased until term. In contrast, in the F-group it declined during the course of pregnancy ($P < 0.0001$).

Comparing both groups, the difference between testosterone levels was statistically, significant in the second half of pregnancy (period 2, 3 and 4). This is in contrast with results obtained by Klinga *et al.* [14] who demonstrated higher testosterone levels in women carrying a male fetus only in the first 20 weeks of pregnancy. No sex related difference was found by other investigators [16–18].

Increased testosterone levels in pregnant women can be accounted for by an estrogen induced increase in SHBG (sex hormone binding globulin) levels [19–21]. As a consequence of the higher binding capacity for testosterone, the fraction of unbound testosterone in plasma declines, resulting in a lower metabolic clearance rate. According to Bamman *et al.* [22] this holds true until week 28 of gestation and unbound testosterone concentrations remain in the normal range during this period. After that time levels of unbound testosterone exceed non-pregnant values, suggesting a fetal source of elevated testosterone in maternal blood. Passage of testosterone from the fetus into the maternal circulation in spite of a gradient in total testosterone concentration in the opposite direction, can be explained by differences at the level of the unbound fraction. Levels of unbound testosterone exhibit a maternal–placental–fetal gradient with higher fetal concentrations compared with maternal concentrations [22]. This can be explained by SHBG concentrations being 10–13 times higher in the maternal than in the fetal compartment [20, 23, 24]. It is the unbound fraction which is potentially capable of crossing the placental barrier. Apart from the fraction which is metabolized in the placenta [25], fetal unbound testosterone has been indicated to be a source of increasing testosterone levels in maternal plasma [8, 22, 23].

In cord blood sex-related differences exist at the level of SHBG, total plasma testosterone and unbound plasma testosterone, all exhibiting higher levels in boys than in girls [23]. Hence, any influence of fetal testosterone upon maternal testosterone will be greater in women carrying a male fetus than in women carrying a female fetus.

The fetal sex-related differences indicate that as a consequence of a maternal–fetal gradient unbound testosterone crosses the placenta from the male fetus toward the maternal circulation, whereas the opposite direction applies to a female fetus. In addition, Forest *et al.* [10] showed that unbound testosterone levels in male newborns were significantly higher than those in normal adult women, whereas in newborn girls they were lower. Further support for our findings can be deduced from data showing that in term male infants testosterone concentrations in the umbilical artery are significantly higher than in the umbilical vein [8, 23, 25]. The observed differences indicate a passage of testosterone from the fetal to the maternal compartment. However, in term female infants both levels are not significantly different [23].

In conclusion, the results of this study document that fetal sex affects maternal testosterone levels in the course of pregnancy. Referring to the results of Bamman *et al.* [22] increasing testosterone levels caused by a male fetus may be explained by a gradient from the fetal to the maternal compartment at the level of plasma unbound testosterone. In contrast, in the female fetus a flow of testosterone in the opposite direction is possibly caused by much lower testosterone and SHBG concentrations compared to those in maternal blood. Extended research is needed to demonstrate the degree to which the fetus influences maternal hormone concentrations.

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