# Plasma Precursors of Estrogen. II. Correlation of the Extent of Conversion of Plasma Androstenedione to Estrone with Age[1]

DAVID L. HEMSELL,[2] J. M. GRODIN,[3] P. F. BRENNER,[3]
P. K. SIITERI, AND P. C. MACDONALD

*Department of Obstetrics and Gynecology, The University of Texas Southwestern Medical School, Dallas, Texas*

**ABSTRACT.** The purpose of the present study was to ascertain the relationship between aging and the extent of conversion of plasma androstenedione to estrone. Studies were carried out in 23 women and 26 men who weighed 130–180 pounds and 150–190 pounds, respectively, in which $^{3}H$ estrone and $^{14}C$-androstenedione were administered intravenously and the transfer constant of plasma androstenedione to estrone as measured in urine, [$\rho$]AE1/BU, was determined.

The results of these studies in both women and men demonstrate that with advancing age, there is a progressive and highly statistically significant increase in the efficiency with which circulating androstenedione is converted to estrone. The magnitude of the increase observed in the older subjects reached 2- to 4-fold that observed in the young adult. (*J Clin Endocrinol Metab* 38: 476, 1974)

THE formation of estrogen from plasma $C_{19}$ steroids by tissues other than ovary, adrenal, or testis has been thoroughly established in recent years (1–9). The extent of the conversion of certain androgens to estrogen is sufficient to be quantitatively important in some conditions. For example, "extraglandular" conversion of plasma androstenedione to estrone accounts for most, if not all, of the estrogen production in postmenopausal women (2–8). Also, estrogen production in normal adult males is principally the consequence of the conversion of plasma androstenedione and testosterone to estrone and estradiol, respectively, by tissues other than the adrenal or testis (8,9). The contribution to total estrogen production by testicular secretion is small (8–11).

In a continuing study of estrogen production from circulating precursors in the human, we have found that the amount of estrogen formed by "extraglandular" tissues is determined by the efficiency with which precursors are converted to estrogens and by the amounts of precursors circulating in blood. During these investigations, it was observed that there was a greater extent of conversion of circulating androstenedione to estrone in postmenopausal women and in older men than there was in young adults. For this reason, a study was conducted to ascertain the relationship between aging and the extent of conversion of androstenedione to estrone. The results indicate that after puberty the efficiency of conversion of plasma androstenedione to estrone increases with age.

## Materials and Methods

### *Subjects*

In view of the likelihood that a variety of metabolic, pathologic, or pharmacologic events may affect the conversion of plasma androstenedione to estrone, we chose women and men for this study whose body weights were 130–186 and 150–190 pounds, respectively. None of the subjects had clinical or laboratory evidence of hepatic disease. All were ambulatory, and none were receiving exogenous hormonal preparations with the exception of subject AR, who had been bilaterally adrenalectomized four years prior to

Received October 8, 1973.

[1] Supported, in part, by USPHS Grant AM-09612 and Contract NICHD-72-2756.

[2] Lt. Col., USAF, M.C. The views expressed here are those of the authors and do not necessarily reflect the views of the United States Air Force or the Department of Defense.

[3] Research Trainees supported by NIH Training Grant HD-00256.

the study and was receiving 37.5 mg of cortisone acetate plus 0.05 mg of 9α flurocortisol-21-acetate daily for maintenance.

*Methods*

*Experimental design.* For the purpose of determining the transfer constant of plasma androstenedione to estrone $[\rho]^{AE1}_{BU}$, the urinary method was used (1). The techniques of purification of tracers and their administration have been described in detail (1,2). The experimental design employed the intravenous administration of an *in vivo* internal standard as the product estrogen hormone, 6,7-$^3$H-estrone, plus the simultaneous administration of the precursor, 4-$^{14}$C-androstenedione. The tracers were adminstered by single bolus injection or by continuous infusion over four hours in 10% ethanol in 5% dextrose as previously described (1,4). After the administration of the isotope-labeled tracers, urine was collected for 72 hr. The 72-hr urine collection was treated with β-glucuronidase. The liberated steroids were extracted with ethyl acetate and subjected to celite column gradient elution chromatography, thin-layer chromatography, acetylation and rechromatography, and finally, recrystallized in the presence of authentic nonradioactive carrier until a constant tritium to carbon-14 ratio was achieved as previously described (1). The transfer constant, $[\rho]^{AE1}_{BU} \times 100$ (percent), was calculated by dividing the tritium to carbon-14 ratio of the infused isotope-labeled tracers (rI), by the tritium to carbon-14 ratio of the isolated urinary estrone, (rE1), *i.e.*, $[\rho]^{AE1}_{BU} = \frac{rI}{rE1} \times 100$. This measurement represents the fraction of $^{14}$C-androstenedione which was metabolized identically to the *in vivo* internal standard, *i.e.*, the administered 6,7-$^3$H-estrone, and thus represents the extent of aromatization of androstenedione at the tissue site(s) of conversion and *not* the extent of conversion to a urinary product.

## Results

*Urinary estrogen metabolites following the simultaneous administration of 6,7-$^3$H-estrone plus 4-$^{14}$C-androstenedione.* The data obtained in the female subjects of this study are presented in Table 1. The $^3$H/$^{14}$C ratios among estrone, estradiol, and estriol in most subjects are remarkably similar indicating that the carbon-14 androstenedione converted to estrogen suffered a metabolic fate similar to that of estrone. The recovery of radioactivity in every instance was sufficient to establish the radiochemical homogeneity of the estrogen metabolites studied.

TABLE 1. Tritium to carbon-14 dpm ratios of urinary estrogens following the intravenous administration of 6,7-$^3$H-estrone + 4-$^{14}$C-androstenedione to female subjects

| Subjects | Age | $^3$H/$^{14}$C ratio* (dpm of urinary metabolites $E_1$ | $E_2$ | $E_3$ | $[\rho]^{AE1}_{BU}$ (%) |
|---|---|---|---|---|---|
| PW | 19 | 36.4 | 33.9 | 33.0 | 1.1 |
| AW | 21 | 40.1 | 39.4 | 40.6 | 1.0 |
| PS | 22 | 40.5 | 32.4 | 43.9 | 1.0 |
| CA | 22 | 41.6 | 40.0 | 42.1 | 1.0 |
| MB | 23 | 54.0 | — | — | 0.7 |
| AW | 23 | 21.4 | 28.0 | 29.0 | 1.9 |
| HC | 25 | 30.8 | 31.8 | 29.5 | 1.3 |
| CK | 25 | 23.6 | 20.9 | 29.1 | 1.7 |
| IL | 26 | 28.2 | 24.9 | — | 1.4 |
| MF | 29 | 35.5 | 20.2 | 37.5 | 1.1 |
| DP | 32 | 24.3 | 21.0 | 25.6 | 1.6 |
| AR | 37 | 23.7 | 20.5 | 24.3 | 1.7 |
| EK | 38 | 19.4 | 19.5 | 24.8 | 2.1 |
| IP | 50 | 16.5 | 14.9 | 13.5 | 2.4 |
| RR | 53 | 16.4 | 16.3 | 17.2 | 2.4 |
| LS | 53 | 11.8 | 12.7 | 13.0 | 3.4 |
| TF | 56 | 17.0 | 23.9 | 23.1 | 2.4 |
| RS | 57 | 13.2 | 12.0 | 13.0 | 3.0 |
| CW | 62 | 26.4 | 21.4 | 23.4 | 1.5 |
| MP | 62 | 13.1 | 11.9 | 13.8 | 3.1 |
| BG | 67 | 11.7 | — | 14.7 | 3.4 |
| MK | 73 | 14.1 | 14.1 | 14.9 | 2.8 |
| OL | 73 | 11.7 | 10.8 | 11.8 | 3.4 |

* For ease of comparison, all tritium to carbon-14 ratios have been corrected to a common injected tracer dose of 28.0 × 10$^6$ dpm of $^3$H-estrone and 70.0 × 10$^6$ dpm of $^{14}$C-androstenedione, (injected ratio = 0.40).

The relationship of age to the transfer constant, $[\rho]^{AE1}_{BU} \times 100$ (percent), is presented in Fig. 1. This regression line, $y = bx + a$, for the correlation of $[\rho]^{AE1}_{BU}$ and age was computed by the method of least squares (12) where $y$ is the transfer constant of plasma androstenedione to estrone, $[\rho]^{AE1}_{BU}$; $x$ is patient age; $b$ is the slope of the line, (0.0404); and $a$ is the $y$ intercept, (0.2913). The correlation coefficient of these two variables is 0.8638 and is statistically significant at 21 degrees of freedom ($p < 0.001$). The

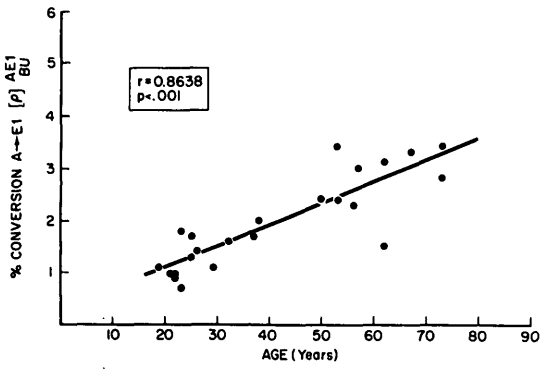


FIG. 1. Correlation between the extent of conversion of plasma androstenedione to estrone and age in 23 women. The calculation of the correlation coefficient, r, for these two variables as well as the determination of the regression line is described in the text.

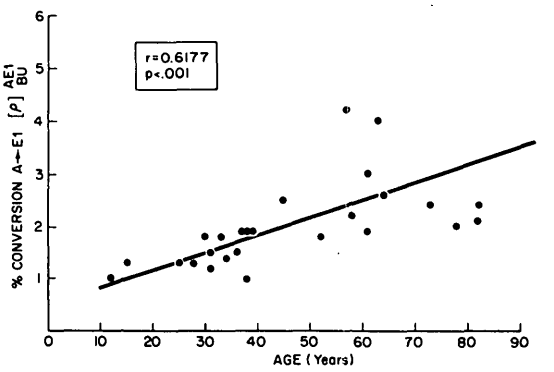


FIG. 2. Correlation between the conversion of androstenedione to estrone and age in 26 men. The calculation of the correlation coefficient, r, for these two variables as well as the determination of the regression line is described in the text.

TABLE 2. Tritium to carbon-14 dpm ratios of urinary estrogens following the intravenous administration of 6,7-$^{3}$H-estrone and 4-$^{14}$C-androstenedione to males

| Subjects | Age | $^{3}H/^{14}C$ ratio* (dpm of urinary metabolites) $E_1$ | $E_2$ | $E_3$ | $[\rho]^{AE1}_{BU}$ (%) |
|---|---|---|---|---|---|
| CM | 12 | 40.0 | 38.9 | 43.2 | 1.0 |
| DM | 15 | 30.4 | 28.7 | 34.8 | 1.3 |
| TB | 25 | 30.5 | 28.2 | 29.7 | 1.3 |
| SS | 28 | 30.3 | — | 32.4 | 1.3 |
| JS | 30 | 24.1 | 24.1 | 25.0 | 1.7 |
| WC | 31 | 33.3 | 33.2 | 33.5 | 1.2 |
| BK | 31 | 24.8 | 25.9 | 23.4 | 1.6 |
| BA | 33 | 22.3 | 28.0 | 25.5 | 1.8 |
| DH | 34 | 31.3 | 28.4 | — | 1.3 |
| PM | 36 | 27.1 | 26.7 | 27.2 | 1.5 |
| PS | 37 | 20.6 | 28.4 | 21.9 | 1.9 |
| FH | 38 | 38.2 | — | — | 1.0 |
| JO | 38 | 20.6 | 20.3 | 21.4 | 1.9 |
| RA | 39 | 20.6 | 36.1 | 21.1 | 1.9 |
| JP | 45 | 16.6 | 16.6 | 17.5 | 2.4 |
| JM | 53 | 23.6 | 19.7 | 22.3 | 1.7 |
| OB | 57 | 9.6 | 9.1 | 10.9 | 4.2 |
| JT | 58 | 18.3 | 18.0 | 19.0 | 2.2 |
| RB | 61 | 13.5 | 13.1 | 12.2 | 3.0 |
| HM | 61 | 23.0 | 22.7 | 22.9 | 1.7 |
| BP | 63 | 10.0 | 9.9 | — | 4.0 |
| JR | 64 | 15.0 | 15.3 | 15.2 | 2.7 |
| WC | 73 | 17.8 | — | 17.0 | 2.3 |
| WR | 78 | 20.9 | 20.4 | 21.0 | 1.9 |
| TJe | 82 | 17.4 | 15.9 | 17.4 | 2.3 |
| TJa | 82 | 20.6 | 20.0 | 17.4 | 1.9 |

* For ease of comparison, all tritium to carbon-14 dpm ratios have been corrected to a common injected tracer dose of $28.0 \times 10^6$ dpm of $^{3}$H-estrone and $70.0 \times 10^6$ dpm of $^{14}$C-androstenedione (injected ratio = 0.40).

slope of the regression line differed significantly from zero ($p < 0.001$).

The results obtained in the male patients are presented in Table 2. The correlation between patient age, $x$, and the transfer constant of plasma androstenedione to estrone, $y$, in the male subjects is shown in Fig. 2. The slope of the regression line, $b$, for these data is 0.0343; and the $y$ intercept, $a$, is 0.4970. The correlation coefficient for these data (0.6177) is significant at 24 degrees of freedom ($p < 0.001$). The slope of the line differed significantly from zero ($p < 0.001$).

## Discussion

The results show that with advancing age there is a progressive increase in the efficiency with which circulating androstenedione is converted to estrone. Previous studies have reported that in women during the postmenopausal life there may be, with aging, an increase in the excretion of urinary estrogens (13). This previously inexplicable finding is now understandable in light of the present observations.

Estrogen production in postmenopausal women as well as elderly men by tissues other than the gonads or adrenal cortices is affected not only by aging processes but also by other metabolic event(s). For example, it has been observed that the conversion of $C_{19}$ plasma steroids to estrogenic products is increased in obese patients and in patients

with liver disease (unpublished observations). An increase in the efficiency of utilization of plasma precursors for estrogen production has been observed in patients with hyperthyroidism by Southren *et al.* (14).

From the present study, it is apparent that there may be an increase in the efficiency of the utilization of circulating estrogen precursors such that a two to four-fold increase in capability for extraglandular estrogen production may occur with aging over that of the young adult male or female.

We have reported previously that the estrogenic hormonal milieu of endometrial neoplasia is most commonly the exclusive production of estrone in "extraglandular" tissues (3,5,6,8). Furthermore, preliminary studies suggest that certain of the constitutional stigmata of endometrial neoplasia (aging, obesity, hepatic disease) are uniquely associated with those metabolic events that lead to increased estrone production via an increased utilization of plasma androstenedione. Paramount among these is aging. The occurrence of endometrial carcinoma in women under 40 yr of age is extremely uncommon, this neoplasia being one predominantly of the postmenopausal years. Evidence has accrued through studies in these laboratories that aging may also act in concert with other constitutional stigmata of endometrial neoplasia, *e.g.*, obesity, to result in even further increases in the efficiency of utilization of plasma androstenedione for estrone production.

The ultimate significance, physiologically, or as a contributing determinant of pathophysiologic states of the increased peripheral aromatization of plasma precursors with aging is yet to be defined. However, it is apparent that declining gonadal function does not necessarily herald a state of static senescence in sex hormone production. Rather, in older individuals, the controlling features of estrogen production are more related to total body metabolic processes and to the secretion of $C_{19}$ prehormones.

## Acknowledgments

The authors wish to acknowledge the very expert technical assistance of Mrs. Patricia Taliaferro and Mr. Frank Hereford as well as the excellent cooperation of the Resident Staff in Obstetrics and Gynecology of Parkland Memorial Hospital in the performance of these studies. In addition, the authors are grateful to Dr. John C. Porter for the statistical analysis of these data.

## References

1. MacDonald, P. C., R. P. Rombaut, and P. K. Siiteri, *J Clin Endocrinol Metab* **27:** 1103, 1967.
2. MacDonald, P. C., J. M. Grodin, and P. K. Siiteri, Excerpta Medica, Int. Congress Series No. 184, Progress in Endocrinology, Proceedings of the Third Int. Congress of Endocrinology (Mexico), p. 770, 1968.
3. MacDonald, P. C., and P. K. Siiteri, Excerpta Medica, Int. Congress Series No. 111, Progress in Endocrinology, Proceedings of the Second Int. Congress of Endocrinology (Milan), p. 151, 1966 (Abstract).
4. Grodin, J. M., P. K. Siiteri, and P. C. MacDonald, *J Clin Endocrinol Metab* **36:** 207, 1973.
5. Siiteri, P. K., J. M. Grodin, D. Hemsell, and P. C. MacDonald, Proc. of 4th Int. Congress of Endocrinology, Excerpta Medica Foundation, 1972 (in press).
6. Siiteri, P. K., and P. C. MacDonald, *In* Geiger, S. R., E. B. Astwood, and R. O. Greep (eds.), *Handbook of Physiology,* New York, The American Physiological Society, 1973 (in press).
7. Longcope, C., *Am J Obstet Gynecol* **111:** 778, 1971.
8. MacDonald, P. C., J. M. Grodin, and P. K. Siiteri, *In* Baird, D. T., and J. A. Strong (eds.), Control of Gonadal Steroid Secretion, Edinburgh University Press, Edinburgh, p. 158, 1971.
9. MacDonald, P. C., H. T. Hutchinson, J. B. Miller, and P. K. Siiteri, Submitted *J Clin Invest,* 1974.
10. Kelch, R. P., M. R. Jenner, R. Weinstein, S. L. Kaplan, and M. M. Grumbach, *J Clin Invest* **51:** 824, 1972.
11. Longcope, C., W. Widrich, and C. T. Sawin, *Steroids* **20:** 439, 1972.
12. Wallis, W. A., and H. V. Roberts, Statistics: A New Approach, The New Press, Glencoe, Ill., 1956.
13. Pincus, G., L. P. Romanoff, and J. Carlo, *J Gerontol* **9:** 113, 1954.
14. Southren, A. L., J. Olivo, G. G. Gordon, J. Brener, and F. Rafii, Proceedings of Endocrine Society, p. 181, 1973 (Abstract).