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ANDROGEN AND ESTROGEN DYNAMICS: STABILITY OVER A TWO YEAR INTERVAL IN PERI-MENOPAUSAL WOMEN

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Summary—As part of a study on hormones and bone density in peri-menopausal women, metabolic clearance rates (MCR), and interconversions of androgens and estrogens and the peripheral aromatization of androgens were measured twice 2 yr apart. Measurements of clearance rates and interconversions were made from blood samples obtained during constant infusions of [^{3}H]androgens and [^{14}C]estrogens. Measurements of peripheral aromatization were made from the estrogen glucuronides in a pooled 4-day urine collection timed from the start of the infusions. The women were divided into 3 groups: Group A ($n = 15$) were having menstrual cycles throughout the 2 yr interval; Group B ($n = 11$) were having menstrual cycles at the time of Study 1 but had been amenorrheic for at least 1 yr at the time of Study 2; Group C ($n = 28$) were amenorrheic for at least 1 yr at the time of Study 1 and had remained amenorrheic through Study 2.

The MCRs for testosterone, androstenedione, estrone and estradiol were not different for Study 1 and Study 2 in any of the groups. The interconversions of the androgens were similar in both studies for all groups. The conversion of estrone to estradiol decreased in Group A, otherwise the interconversions of the estrogens did not vary between the studies for the other groups. The peripheral aromatization of androstenedione, but not of testosterone, was significantly greater at study 2 compared to Study 1 for all groups.

We conclude that the MCRs and interconversions of androgens and of estrogens are stable over time, but that the peripheral aromatization of androstenedione increases over a 2 yr interval. This increase may be menopausal and/or age related.

INTRODUCTION

In order to examine the effects of time, changes in hormonal status or disease states on steroid dynamics, most investigators have used the vertical rather than the horizontal approach. That is, the effects of time have been examined in groups of subjects of differing age rather than in the same subjects at differing ages [1–4]. Similarly, the effects of the menopause of disease have been examined in groups of subjects [3, 5, 6] rather than in the same subject at different times. Exceptions to this have been studies of steroid dynamics in a few subjects with thyroid dysfunction [7–9] or with obesity and subsequent weight loss [10]. These studies have implicated changes in thyroid status and obesity as being responsible for any changes in steroid dynamics. However, there have been few studies [11] as to the reproducibility of steroid dynamic studies over time in the same individuals with or without associated changes in endocrine function.

As part of a study on the relationship between hormones and bone density, we measured androgen and estrogen dynamics in a group of peri-menopausal women twice, 2 yr apart. Our results, which are reported here, indicate that there was no major difference in metabolic clearance rates (MCR) or interconversions, but there was an increase in the peripheral aromatization of androstenedione.

EXPERIMENTAL

Fifty-four women were studied twice, 2 yr apart. For the purpose of these analyses, they were divided into 3 groups: Group A ($n = 15$) were having menstrual cycles throughout the 2 yr interval; Group B ($n = 11$) were having menstrual cycles at the time of Study 1 but had been amenorrheic for at least 1 yr at the time of Study 2; Group C ($n = 28$) were amenorrheic for at least 1 yr at the time of Study 1 and had remained amenorrheic through Study 2.

Their mean ages and weights are shown in Table 1. All were in good health, and none was taking any hormonal medication. All had given informed consent for the studies, which were approved by the appropriate committees of both Indiana University

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Table 1. Age and weight of the subjects at the time of the two studies 2 yr apart

		n	Age (yr)	Weight (kg)
Group A,	Study 1	15	47.4 ± 0.7[a]	66.8 ± 2.5[b]
	Study 2	15	49.9 ± 0.6	68.4 ± 2.5
Group B,	Study 1	11	50.7 ± 1.0	62.9 ± 3.6
	Study 2	11	52.8 ± 1.0	64.9 ± 4.3
Group C,	Study 1	28	52.9 ± 0.5	60.7 ± 1.8
	Study 2	28	54.9 ± 0.5	61.5 ± 1.9

[a]Mean ± SE. [b]Weight for Study 1 significantly less than for Study 2. $P = 0.0381$.

and the University of Massachusetts Medical School. All studies were done in the General Clinical Research Center at Indiana University. All women were part of an ongoing study on hormonal status and osteoporosis, and some of these findings have been reported [12].

The women were evaluated with a detailed history, including information on their menstrual periods and a brief physical examination on their initial visit, at which time the first radiolabeled steroid infusion for the dynamic studies was done.

The women were seen at 4 month intervals for interim evaluations and after 2 yr the dynamic studies were repeated.

On both occasions MCRs and steroid conversions were measured using constant infusions of [^{3}H]androgens and [^{14}C]estrogens as previously described [13–15]. At 0800 h while supine and fasting, the women were given a priming dose of [^{3}H]androgen/[^{14}C]estrogen, followed by a constant infusion of the same [^{3}H]androgen/[^{14}C]estrogen for 210 min. The steroid pairs consisted of [^{3}H]A/[^{14}C]E_1 or [^{3}H]T/[^{14}C]E_2, the priming dose was 20 μCi ^{3}H/0.8 μCi ^{14}C, and the infusion dose was 50 μCi ^{3}H/2.0 μCi ^{14}C, all administered in a total of 25 ml 8% ethanolic saline via an arm vein. Heparinized venous blood samples (30 ml) were obtained from the opposite arm at 150, 180, and 210 min. The samples were centrifuged, and the plasma was frozen and stored at −15°C until analyzed. All urine was collected for 96 h and stored frozen at −15°C until analyzed.

Reagents were prepared as previously described [13–15]. [7-^{3}H]T (SA, 25 Ci/mmol), [4-^{14}C]T (SA, 57.5 mCi/mmol), [4-^{14}C]A (SA, 54 mCi/mmol), [4-^{14}C]E_2 (SA, 54 mCi/mmol), and [4-^{14}C]E_1 (SA, 54 mCi/mmol) were obtained from New England Nuclear Corp. (Boston, Mass.). [7-^{3}H]A was formed from [7-^{3}H]T by Jones' oxidation [16]. The [7-^{3}H]A so formed was purified by thin-layer chromatography and was more than 98% pure by reverse isotope dilution and crystalization. All labeled androgens and estrogens were purified, and the purity was checked before use [17]. Nonradioactive E_1 and E_2 were obtained from Steraloids Co. (Wilton, N.H.) and crystalized from methanol before use. Alumina HF254 (basic type E) and silica gel HF254 were obtained from Brinkmann (Westbury, N.Y.). Whatman No. 2 paper was used for paper chromatography (Whatman, Clifton, N.J.).

Steroid extraction, chromatographic purifications, preparation of derivatives, and measurements of radioactivity were performed as previously described [13, 17]. Radioactivity was determined using a Packard liquid scintillation spectrophotometer (Packard, Downers Grove, Ill.).

For the infused androgens the ^{3}H disintegrations per min, uncorrected for recovery, ranged from 370 dpm in the product to 9000 dpm in the precursor. These samples were counted for 50 min, and the counting errors were less than 1.5% using the analysis of Horton and Tait[18]. For the infused estrogens the ^{14}C disintegrations per min, uncorrected for recovery, ranged from 20 dpm in the product to 450 dpm in the precusor. These samples were counted for 100 min, and the counting errors were less than 5% for the products and less than 1% for the precursor steroids.

For the infusions of [^{3}H]androgen/[^{14}C]estrogen the concentrations of radioactivity as infused precursor and as product steroids were compared as a function of time of blood sampling. When these data were analyzed using linear regression and analysis of variance, there was no significant trend in the steroid concentrations from 150–210 min, indicating that an isotopic steady-state for both precursor and product steroids had been reached and was maintained [19].

The MCRs were calculated as rate of infusion in the peripheral venous blood/mean concentration of radioactivity in venous blood [20, 21]. The interconversions between the androgens and between the estrogens were defined as the percentage of precursor infused that is measured as product in the blood [20, 21]. They were calculated as $[p]_{BB}^{Prec.\ Prod} = 100*(X^{Pro} \times MCR^{Pro})/r^{Pre}$, where r^{Pre} is the infusion rate of precursor, and X^{Pro} is the mean concentration of precursor radioactivity measured as product.

The urine samples were analyzed as described previously [22, 23]. Unconjugated steroids were extracted using cyclohexane-ethylacetate (1:2, v/v) and the urine was incubated with β-glucuronidase. The hydrolyzed steroids were extracted with cyclohexane-ethylacetate (1:2, v/v) and purified by alkaline partition and multiple chromatographic and derivatization steps. The E_1 and E_2 radioactivity were measured using a liquid scintillation spectrometer. In these samples the ^{3}H disintegrations per min ranged from 150–500 dpm, and the ^{14}C disintegrations per min ranged from 3000–8000 dpm. Each sample was counted for 50 min, and the counting error was less than 2%. $[p]_{BM}^{And.\ Est}$ (the percentage of androgen infused converted to estrogen) is calculated as $[([^3H]\text{estrogen}/[^{14}C]\text{estrogen})_{urine}/([^3H]\text{androgen}/[^{14}C]\text{estrogen}_{infused})] \times 100$ [22].

The data for each group for Study 1 were compared to the respective data for Study 2 by paired-t-test. The data for each study were also compared separately for all groups using a one-way analysis of variance and Student–Newman–Keuls test for multi-

ple comparisons between means (Statistical Package for the Social Sciences, SPSSX) [24].

RESULTS

As shown in Table 1, the studies were done about 2 yr apart and in Group A only, there was a slight but significant, $P < 0.05$, weight gain in the 2 yr interval between studies. The mean age of the women in Group C was significantly, $P < 0.05$, greater than the other 2 groups and the women in Group B were significantly, $P < 0.05$, older than those in Group A.

The MCRs, Table 2, were not significantly different for any of the groups comparing Study 1 with Study 2. There was also no intergroup difference in the MCRs in Study 1.

The mean interconversions between the androgens, Table 3, were not different between studies nor between groups. The mean conversion of estradiol to estrone also was not different but for the mean conversion of estrone to estradiol, $[p]_{BB}^{E1,E2}$, there was a small, but significant difference between Studies 1 and 2. There were no inter-group differences.

The mean values for the aromatization of testosterone to estradiol, Table 4, did not differ between studies or between groups. However, in all groups there was a significant increase, $P < 0.02$, in the peripheral aromatization of androstenedione between Study 1 and Study 2. There were no inter-group differences.

DISCUSSION

Most studies of androgen and estrogen dynamics have involved measurements of clearance rates and conversions made once in each subject with the assumption that there will be little change with time.

Table 4. Peripheral aromatization ($[p]_{BM}$) measured twice 2 yr apart

		n	$[p]_{BM}^{A,E1}$ [a] (%)	$[p]_{BM}^{T,E2}$ (%)
Group A	Study 1	15	2.03 ± 0.17[b,c]	0.42 ± 0.05
	Study 2	15	2.50 ± 0.17	0.54 ± 0.08
Group B	Study 1	11	2.11 ± 0.24	0.41 ± 0.06
	Study 2	11	2.54 ± 0.25	0.48 ± 0.06
Group C	Study 1	28	2.26 ± 0.12	0.43 ± 0.03
	Study 2	28	2.56 ± 0.13	0.42 ± 0.05

[a] $[p]_{BM}^{A,E1}$ is the percent of androstenedione (A) infused into the blood and converted to estrone (E1) in the body. $[p]_{BM}^{T,E2}$ is the percent of testosterone (T) infused into the blood and converted to estradiol in the body [5, 22]. [b] Mean ± SE. [c] Study 1 significantly less than Study 2, $P < 0.02$, for all groups.

Our present data indicate that these assumptions were correct and that the MCRs for androgens and estrogens do not change significantly over a 2 yr period when carried out under the same conditions in normal women. The data also indicate that the change from having menstrual cycles to the postmenopausal state does not alter the MCRs of androgens or estrogens. This study carried out in the same women thus largely confirms what has been reported previously in separate groups of women [3, 25]. However, we did not find any decrease in the MCR of estradiol in the group of women who became postmenopausal during the interval between studies.

Although the interconversions of the androgens were not different when studied at 2 yr intervals, we did find a decrease in the conversion of estrone to estradiol in the group of women who were having menstrual cycles for both study times. The biologic significance and cause of this decrease remains uncertain, especially in view of the constancy of the reverse conversion, estradiol to estrone.

We and others[1, 22] have noted that the peripheral aromatization of androstenedione is increased in older as opposed to younger women. Although Hem-

Table 2. Metabolic clearance rates for the subjects measured twice 2 yr apart

		n	Testosterone (1/day)	Androstenedione (1/day)	Estrone (1/day)	Estradiol (1/day)
Group A	Study 1	15	472 ± 37[a]	1892 ± 115	1579 ± 125	1115 ± 82
	Study 2	15	523 ± 53	2063 ± 102	1723 ± 99	1239 ± 101
Group B	Study 1	11	479 ± 55	1789 ± 118	1411 ± 98	1029 ± 75
	Study 2	11	467 ± 73	1781 ± 75	1562 ± 143	1163 ± 98
Group C	Study 1	28	434 ± 22	1711 ± 90	1435 ± 86	980 ± 47
	Study 2	28	453 ± 25	1708 ± 67	1462 ± 73	1067 ± 70

[a] Mean ± SE.

Table 3. Interconversions ($[p]_{BB}$) of androgens and of estrogens measured twice 2 yr apart

		n	$[p]_{BB}^{A,T}$ [a] (%)	$[p]_{BB}^{T,A}$ (%)	$[p]_{BB}^{E1,E2}$ (%)	$[p]_{BB}^{E2,E1}$ (%)
Group A	Study 1	15	3.8 ± 0.2[b]	10.7 ± 0.9	7.5 ± 0.9[c]	17.1 ± 1.3
	Study 2	15	3.9 ± 0.6	14.0 ± 1.8	5.2 ± 0.7	16.7 ± 1.3
Group B	Study 1	11	3.9 ± 0.3	9.9 ± 0.9	8.2 ± 1.1	18.0 ± 1.9
	Study 2	11	3.4 ± 0.3	10.8 ± 0.6	5.8 ± 0.6	19.9 ± 1.7
Group C	Study 1	28	3.9 ± 0.4	17.7 ± 3.0	6.0 ± 0.4	19.3 ± 1.1
	Study 2	28	4.0 ± 0.4	13.4 ± 1.2	6.4 ± 0.6	19.5 ± 1.2

[a] $[p]_{BB}^{A,T}$ is the percent of androstenedione (A) which is infused into and measured as testosterone (T) in the blood. $[p]_{BB}^{E1,E2}$ is the percent of estrone which is infused into and measured as estradiol in the blood. $[p]_{BB}^{T,A}$ and $[p]_{BB}^{E2,E1}$ are the percentages for the reverse reactions [20, 21]. [b] Mean ± SE. [c] Study 1 significantly, $P = 0.046$, greater than Study 2.

sell *et al.*[26] ascribed this to aging *per se*, we have ascribed it to events occurring at the time of the menopause [22]. However, our present data indicate that all women studied had an increase in androstenedione aromatization in the 2 yr interval no matter what changes might have occurred, or not occurred, in their menstrual status. We have shown that peripheral aromatization appears to be stable in humans [27] and in non-human primates where repeated measurements are made [28]. However, those studies involved only a short interval repeat measurements, and stability over a longer time interval has not been investigated in normal subjects, to our knowledge. The mean increase in $[p]_{BM}^{A,E1}$ was greatest in Group A women, $17 \pm 4\%$, and least in the Group C women, $9 \pm 4\%$, so it is unlikely that ageing was the sole cause of the increase, but that a shift in menstrual status played a role as well. Although we noted no significant change in $[p]_{BM}^{T,E2}$ between the studies, there was a slight increase in Group A but not in Group C over the 2 yr interval, suggesting again that changing menstrual status may be more important than age.

Since the infusions and analyses were done 2 yr apart, there is the possibility that changes in techniques could have perturbed the results. However, all studies were done under the same conditions by the same individuals, and our analytical techniques did not change. It is, therefore, unlikely that methodological "drift" was responsible for our results.

Thus, we find that most measurements of androgen and estrogen dynamics are stable over at least a 2 yr period, even if this interval involves a shift in menstrual status. However, the peripheral aromatization of androstenedione, at least, is not stable over this time period in the peri-menopausal period. While age may play a role, it would appear that the change in menstrual status was also important in the increase in androstenedione aromatization.

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REFERENCES

1. Siiteri P. K. and MacDonald P. C.: *Role of extraglandular estrogen in human endocrinology* American Physiology Society, Washington D.C. (1973) pp. 615–629.
2. Baker H. W. G., Burger H. G., Kretser D. M. D., Hudson B., O'Connor S., Wang C., Mirovics A., Court J., Dunlop M. and Rennie G. C.: Changes in the pituitary-testicular system system with age. *Clin. Endocr.* **5** (1976) 349–372.
3. Longcope C., Jaffee W. and Griffing G.: Metabolic clearance rates of androgens and estrogens in aging women. *Maturitas* **2** (1980) 283–390.
4. Longcope C., Bourget C. and Flood C.: The production and aromatization of dehydroepiandrosterone in postmenopausal women. *Maturitas* **4** (1982) 325–332.
5. MacDonald P. C., Edman C. D., Hemsell D. L., Porter J. C. and Siiteri P. K.: Effect of obesity on conversion of plasma androstenedione to estrone in postmenopausal women with and without endometrial cancer. *Am. J. Obstet. Gynec.* **130** (1978) 448–455.
6. Reed M. J., Beranek P. A., Ghilchik M. W. and James V. H. T.: Estrogen production and metabolism in normal postmenopausal women and postmenopausal women with breast or endometrial cancer. *Eur. J. Cancer Clin. Oncol.* **22** (1986) 1395–1400.
7. Southern A., Olivo J., Gordon G. G., Vittek J., Brener J. and Rafii F.: The conversion of androgens to estrogens in hyperthyroidism. *J. Clin. Endocr. Metab.* **38** (1974) 207–214.
8. Ridgway E. C., Longcope C. and Maloof F.: Metabolic clearance and blood production rates of estradiol in hyperthyroidism. *J. Clin. Endocr. Metab.* **41** (1975) 491–497.
9. Ridgway E. C., Maloof F. and Longcope C.: Androgen and oestrogen dynamics in hyperthyroidism. *J. Endocr.* **95** (1982) 105–115.
10. Siiteri P. K., Williams J. E. and Takaki N. K.: Steroid abnormalities in endometrial and breast carcinoma: a unifying hypothesis. *J. Steroid Biochem.* **7** (1976) 897–903.
11. Longcope C.: Peripheral aromatization: studies on controlling factors. *Steroids* **50** (1987) 253–267.
12. Slemenda C., Hui S. L., Longcope C. and Johnston C. C.: Sex steroids and bone mass; a study of changes about the time of the menopause. *J. Clin. Invest.* **80** (1987) 1261–1269.
13. Longcope C., Pratt J. H., Schneider S. and Fineberg E.: Estrogen and androgen dynamics in liver disease. *J. Endocr. Invest.* **7** (1984) 629–634.
14. Johnston Jr C. C., Hui S. L., Witt R. M., Appledorn R., Baker R. S. and Longcope C.: Early menopausal changes in bone mass and sex steroids. *J. Clin. Endocr. Metab.* **61** (1985) 905–911.
15. Longcope C. and Johnston Jr C. C.: Androgen and estrogen dynamics in pre- and postmenopausal women: a comparison between smokers and nonsmokers. *J. Clin. Endocr. Metab.* **67** (1988) 379–383.
16. Bardin C. W. and Lipsett M. B.: Testosterone and androstenedione blood production rates in normal women and women with idiopathic hirsutism or polycystic ovaries. *J. Clin. Invest.* **46** (1967) 891–902.
17. Longcope C., Kato T. and Horton R.: Conversion of blood androgens to estrogens in normal adult men and women. *J. Clin. Invest.* **48** (1969) 2191–2201.
18. Horton R. and Tait J. F.: *In Vivo* conversion of dehydroisoandrosterone to plasma androstenedione and testosterone in man. *J. Clin. Endocr. Metab.* **27** (1967) 79–88.
19. Longcope C. and Tait J. F.: The validity of metabolic clearance and interconversion rates of estrone and 17-estradiol in normal adults. *J. Clin. Endocr. Metab.* **32** (1971) 481–490.
20. Baird D., Horton R., Longcope C. and Tait J. F.: Steroid prehormones. *Perspect. Biol. Med.* **11** (1968) 384–421.
21. Baird D. T., Horton R., Longcope C. and Tait J. F.: Steroid dynamics under stead-state conditions. *Recent Prog. Horm. Res.* **25** (1969) 611–664.
22. Longcope C.: Methods and results of aromatization studies *in vivo*. *Cancer Res.* **42** (1982) 3307s–3311s.
23. Longcope C., Femino A. and Johnston J. O.: Androgen and estrogen dynamics in the female baboon (*Papio anubis*). *J. Steroid Biochem.* **31** (1988) 195–200.
24. SPSSX, Users Guide (2nd edn). McGraw-Hill N.Y. (1986).
25. Longcope C.: Metabolic clearance and blood production rates of estrogens in post-menopausal women. *Am. J. Obstet. Gynec.* **111** (1971) 778–791.
26. Hemsell D. L., Grodin J. M., Brenner P. F., Siiteri P. K. and MacDonald P. C.: Plasma precursors of estrogen.

II. Correlation of the extent of conversion of plasma androstenedione to estrone with age. *J. Clin. Endocr. Metab.* **38** (1974) 476–479.
27. Pratt J. H. and Longscope C.: Effect of adrenocorticotropin on production rates and metabolic clearance rates of testosterone and estradiol. *J. Clin. Endocr. Metab.* **47** (1978) 307–313.
28. Franz C. and Longcope C.: Androgen and estrogen metabolism in male rhesus monkeys. *Endocrinology* **105** (1979) 869–874.