

# Effects of Aging and Obesity on Aromatase Activity of Human Adipose Cells\*

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**ABSTRACT.** Adipose tissue is the principal site of estrogen formation in postmenopausal women; with advancing age as well as with increased body weight, there is an increase in the fractional conversion of circulating androstenedione to estrone. We have studied the effects of aging as well as body weight on aromatase activity of adipose tissue specimens taken from 50 women of various ages and weights. Since aromatase activity of adipose tissue is detectable primarily in stromal cells, these cells were incubated with [ $^3\text{H}$ ]androstenedione (150 nM), and estrogen formation was assayed by measuring the incorporation of tritium into [ $^3\text{H}$ ]water. The aromatization rate, when normalized on the basis of equal numbers of cells, increased with increasing age ( $P < 0.03$ ;  $r = 0.43$ ). In contrast, when expressed as a function of body weight, no change in aromatase activity of adipose stromal cells were found. Aromatization of androstene-

dione by cells from young women who had undergone oophorectomy was not increased compared with that of cells from young women with normal ovarian function, indicating that the onset of menopause *per se* and the accompanying increase in circulating gonadotropin levels were not causative factors in the increased aromatase activity of adipose stromal cells. We conclude, therefore, that increased estrone production associated with aging may result from an increase in the specific activity of the aromatase enzyme in adipose stromal cells and is not affected by changes in gonadotropin concentrations associated with menopause. On the other hand, the increase in estrogen formation as a function of obesity is probably due to increased numbers of adipose cells, rather than to an increase in the specific activity of aromatase in those cells. (*J Clin Endocrinol Metab* 60: 174, 1985)

THE principal estrogen formed in postmenopausal women is estrone ( $\text{E}_1$ ), which is derived from the extraglandular aromatization of circulating androstenedione in adipose tissue (1). The results of various *in vivo* studies indicate that the fractional conversion of androstenedione to  $\text{E}_1$  increases with aging and obesity (2-6). The incidence of endometrial cancer also is known to increase with aging and obesity. The estrogen produced in adipose tissue, therefore, is believed to play an important role in the pathogenesis of endometrial cancer. The objective of the present study was to determine whether the increases in estrone production that occur with aging and obesity are the result of increased specific activity of the aromatase enzyme complex in adipose tissue. In previous studies we found that aromatase activity is detected primarily in the stromal cells of human adipose tissue; aromatase activity in intact adipocytes is either low or undetectable (7, 8). Consequently, in the present study, aromatase activity was assayed in stromal cells

prepared from sc adipose tissue obtained from 50 women of various ages undergoing surgery. The results indicate that the increased estrogen formation associated with aging results from an increase in the specific activity of the enzyme in adipose cells, whereas that associated with obesity does not.

## Materials and Methods

### Source and preparation of cells

Abdominal sc adipose tissue was obtained from women undergoing gynecological surgery, who gave consent in writing. The consent form and protocol were approved by the Human Research Review Committee of The University of Texas Health Science Center at Dallas, TX. Adipose tissue obtained at the time of surgery was minced and incubated for 60 min at 37 C in Krebs bicarbonate buffer that contained BSA (Pentex fraction V, Miles Laboratories, Elkhart, IN; 4%) and collagenase (Worthington Biochemical Corp., Freehold, NJ; 1 mg ml $^{-1}$ ) (7). The digested tissue was filtered through nylon mesh, and the stromal cells and adipocytes were isolated by centrifugation (400  $\times$  g) for 5 min and washed twice by resuspension in normal saline and recentrifugation. Stromal cells then were resuspended in Waymouth's enriched medium containing fetal calf serum (Grand Island Biological Co., Grand Island, NY; 15%), and the number of cells was determined by counting in a hemacytometer. Fresh suspensions of stromal cells ( $10^6$  cells)

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were placed in 35-mm culture dishes (Falcon Plastics, Cockeysville, MD) for assay of aromatase activity at 37 C in a humidified atmosphere of 95% air-5% CO<sub>2</sub>.

#### Preparation of radiolabeled substrates and assay of aromatase activity

[1-<sup>3</sup>H]Androstenedione was prepared from [1,2-<sup>3</sup>H]androstenedione (New England Nuclear, Boston, MA), as described previously (7, 9). Aromatase activity was assayed after the addition of [1-<sup>3</sup>H]androstenedione to the medium (150 nM). This concentration of substrate is 6-fold greater than the apparent Michaelis constant ( $K_m$ ) for androstenedione of adipose stromal cells (7, 8). Incubations were continued for 18 h. At the end of the incubation period, the medium was removed, and the incorporation of tritium from [1-<sup>3</sup>H]androstenedione into [<sup>3</sup>H]water was assayed, as described in detail previously (7). Each data point on the figures represents the mean of results obtained using either duplicate or triplicate sets of dishes.

#### Statistical analysis

The significance of the correlation coefficients was tested using an *r* test table. The significance of differences between the means of groups of data was tested by Student's *t* test.

### Results

In a previous publication (8), we reported results of studies in which the maximum velocity ( $V_{max}$ ) and  $K_m$  for the conversion of [1-<sup>3</sup>H]androstenedione to E<sub>2</sub> by freshly prepared human adipose stromal cells were determined. The  $V_{max}$  was 0.85 pmol 18 h<sup>-1</sup> 10<sup>6</sup> cells<sup>-1</sup>, and the apparent  $K_m$  was 30 nM, similar to the value previously reported for the  $K_m$  for androstenedione of adipose stromal cells in confluent monolayer culture (7). For this reason, in the present studies, an incubation time of 18 h was chosen, and a [1-<sup>3</sup>H]androstenedione concentration of 150 nM was used, which is 4 times the value of the apparent  $K_m$ .

To determine whether there was a correlation between aromatase activity of freshly prepared adipose tissue stromal cells and the age of the subject, stromal cells were incubated with [1-<sup>3</sup>H]androstenedione (150 nM) for 18 h (Fig. 1). The rate of aromatization of androstenedione by adipose stromal cells increased as a function of age ( $P < 0.03$ ;  $r = 0.43$ ). The mean aromatase activity of cells from all premenopausal women was 0.73 pmol 18 h<sup>-1</sup> 10<sup>6</sup> cells<sup>-1</sup>, and that of cells from all postmenopausal subjects was 1.39 pmol 18 h<sup>-1</sup> 10<sup>6</sup> cells<sup>-1</sup>. There was a significant difference in the aromatase activities of stromal cells obtained from premenopausal and postmenopausal subjects ( $P < 0.01$ ). It is of interest that the aromatase activity of stromal cells obtained from five women under the age of 45 yr who had undergone a previous surgically induced menopause (oophorectomy)

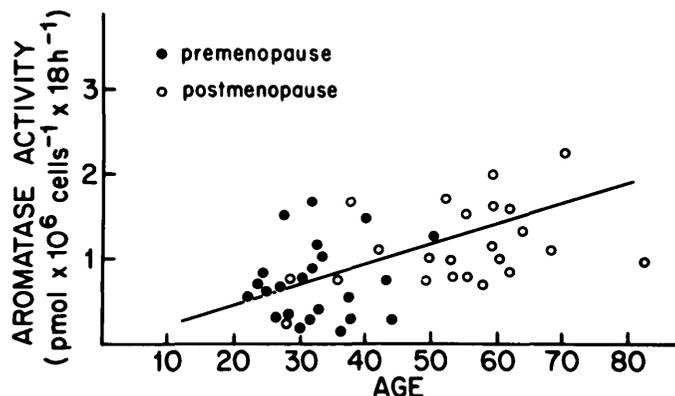


FIG. 1. Aromatase activity of freshly prepared adipose tissue stromal cell suspensions as a function of age. Aromatase activity of cells of premenopausal women (●) compared with that of cells of postmenopausal women (○) is shown. Of the women indicated by the open circles, the five youngest had a surgical menopause. Aromatase activity was determined as described in *Materials and Methods*. The data are the means of values obtained from assays of cells of two or three replicate dishes.

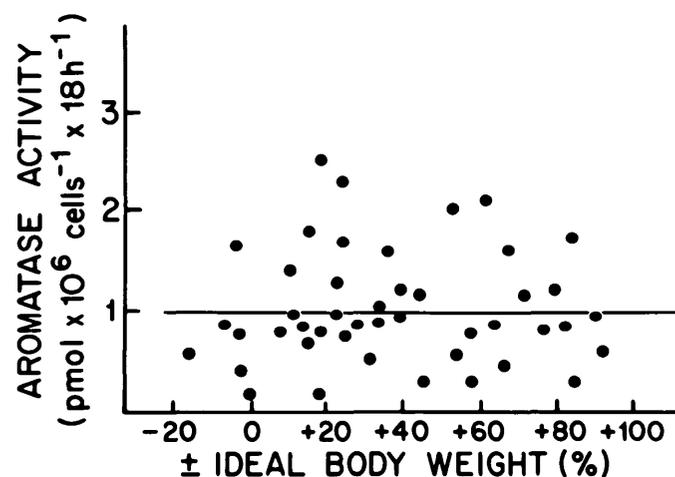


FIG. 2. Aromatase activity of freshly prepared adipose tissue stromal cell suspensions calculated as a function of percentage above or below ideal body weight (as determined from Metropolitan Life Insurance tables). Stromal cell suspensions were incubated with [1-<sup>3</sup>H]androstenedione (150 nM) for 18 h. Aromatase activity was determined as described in *Materials and Methods*. The data are the means of values obtained from assays of cells of two or three replicate dishes.

and were not being treated with estrogen was not significantly different from that of an age-matched group of premenopausal women [ $0.9 \pm 0.3$  and  $0.7 \pm 0.1$  pmol 18 h<sup>-1</sup> 10<sup>6</sup> cells<sup>-1</sup>, respectively (mean  $\pm$  SEM)].

Since increased estrogen formation is correlated positively with increased body weight (5, 6), we sought to determine whether aromatase activity of stromal cell suspensions (10<sup>6</sup> cells/incubation) prepared from adipose tissue from the same group of women presented in Fig. 1 might be correlated with body weight. When aromatase activity of stromal cells was plotted as a function of the percentage above or below ideal body weight (as determined from Metropolitan Life Insurance tables), there

was a significant lack of correlation between aromatase activity and body weight (Fig. 2;  $P > 0.2$ ;  $r = 0.023$ ).

### Discussion

The results of previous studies indicate that there is an increase in the fractional conversion of circulating androstenedione to  $E_1$  as a function of aging (1). These studies suggest that there may be an increase in the efficiency of utilization of circulating estrogen precursors, such that there is a 2- to 4-fold increase in extraglandular estrogen production associated with aging. Since the principal estrogen formed in postmenopausal women is  $E_1$ , which is derived primarily from the extraglandular aromatization of circulating androstenedione in adipose tissue (1), we sought to determine whether the increased aromatization of androstenedione with aging might be explained, in part, by an increase in the activity of the aromatase enzyme in adipose tissue of patients as a function of age.

We found that aromatase activity of stromal cells prepared from adipose tissue of women was increased as a function of age ( $P < 0.01$ ). Also, aromatase activity of stromal cells of postmenopausal women was significantly increased compared to that of stromal cells from premenopausal women. Whether the difference in extraglandular estrogen formation in postmenopausal and premenopausal subjects is a result of the hormonal changes associated with menopause or of the endocrine and metabolic changes associated with aging is not clear. The results of the present study suggest that the difference in aromatase activity may be due to the aging process rather than the hormonal changes associated specifically with menopause; the mean aromatase activity of stromal cells of five women (<45 yr of age) who had undergone surgically induced menopause did not differ significantly from that of age-matched premenopausal women. Furthermore, aromatase activity in men increases as a function of aging (1). Also, when we investigated the effects of gonadotropins (which are markedly elevated in postmenopausal women) known to induce aromatization in gonadal tissues (9-13), we were unable to demonstrate any effects on aromatase activity in adipose stromal cells maintained in culture (14).

We conclude that increased  $E_1$  production associated with aging may result from an increase in the specific activity of the aromatase enzyme in adipose stromal cells and is not affected by changes in gonadotropin concentrations associated with menopause. Significantly, when cells were maintained in culture until confluent, the differences in aromatase activity that were related to differences in the ages of the subjects were eliminated (data not shown). This result suggests that the increase in the aromatase specific activity of adipose stromal cells

with aging is mediated by a factor that changes as a function of the aging process.

The finding that the specific activity of aromatase of freshly prepared stromal cells was not related to the body weight of the individual suggests that the increase in estrogen formation as a function of obesity *in vivo* is a result of an increase in the number of adipose tissue cells rather than an increase in the intrinsic aromatase activity of the cells *per se*. It has been shown that with increasing obesity, there is an increase not only in the size of the adipocytes but also in the numbers of adipocytes and adipose stromal cells (15, 16). Thus, the increase in aromatase activity with obesity could simply reflect the increased numbers of these cells.

Although aromatase activity of adipose stromal cells is not affected by gonadotropins, it is stimulated by glucocorticoids (14) and ACTH and isoproterenol (17) and inhibited by epidermal growth factor and platelet-derived growth factor (Mendelson, C. R., M. E. Smith, and E. R. Simpson, unpublished observations). Whether one or more of these factors are responsible for the increase in aromatase activity of adipose stromal cells as a function of aging remains to be determined.

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