****

Batch Name: 166355

Batch Creator: SWORD 1

Batch Creation Date: 6/15/2009

Batch Creation Time: 14:57:39

Number of Pages: 10 [All]

Printed by: SWORD 1

Print Date: 6/15/2009

Print Time: 15:1:32

****

166355

by sigerson.sword.org (8.13.4/8.13.4/Debian-3) with ESMTP id n5FGVOAj002901
by spamfirewall.muohio.edu (Spam Firewall) with SMTP id 60A56F18DC07
Content-transfer-encoding: 7BIT
(Sun Java(tm) System Messaging Server 6.3-8.01 (built Dec 16 2008; 64bit))
id <0KLA00300G7IHB00@sms3.wright.edu> for requests@sword.org; Mon,
15 Jun 2009 12:27:24 -0400 (EDT)
by sms3.wright.edu
(Sun Java(tm) System Messaging Server 6.3-8.01 (built Dec 16 2008; 64bit))
with ESMTP id <0KLA000D2GDN4220@sms3.wright.edu> for requests@sword.org; Mon,
15 Jun 2009 12:27:23 -0400 (EDT)
Date: Mon, 15 Jun 2009 12:27:26 -0400
From: Fordham Interlibrary Loan <fill@www.libraries.wright.edu>
Subject: Please fill request
To: requests@sword.org
Rule breakdown below
pts rule name description
---- ---------- ----------

This request has been forwarded from ILL by barb.

Please fill this request for FORDHAM HEALTH SCIENCES LIBRARY

166355

Call Number: 81354130506

Journal Title: Acta Endocrinologica
Journal Vol: 80
Journal Issue: 3
Journal Year: 1975
Article Title: VARIATIONS IN THE CONCENTRATION OF TESTOSTERONE IN PERIPHERAL VENOUS PLASMA FROM HEALTHY WOMEN
Article Author: John P. P. Tyler
Article Pages: 542-550

Customer Information:

Name: Glaser, Rebecca
Status: Faculty
Address: SOUTHVIEW (via Kettering Hosp),
Site:
E-Mail Address: rglaser@woh.rr.com
Phone: 937-885-4555
Department: School of Medicine

9 pp scanned 06-15-09

ACTA ENDOCRINOLOGICA
80 (1975) 542–550

Department of Obstetrics and Gynaecology
and WHO Collaborating Centre for Clinical Research
in Human Reproduction, King's College Hospital
Medical School, Denmark Hill, London, S. E. 5

VARIATIONS IN THE CONCENTRATION OF TESTOSTERONE IN PERIPHERAL VENOUS PLASMA FROM HEALTHY WOMEN

By

John P. P. Tyler, John R. Newton
and William P. Collins

ABSTRACT

The concentration of testosterone (ng/100 ml; mean ± SD) has been determined in peripheral venous plasma at 4 hourly intervals for 24 h from 9 non-pregnant women (39.8 ± 11.8) and 9 during late pregnancy (70 ± 32). In addition, the level of this hormone has been measured in samples of plasma removed daily (between 08.30 and 10.00 h) throughout 9 ovarian cycles (41.6 ± 11.8), and weekly from 10 women during gestation (57.6 ± 14.7).

The results show that there is considerable individual variation in both the concentration and pattern in serial samples over the respective time scales. The values of the arithmetic mean from non-pregnant subjects show that there is a tendency for the concentration to be lower between 20.00 and 04.00 h. However, the differences between the values at defined times over a 24 h period are not statistically significant. During the ovarian cycle 3 subjects had peak values during the peri-ovulatory phase, 2 in the luteal, 1 in the follicular and 3 showed no consistent pattern. There was no significant difference in values (40.9 ± 11.8, 41.9 ± 11.9 and 41.8 ± 11.9) for the 3 phases of the cycle. There is a significant rise in the concentration of plasma testosterone during pregnancy ($P < 0.0005$), but the pattern of the arithmetic mean is not related to the corresponding values for progesterone or oestradiol. During late pregnancy (34 weeks to term) there is a change in the nycterohemeral pattern, with the highest mean values occurring between 16.00 and 04.00 h. The results are discussed.

Although the technique of radioimmunoassay has become established as the method most favoured for the determination of testosterone in biological fluids, the opportunity that it affords for extensive studies on reproductive endocrinology has not been exploited. Consequently there has not been the expected increase in knowledge regarding the physiological role of this compound in women.

There are many interrelated reasons for the lack of precise information regarding the pattern and significance of changes in the concentration of testosterone in peripheral venous plasma from women throughout the reproductive cycle. Frequently the mean and range of values have been determined from the analysis of single samples of plasma from relatively few women, who were presumed to have no endocrine dysfunction. Furthermore, the time at which the samples were removed has been invariably related to the process of menstruation, rather than to changes in the level of other hormones from either the pituitary or the gonads.

An attempt has been made to overcome some of these problems, and the present study has involved the determination of testosterone, oestradiol, progesterone, follicle stimulating hormone (FSH), luteinising hormone (LH) and cortisol in serial samples of peripheral venous plasma from well defined groups of women. In this paper results are presented on the nycterohemeral variation of plasma testosterone in 18 subjects, on the daily variation throughout 9 ovarian cycles and 10 uncomplicated pregnancies studied at weekly intervals from the 26th week to term.

EXPERIMENTAL

Subjects

Thirty-seven women (aged 26 to 34 years) volunteered to participate in the study. The non-pregnant subjects had a history of reasonably regular menstrual cycles, and no apparent general medical or gynaecological disease. Nine were admitted to hospital, and peripheral venous blood was removed every 4 h throughout a 24 h period. The samples were taken through an indwelling catheter that had been inserted into an antecubital vein. A further 9 volunteers were bled by venepuncture between 08.30 and 10.00 h every day throughout a complete menstrual cycle. The results from the analyses of the latter samples demonstrated that every subject had a biphasic pattern of oestradiol, a midcycle peak of LH, and levels of progesterone that were greater than 5 ng/ml during the luteal phase of the cycle. Ten pregnant subjects were chosen at random from an antenatal clinic that was held in the morning. Blood samples were taken at fortnightly intervals from weeks 26 to 36 after the last menstrual period and then every week until term. An additional 9 subjects, who were at least 34 weeks pregnant, had blood samples removed every 4 h (at the same times as the non-pregnant women) over a 24 h period.

Method

The concentration of testosterone in peripheral venous plasma was determined in a liquid phase radioimmunoassay system. Tritiated testosterone (S. A. 100 Ci/mmol) was used as the labelled antigen, and the antiserum was raised against testosterone-3-carboxymethyl oxime-bovine serum albumin. The steroid bound to antibodies was precipitated with a mixture of ammonium and calcium sulphates, and the precipitate was resuspended for liquid scintillation counting in the assay tube. Under these conditions the cross-reaction with 5α-dihydrotestosterone was less than 5 %, and the overall cost of each determination was reduced by up to 70 %. The method has been described and evaluated in detail (*Tyler et al.* 1973).

RESULTS

Nycterohemeral variation: non-pregnant women

The individual values for the 9 subjects are listed in Table 1, and the pattern of the arithmetic mean ± SE (NP) is shown in Fig. 1. The lowest mean value at 04.00 h is 77 % of the maximum value at 08.00 h, and there is a progressive decrease in the mean concentration throughout the day. There is no significant difference (Student's t-test) between the concentrations at any of the defined times. The overall concentration (mean ± SD) is 39.8 ± 11.8 ng/100 ml.

Table 1.
The concentration (ng/100 ml) of testosterone in serial samples of peripheral venous plasma from adult women.

Subject	Time (h) 08.00	12.00	16.00	20.00	24.00	04.00	08.00
1	41	35	26	51	37	28	-
2	38	37	66	46	48	34	52
3	66	63	–	41	34	80	35
4	41	35	27	26	25	29	41
5	42	–	37	33	37	38	38
6	45	39	57	–	39	26	62
7	42	45	46	35	27	26	27
8	63	76	56	43	52	24	57
9	39	37	39	–	34	37	35
Mean	46.3	45.9	44.2	39.3	37.0	35.7	43.4
± Standard deviation	10.5	15.3	14.6	8.5	8.7	17.3	12.2
Coefficient of variation (%)	22.0	33.0	32.0	21.0	23.0	48.0	28.0

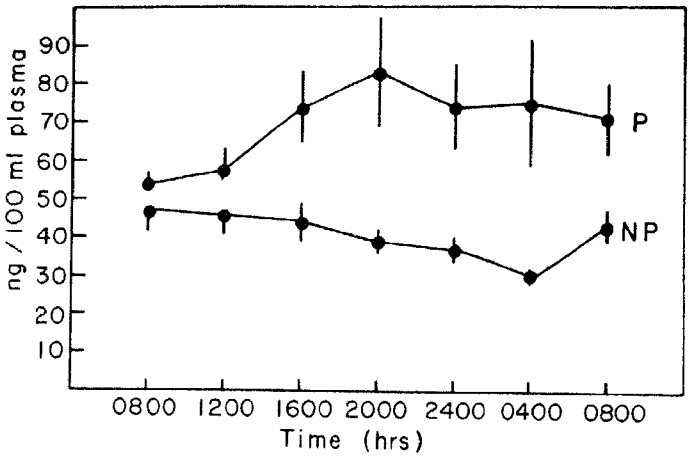


Fig. 1.
The concentration (mean ± SE) of testosterone in peripheral venous plasma throughout a 24 hour period. P is the pattern from 9 women during the 3rd trimester of pregnancy, and NP the corresponding pattern from 9 non-pregnant subjects.

Daily variation during the ovarian cycle

The mean and range of values for plasma testosterone from 9 subjects throughout one complete ovarian cycle is shown in Fig. 2. The sample col-

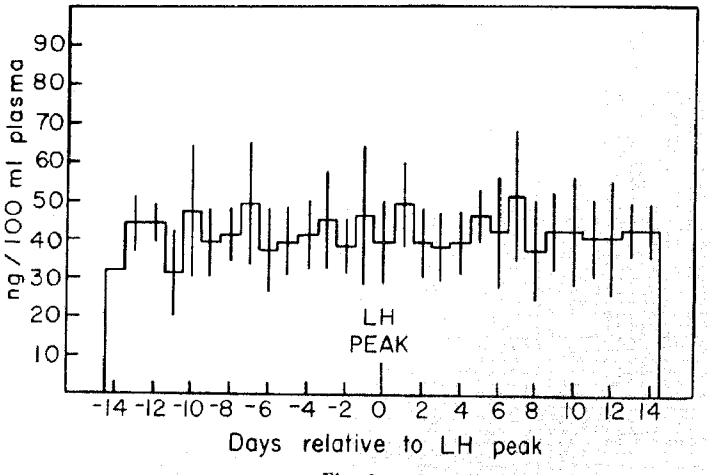
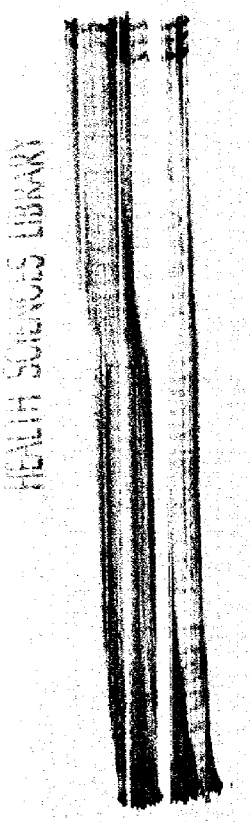


Fig. 2.
The concentration (mean ± SD) of testosterone in daily samples of peripheral venous plasma throughout 9 ovarian cycles.

lection was started on the first or second day of menstrual bleeding, and continued until the first day of the subsequent cycle. The values, however, are plotted relative to the peak concentration of LH (Day 0) in the same samples of plasma. Accordingly, days –4 to –14 refer to the follicular phase of the ovarian cycle, and days 4 to 14 to the luteal phase. The period –3 to 3 is designated as the peri-ovulatory phase.

The concentration (ng/100 ml; mean ± SD) of testosterone in peripheral venous plasma during the follicular phase is 40.9 ± 11.8, during the peri-ovulatory phase 41.9 ± 11.9, and throughout the luteal phase 41.8 ± 11.9. There is no significant difference in the values during the three periods. The mean concentration for the complete study is 41.6 ± 11.8.

If the pattern for every cycle is viewed independently, then 3 subjects had peak values in the peri-ovulatory phase, 2 in the luteal, and 1 in the follicular phase. However, the raised values are not significantly different from the values during the other parts of the same cycle. In the remaining 3 subjects the results fluctuated in a random manner.



Changes throughout gestation

The concentration (mean ± SD) of plasma testosterone throughout pregnancy in 10 subjects is shown in Fig. 3. There is no apparent correlation between the level of testosterone and the number of weeks of gestation. However, the overall mean concentration during pregnancy (57.6 ± 14.7 ng/100 ml) is signi-

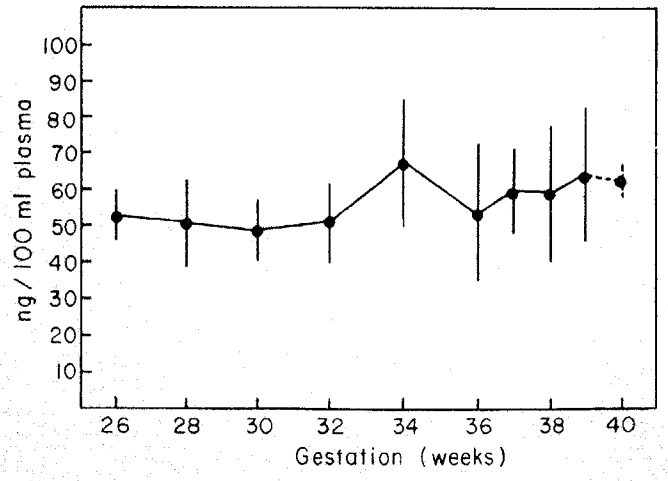


Fig. 3.
The concentration (mean ± SD) of testosterone in peripheral venous plasma from 10 women throughout pregnancy.

ficantly higher ($P < 0.0005$, Student's t-test) than in non-pregnant women. There is no correlation between the concentration of testosterone in peripheral venous plasma from the mother, and the birth weight or sex of the baby.

Nycterohemeral variation: late pregnancy

The individual values for testosterone in plasma samples removed every 4 h from 9 pregnant subjects prior to labour are shown in Table 2. The pattern of the arithmetic mean ± SE (P) is shown in Fig. 1. The lowest mean concentration is 65 % of the maximum value at 20.00 h. There is no significant difference between the concentrations of plasma testosterone at different times, and the individual variation in both level and pattern is considerable. The overall concentration (mean ± SD) is 70 ±32 ng/100 ml.

DISCUSSION

There are conflicting reports in the literature regarding changes in the concentration of testosterone in serial samples of peripheral venous plasma from healthy women. For example, previous studies on samples removed throughout

Table 2.
The concentration (ng/100 ml) of testosterone in serial samples of peripheral venous plasma from women during the third trimester of pregnancy.

Subject	Time (h) 08.00	12.00	16.00	20.00	24.00	04.00	08.00
1	73	89	104	154	89	118	77
2	55	56	81	68	59	55	73
3	54	46	53	62	53	56	64
4	45	48	56	128	108	59	113
5	42	41	51	45	42	50	47
6	57	87	119	54	143	58	113
7	55	45	48	40	65	34	48
8	51	47	102	59	52	191	54
9	52	58	55	133	53	54	50
Mean ± Standard deviation	53.8	57.1	74.3	82.6	73.8	75.0	71.0
Coefficient of variation (%)	8.7	18.2	27.6	43.2	33.2	49.2	26.1
	16.2	31.8	37.1	52.3	45.0	65.8	36.8

the ovarian or menstrual cycle have shown that the highest values may occur during the follicular phase (*Lloyd et al.* 1966), the peri-ovulatory phase (*Judd & Yen* 1973; *Abraham* 1974), or the luteal phase (*Coyotupa et al.* 1972). The results from the present study on 9 subjects have confirmed that the pattern is variable, and that the fluctuations are small. Only 1 of the subjects had peak levels during the follicular phase, 3 had raised values during the peri-ovulatory period, and 2 during the luteal phase. The 3 remaining subjects showed no obvious pattern. A statistical analysis of the results established that there was no significant difference between any of the defined phases of the ovarian cycle. Accordingly, it is concluded that the findings reported by previous investigators may have depended primarily upon the number of subjects and cycles that were studied.

Complementary studies on sex hormone binding globulin (SHBG) have shown that there is no significant change in the level of this protein in peripheral plasma throughout the ovarian cycle (*Anderson* 1974). This finding implies that the concentration of apparent free testosterone probably follows a similar pattern to that of total testosterone.

To date there is no information regarding changes in the concentration of testosterone in peripheral plasma throughout the day and night in women. Numerous studies on this aspect have been undertaken on groups of men, and the majority of the results have shown higher concentrations in the early morning (04.00 to 08.00 h). However, there is variation between individuals in the amplitude and duration of the rhythm and it is difficult to prove that significant differences do occur (*Boon et al.* 1972). The results from the present study on serial samples from 9 non-pregnant women have shown a similar pattern, in that there is a tendency for the mean values to decrease during the day to a value at 04.00 h that is 77 % of the maximum value at 08.00 h. This trend is similar to that observed for cortisol (the lowest mean value is 19 % of the maximum) and androstenedione (*James & Andre* 1974), and may well reflect changes in the contribution of testosterone and precursors from the adrenal cortices.

During pregnancy the foetus and placenta contribute to the concentrations of various hormones in maternal plasma, and there is general agreement that the total amount of testosterone is increased (*Osborn & Yannone* 1971). The complete explanation for this rise is not known, but it has been postulated that the increased production of oestrogens is associated with a rise in the concentration of SHBG. The results from the present study show that although the mean concentration of testosterone throughout pregnancy is elevated when compared with the values from non-pregnant women ($P < 0.0005$), there is not a progressive increase, and the pattern is different from that of progesterone or oestradiol. These findings are similar to those reported by *Mizuno et al.* (1968), although their values were considerably higher. The finding that the

mean concentration of testosterone in 2 of the 9 subjects was within the range of values for non-pregnant women is consistent with a report by *Bachmann et al.* (1974), who found a similar phenomenon in 2 out of 6 subjects. The pattern of testosterone in peripheral venous plasma throughout the day and night is more variable during late pregnancy, and is different to that defined in non-pregnant women. The trend for the arithmetic mean is similar to that obtained for progesterone (*Runnebaum et al.* 1972), which possibly indicates a contribution from the foeto-placental unit.

The results detailed in this paper show that there is a marked individual variation in the concentration of testosterone in peripheral venous plasma. However, when the arithmetic mean values from at least 9 subjects are plotted, trends may be seen throughout the day and night in non-pregnant and pregnant women, and throughout the course of pregnancy. The values during the ovarian cycle remain remarkedly constant compared with the wide fluctuations in the concentrations of other gonadal steroids. These findings may be explained with reference to studies of the dynamic aspects of androgen metabolism. For example, it has been shown that up to 60 % of the testosterone in peripheral venous plasma may originate from the extraglandular conversion of androstenedione (*Horton & Tait* 1967). Furthermore both testosterone and androstenedione are secreted by the ovaries and adrenal cortices. The mean blood production rate of androstenedione has been estimated as 3.3 mg/24 h and that of testosterone as 0.23 mg/24 h (*Bardin & Lipsett* 1967). The relative contribution of the ovaries and adrenal cortices is difficult to determine over a given period of time, but studies involving the analysis of ovarian venous blood have indicated that only small amounts of testosterone are secreted, and that androstenedione is the major C_{19} steroid secreted from the ovary (*de Jong et al.* 1974). Other investigations on a woman who had been adrenalectomised (*Abraham & Chakmakjian* 1973) and on patients whose adrenals had been suppressed (*Abraham* 1974) suggest that up to 60 % of the androstenedione in peripheral venous plasma may be of adrenal origin.

It is concluded that the measurement of testosterone in peripheral venous plasma from women provides a reasonable index of androgen status, but may not reflect acute changes in ovarian physiology.

ACKNOWLEDGMENTS

The authors are grateful to colleagues who helped select the subjects and collect the samples. The work was supported by the Wates Foundation, and forms part of a thesis by J. P. P. T. for the degree of Ph. D. (University of London).

REFERENCES

Abraham G. E.: J. clin. Endocr. *39* (1974) 340.
Abraham G. E. & Chakmakjian Z. H.: J. clin. Endocr. *37* (1973) 581.
Anderson D. C.: Clin. Endocr. *3* (1974) 69.
Bachmann R., Gennser B., Hökfelt B., Nilsson K. O. & Sternby N. H.: Acta endocr. (Kbh.) *76* (1974) 747.
Bardin C. W. & Lipsett M. B.: J. clin. Invest. *46* (1967) 891.
Boon D. A., Keenan R. E. & Slaunwhite W. R.: Steroids *20* (1972) 269.
Coyotupa J., Parlow A. F. & Abraham G. E.: Analytical Letters *5* (1972) 329.
de Jong F. H., Baird D. T. & van der Molen H. J.: Acta endocr. (Kbh.) *77* (1974) 575.
Horton R. & Tait J. F.: J. clin. Endocr. *27* (1967) 79.
James V. H. T. & Andre C. M. In: Curry A. S. and Hewitt J. V., Eds. The Biochemistry of Women; Clinical Concepts. C. R. C. Press, Ohio (1974) 23.
Judd H. L. & Yen S. S. C.: J. clin. Endocr. *36* (1973) 475.
Lloyd C. W., Lobotsky J., Segre E. H., Kobayashi T., Taymor M. L. & Batt R. E.: J. clin. Endocr. *26* (1966) 314.
Mizuno M., Lobotsky J., Lloyd C. W., Kobayashi T. & Murasawa Y. J.: J. clin. Endocr. *28* (1968) 1133.
Osborn R. H. & Yannone M. E.: Obstet. and Gynacc. Surv. *26* (1971) 195.
Runnebaum B., Bierwirth B., Münstermann A. M. & Zander J.: Acta endocr. (Kbh.) *69* (1972) 731.
Tyler J. P. P., Hennam J. F., Newton J. R. & Collins W. P.: Steroids *22* (1973) 871.

Received on December 4th, 1974.