J. steroid Biochem. Vol. 24, No. 5, pp. 1033–1039, 1986
Printed in Great Britain.

0022-4731/86 $3.00 + 0.00
Pergamon Journals Ltd

AROMATASE ACTIVITY AND CONCENTRATIONS OF CORTISOL, PROGESTERONE AND TESTOSTERONE IN BREAST AND ABDOMINAL ADIPOSE TISSUE

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(*Received* 26 *July* 1985)

Summary—Aromatase activity and concentrations of cortisol, progesterone and testosterone were measured in samples of breast and abdominal adipose tissue obtained from both pre- and postmenopausal subjects. Enzyme activity was determined by the incorporation of tritium from [1β-^{3}H]androstenedione into water and found to be in close agreement to that measured when tritium labelled oestrone (E_1) and oestradiol (E_2) were isolated. No significant difference in enzyme activity was noted between breast and abdominal adipose tissue. Increased aromatase activity was not observed in adipose tissue taken from a subject with endometrial cancer. Cortisol concentrations were found to be significantly higher ($P < 0.05$) in abdominal as compared to breast tissue. Without attaining statistical significance progesterone concentrations were higher in abdominal as compared to breast adipose tissue. Aromatase activity was not related to either cortisol or testosterone tissue concentration, but an inverse relationship between progesterone concentration and aromatase activity was observed ($r = 0.542$, $P < 0.02$). On the basis of results obtained a hypothesis for the increased conversion of androgen to oestrogen as seen after the menopause has been proposed.

INTRODUCTION

Of all the various body sites shown to be capable of the aromatisation of androgen to oestrogen, adipose tissue is quantitatively the most important. Depending on the stage of the menstrual cycle peripheral conversion of androgen may account for as much as 50% of the total oestrogen produced in young women [1]. In the postmenopausal woman adipose tissue is considered to be the principal site of oestrogen production [2]. Unlike the ovary in which the primary oestrogen formed is oestradiol, the principal oestrogen produced by adipose tissue is oestrone. The substrate for oestrone formed in this way is androstenedione secreted principally from the adrenal cortex.

The fractional conversion of androstenedione to oestrone has been shown to increase as a function of body weight [3, 4] and also age [5]. In this context the specific activity of the aromatase enzyme responsible for this conversion would appear, in the female subject, to increase with age but not with obesity [6]. Thus increased oestrogen production with body weight may be attributable to an increase in total body adipose tissue capable of this conversion. Although increased adipose tissue oestrogen production in women with, as opposed to without endometrial neoplasia has not been demonstrated [7], an age or obesity related increase has been associated with cancer of the breast and endometrium [5, 8–11].

The factors that control peripheral aromatase activity are not well understood. Glucocorticoids have been shown to increase aromatase activity in both cultured adipose tissue stromal cells [12, 13] and adipose tissue explants [14]. It has been postulated that women with breast cancer may have developed the disease after being exposed to stressful situations [15]. The increased risk that obese women have for developing breast cancer has been well documented [16], and there is some evidence that plasma levels of adrenocorticotrophin (ACTH), which controls the secretion of cortisol and androstenedione, are increased in obese subjects [17]. Thus stress or obesity could result not only in the production of increased amounts of substrate, androstenedione, but also an increase in the extent to which androstenedione is aromatised. To support the latter suggestion there is a good correlation between cortisol production rate and body weight [18]. Similarly, if cortisol did stimulate aromatase activity, an age related increase in plasma free cortisol concentrations [19] could account for the age related increase in the conversion of androstenedione to oestrone as measured *in vivo* [5]. Testosterone and progesterone have also been implicated in the control of peripheral aromatase [20–22]. It has therefore been the aim of the present study to investigate aromatase activity in both breast and abdominal adipose tissue in relation to tissue concentrations of cortisol, progesterone, and testosterone. On the basis of results obtained, a mechanism for the rise in peripheral androgen conversion to oestrogen seen after the menopause, has been suggested.

EXPERIMENTAL

Tissue samples

Samples of abdominal and breast adipose tissue were obtained following hysterectomy or reduction

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mammoplasty from both pre- and post-menopausal subjects. Tissues were transported to the laboratory on solid carbon dioxide and stored at −20°C until required for assay.

Chemicals

[1,2-^{3}H]Androstenedione (46 Ci/mmol), [1,2,6,7-^{3}H]androstenedione (85 Ci/mmol), [4-^{14}C]androstenedione (50mCi/mmol),[4-^{14}C]oestrone (50mCi/mmol) and [4-^{14}C]oestradiol (50mCi/mmol) were obtained from N.E.N. [1,2,6,7-^{3}H]cortisol (85Ci/mmol) and [1,2,6,7-^{3}H]progesterone (99Cimmol) were obtained from Amersham International PLC, U.K. Unlabelled steroids and cofactors were obtained from the Sigma Chemical Company. Antibody for progesterone measurements was obtained from the World Health Organization and used at a final dilution of 1:40,000. Cortisol antibody was supplied courtesy of Professor W. R. Butt and used at a final dilution of 1:16,000. Reagents for the measurement of testosterone concentrations were purchased from St Thomas' Hospital London.

Measurement of aromatase activity

Aromatase activity was measured in samples of adipose tissue using the method of Siiteri and Thompson[23] later modified by Ackerman *et al.*[24]. In brief this method determines the amount of tritiated water formed when tritium is lost from the C-1β position of androstenedione during its aromatisation to oestrone.

Preparation of substrate

[1-^{3}H]androstenedione was produced by a procedure involving the overnight hydrolysis of [1,2-^{3}H]androstenedione as described by Ackerman *et al.*[24]. Our study indicated that 68.5 ± 1.1% (SE, $n = 7$) of the label remained linked to the steroid after hydrolysis.

Determination of tritium lost to water

In order to determine the proportion of tritium lost to water during the aromatisation of androstenedione, incubations were carried out using 1 ml aliquots of placental homogenates as the source of aromatase. To these [4-^{14}C]androstenedione and [1-^{3}H]androstenedione as prepared above were added in a known isotopic ratio together with cofactors for the generation of NADPH [25]. Incubations were carried out for 1 h at 37°C and terminated by the addition of 5 ml diethylether in which steroids were subsequently extracted. The principal product oestrone was purified by two successive TLC separations using the systems toluene–ethylacetate, 60:40 (v/v) and dichloromethane–ethylacetate, 80:20 (v/v). This was followed by overnight acetylation using 100 μl pryridine and 100 μl acetic anhydride. Oestrone acetate was isolated using a final TLC separation in the system DCM–ether 98:2(v/v). Determination of the isotope ratio of E_1 acetate formed indicated that 69.7 ± 0.5% (SE, $n = 4$) of the tritium bound to androstenedione is lost to water during aromatisation.

In experiments conducted to validate the use of this method for the measurement of aromatase activity in adipose tissue, [1,2,6,7-^{3}H]androstenedione was also used as substrate. Employing the methodology described above the proportion of tritium lost to water from this compound was determined to be 12.3 ± 1.2 (SE, $n = 4$).

Incubation of adipose tissue homogenates with labelled steroids

Tissues in which aromatase activity was to be measured were minced finely and homogenised using a "Polytron" disintegrator. Short bursts of homogenisation (5 s) followed by a period of cooling were used to minimise damage to the enzyme. The resulting homogenate was centrifuged at 1500 *g* for 5 min and the subnatant removed from below the fat layer.

To measure the production of [^3H_2O], 10 μl of ethanolic solution containing [1-^{3}H]androstenedione (1 μCi, 150 pmol) was added to 1 ml of homogenate. Reactions were initiated by the addition of cofactors for the generation of NADPH and carried out over a 3 h period at 37°C. For the purpose of validation of this method, incubations were also carried out following the addition of 10 μl of an ethanolic solution containing [1,2,6,7-^{3}H]androstenedione (1 uCi, 30–150 pmol) under otherwise similar conditions.

Analysis for [^{3}H]water and radiolabelled E_1 and E_2

At the end of the reaction period, tubes in which [^3H_2O] production was to be assessed were placed in an ice–water bath and left to stand for 15 min, 5 ml of ether was then added and all tubes were mixed. Homogenates in which the amount of labelled steroid produced was to be measured were extracted with ether following the addition of 0.01 μCi [4-^{14}C]E_1 and 0.01 μCi [4-^{14}C]E_2, as recovery markers. These extracts were then processed as described above for placental tissues. E_1 and E_2 acetates isolated in this way were pooled and counted. Ether extracts from [^3H_2O] determinations were discarded. To the remaining aqueous layer 0.5 ml of 30% trichloroacetic acid was added and the mixture shaken. The precipitated protein was sedimented by centrifugation for 10 min at 1500 *g*, and 1 ml of the resulting supernatant was removed to which was added 1 ml of a solution containing 0.5% dextran and 5% charcoal. After mixing, the charcoal was centrifuged (20 min, 2000 *g*) and a 1 ml aliquot of the clear supernatant was taken for counting.

After allowing for the amount of tritium lost to water during the aromatisation of [1,2,6,7-^{3}H]androstenedione, the amount of [^{3}H]E_1 and the [^{3}H]E_2 formed was calculated as described by Folkerd *et al.*[7]. The amount of [^3H_2O] formed was initially corrected for losses incurred during incu-

bation and processing, shown to be 19 ± 0.8% (SE, $n = 10$) when [^3H_2O] was taken through the assay. The values were also corrected by multiplying by 3 to correct for dilution of initial medium and by dividing by 0.697, the fraction of radioactivity that appeared as ^{3}H-water for each molecule converted to oestrogen. This value was then used to calculate the amount of androstenedione converted to oestrogen. Tissue protein concentrations were measured by the method of Lowry *et al.*[26] and aromatase activity was finally expressed as fmol or pg product formed per mg protein per hour.

Extraction of cortisol, progesterone and testosterone from adipose tissue

These three steroids were measured in tissues homogenised in ethanol. To an aliquot of homogenate (0.3–0.5 mg/ml, 100 mg/ml) 50 μl of ethanol containing 4000 dpm of [1,2,6,7-^{3}H]cortisol or [1,2,6,7-^{3}H]progesterone or [1,2,6,7-^{3}H]testosterone was added. After mixing, tubes containing the above were allowed to stand for 30 min at 4°C. All tubes were then centrifuged for 15 min at 1000 *g* following which the supernatant was aspirated with a Pasteur pipette and dried down under nitrogen.

Residues in which cortisol was to be measured were applied to TLC plates and run in the system chloroform–methanol, 90:10, v/v. Plates were then removed and re-run in the system ethylacetate–ethanol, 97:3, v/v. Areas corresponding to a [^{3}H]cortisol marker were identified by the use of a radiochromatogram imaging system (β-graph), cut out and eluted in methanol (1 ml). Aliquots of the methanol eluate were then taken to determine the recovery of tritiated cortisol originally added and cortisol by Radioimmuoassay (RIA). Residues of ethanolic extracts in which progesterone and testosterone were to be measured were applied to TLC plates and run in the system dichloromethane–dioxane, 94:6, v/v. Areas corresponding to radiolabelled marker steroids on β-graph were cut out and eluted overnight in ether (5 ml). Following the addition of 1 ml phosphate buffered saline (pH 7.0) to ether residues, aliquots were taken for recovery estimation and the measurement of steroid concentrations by RIA.

RESULTS

Aromatase activity

On incubating breast adipose tissue homogenates with either [1,2,6,7^{3}H]androstenedione or [1-^{3}H]androstenedione over a concentration range 0–150 pmol/ml the rate of production of oestrone and oestradiol was compared to that of [^3H_2O]. Figure 1 shows that the rate of aromatisation determined by both methods is in close agreement. By the use of [^3H_2O] method for the measurement of aromatase activity in adipose tissue homogenates, linearity over a protein concentration range 2–9 mg/ml homogenate and up to 4 h of incubation could be demonstrated (Fig. 2).

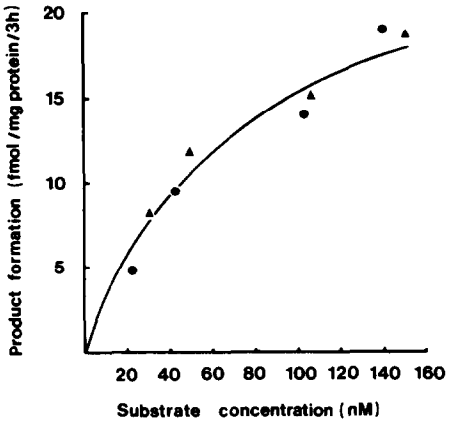

Fig. 1. Comparison of aromatase activity determined by the measurement of radiolabelled metabolites, E_1 and E_2 (▲) and the amount of ^3H_2O (●) produced at varying concentrations of substrate, androstenedione. Each point is the mean of four estimations.

Since aromatase activity in placental tissues is not affected by freezing [23], the need to use fresh tissues for this study was investigated. On removal from the subject a portion of abdominal adipose tissue was placed on ice at 4°C. Both sections of tissue were then transported to the laboratory where aromatase activity was measured according to the [^3H_2O] method described. No significant difference in aromatase activity was noted between both treatments. The practice of freezing tissues immediately after their removal from the subject was subsequently adopted since it was felt that further steroid metabolism during their transportation to the laboratory would be reduced.

Using the tritium water method described, aromatase activity was determined in 7 samples of breast and 21 samples of abdominal adipose tissue. The respective arithmetic mean rates of conversion for breast and abdominal tissue were 11.7 pg/mg protein/3 h (range 5.1–27.0) and 18.4 pg/mg protein/3 h (range 1.5–164). However, allowing for a skewed distribution of data, geometric mean values of 12.0 and 10.1 pg/mg protein/3 h are obtained for abdominal and breast adipose tissue respectively. One of the samples of abdominal tissue was obtained from a patient known to have endometrial cancer, enzyme activity was measured to be 12.6 pg/mg protein/h. Aromatase activity was observed in every sample considered. The mean percent conversion of androstenedione to oestrone in water blank estimations carried out at the same time as the test incubations but in the absence of tissue was 0.027 ± 0.011 (SD, $n = 20$). The mean percent conversion on the presence of tissue was 0.175%. All estimates of aromatase activity were made in duplicate and corrected for the blank measure at the same time. The coefficient of variation (CV) for duplicate sample estimations was 12.2%.

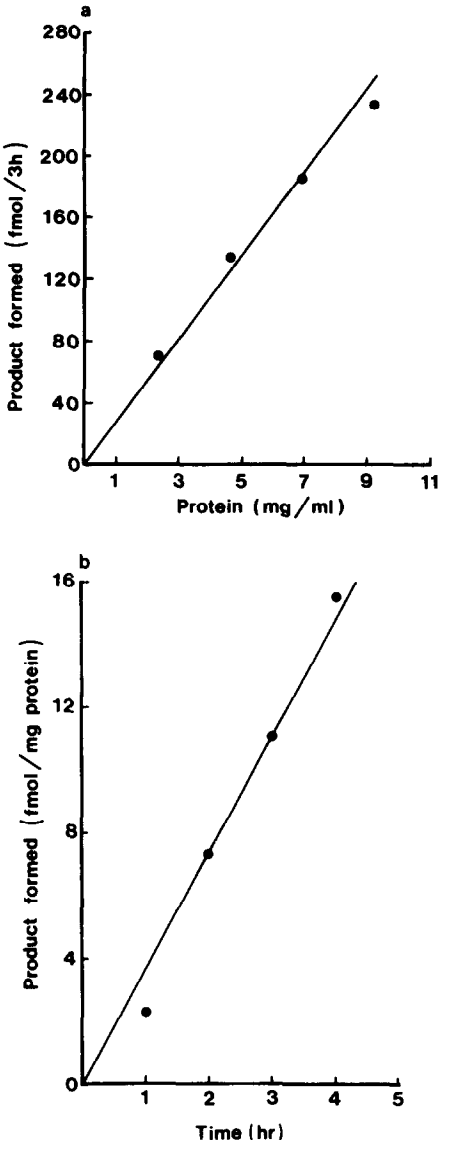

Fig. 2. The effect of (a) tissue protein concentration and (b) time of incubation on the amount of androstenedione converted to oestrogen by adipose tissue.

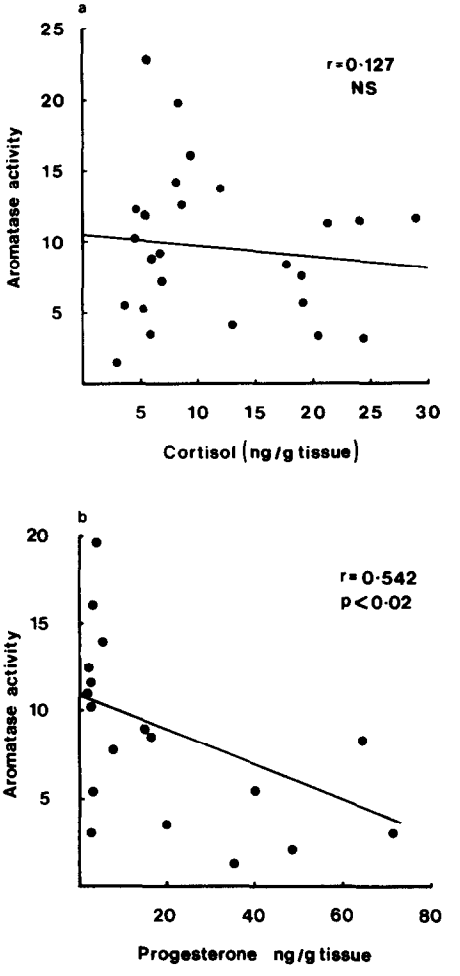

Fig. 3. Correlation between concentrations of (a) cortisol (b) progesterone and aromatase activity in abdominal and breast adipose tissue. Aromatase activity is expressed as pg oestrogen formed per mg protein per 3 h.

Tissue steroid concentrations

Concentrations of cortisol, testosterone and progesterone were measured in samples in which aromatase had previously been determined. Over the range of tissue concentrations employed for this study, the intra-assay coefficient of variation, cold recovery and linearity for each steroid is summarised in Table 1. For all fat samples considered the mean and range for concentrations of all three steroids measured is presented in Table 2. Although the number of breast tissues analysed was small ($n = 7$), concentrations of cortisol were significantly higher in abdominal as compared to breast adipose tissue ($P < 0.05$). Without attaining a statistical significance, mean concentrations of progesterone were also considerably higher in abdominal as compared to breast tissue.

Table 1. Summary of data obtained for steroid assay validation

	Cortisol	Progesterone	Testosterone
CV for duplicate extractions (%)	12.7	6.1	7.2
Linearity pg =	$11.7x + 4.53$	$1.3x + 7.4$	$0.16x + 1.4$
Correlation coefficient	0.994	0.983	0.938
Cold recovery			
Steroid added (range pg/0.5 ml)	20–320	20–160	20–160
Mean recovery (%)	96.3	98.6	94.5

Table 2

	Testosterone	Cortisol	Progesterone
B	—	6.2, 4.8–9.3 ($n = 7$)	10.0, 2.2–19.0 ($n = 5$)
A	1.39, 0.77–3.9 ($n = 7$)	13.4, 3.3–28.9 ($n = 20$)	17.4, 1.4–71.0 ($n = 14$)

Mean and range for concentrations of testosterone, cortisol and progesterone expressed as nanograms steroid per gram tissue in both breast (B) and abdominal (A) adipose tissue. Concentrations of cortisol were significantly higher ($P < 0.05$) in abdominal compared to breast adipose tissue.

Correlation between aromatase activity and tissue steroids

Figure 3 shows that the correlation between aromatase activity and concentrations of cortisol and progesterone in both breast and abdominal adipose tissue. A correlation between cortisol and testosterone and aromatase activity could not be demonstrated. However, a statistically significant ($r = 0.542$, $P < 0.02$) inverse relationship is observed between aromatase activity and progesterone concentrations.

DISCUSSION

Evidence presented in this paper confirms the findings of Ackerman *et al.*[24] that measurement of aromatase activity in fat tissue by the determination of [^3H_2O] production correlates well with that measured when radiolabelled oestrogens are isolated. Values obtained for both breast and abdominal fat are also in close agreement with those previously reported [7, 27] although activity was measured in every sample of breast fat in the present study. In one of the samples of abdominal fat obtained from a woman known to have endometrial cancer the value for aromatase activity obtained fell within the same range as those obtained from subjects without endometrial neoplasia. A similar finding to this was reported in an earlier study from this laboratory [7].

Data presented in Table 2 indicate that considerable quantities of steroids may be stored in both breast and abdominal fat. The concentrations of cortisol, progesterone and testosterone reported are similar to those observed by Feher *et al.*[28]. Our study indicates however, that mean concentrations of both cortisol ($P < 0.05$) and progesterone are higher in abdominal than breast fat. In view of previous concepts concerning the uptake of steroid by adipose tissue [29, 30] such a difference is unexpected. However, more recently a number of workers have been able to demonstrate the presence of high affinity binding proteins or receptors for both glucocorticoids and progestins in adipose tissue [31, 32]. A higher receptor concentration in abdominal as opposed to breast fat may therefore explain these results.

Several studies have been reported in which the synthetic glucocorticoid dexamethasone, has been shown to be capable of inducing aromatase activity within adipose tissue [12–14]. These observations were extended to show that under appropriate conditions, physiological concentrations of the endogenous glucocorticoid, cortisol, could also stimulate aromatase activity in explants of adipose tissue [14]. It has been suggested that the action of dexamethasone and cortisol is mediated via interaction with the glucocorticoid receptor [12]. In granulosa [33] and Sertoli cells [34] aromatase activity is stimulated by follicle stimulating hormone (FSH). In contrast aromatase activity of adipose tissue was shown not to be affected by FSH [12]. Lack of FSH receptor in adipose tissue has more recently been demonstrated [35]. Because of the findings reported above that both synthetic glucocorticoid and cortisol significantly enhance aromatase activity in adipose tissue explants the correlation between cortisol concentrations and enzyme activity in tissues obtained during the present study has been considered. It is apparent from data presented in Fig. 3 that no significant correlation exists. Recent *in vivo* investigations conducted within this laboratory have failed to demonstrate an increased conversion of androstenedione to oestrone in a subject treated with dexamethasone and also in another subject where endogenous cortisol concentrations were raised following the injection of synacthen [36]. In order to explain this discrepancy between *in vivo* and *in vitro* results, we have suggested [36] that the higher tissue concentration of cortisol may be associated with high concentrations of cortisol metabolites, such as 5α-dihydrocortisol which, like the 5α-reduced C_{19} steroids [23], may act as aromatase inhibitors. As an alternative explanation, when adipose tissue cells or explants are kept in culture medium a receptor mechanism may be revealed allowing the expression of new enzyme protein following stimulation with glucocorticoid.

Progesterone has also been implicated in the control of peripheral aromatase activity. However, as to whether inhibition [21] or induction of activity [22] occurs in response to progesterone remains controversial. Our study has shown that when tissue concentrations of progesterone are related to a aromatase activity a significant negative correlation exists ($P < 0.02$). Incubations conducted in which progesterone was added to adipose tissue homogenates to give a concentration 10-fold in excess of the substrate, androstenedione, indicated that this apparent inhibition was not due to a direct effect on the enzyme (unpublished observations). Although unlike granulosa cell aromatase, FSH appears not to be involved in the regulation of aromatase activity in adipose tissue, the mechanism for progesterone inhibition may be similar in both tissues. Schreiber *et al.*[37] found that aromatase activity in rat ovarian granulosa cells was inhibited by several progestins and that the "potency" of these compounds corresponded to their relative affinity of binding to progesterone receptor. Recently, steroid hormones have been shown to increase the activity of enzymes in-

volved in the production of cyclic nucleotides [38, 39]. Reciprocal biological effects have been attributed to cyclic AMP and cyclic GMP [40]. Within adipose tissue cAMP has been shown to induce aromatase activity [41], cGMP production in response to progesterone stimulation [39] may be responsible for the inhibitory effects observed.

Due to the restricted number of samples considered within the present study, a formal comparison of both aromatase activity and progesterone concentrations in samples obtained from premenopausal subjects throughout the menstrual cycle and postmenopausal patients has not been presented. However, a preliminary comparison of data available indicates that progesterone concentrations are lower in samples of tissue obtained from proliferative phase and post menopausal subjects in comparison to adipose tissue obtained from subjects within the secretory phase at the time of operation. Conversely, highest aromatase activities were observed in the post menopausal group and lowest in samples obtained from subjects within the secretory phase of the menstrual cycle. Removal of the inhibitory action of progesterone on cessation of ovarian function may in part explain the rise in peripheral conversion of androgen to oestrogen reported to occur after the menopause [42].

Initial work concerning the role that androgens play in the regulation of peripheral aromatase activity was devoted to studies of the enzyme in placenta. Siiteri and Thompson [23] were able to show that a number of 5α-reduced androgens inhibited the conversion of androstenedione to oestrone during short term incubations. It was considered that the effect of these non-aromatisable androgens was that of direct inhibition by binding to cytochrome P-450, an essential component of the aromatase system. More recently the 5α-reduced metabolite of testosterone, 5α-dihydrotestosterone (DHT), has been shown to stimulate oestradiol production when added to culture medium containing placental tissue fragments [43]. Aromatase activity within the hypothalamus-preoptic area (HPOA) of rat brain has also been shown to be stimulated following the implantation of capsules containing testosterone or DHT. From information gained using antiandrogens, these authors concluded that the control of HPOA aromatase activity by androgens is receptor mediated [44]. The findings reported in our present study that no correlation exists between tissue concentrations of testosterone and aromatase activity may indicate that this mechanism is inoperative or absent in adipose tissue.

The results presented within this paper point only to an involvement of progesterone in the control of aromatase within adipose tissue. It has been suggested that within the ovary progesterone inhibits follicular development and that this effect may be due to inhibition of granulosa cell oestrogen production [45]. Conversely, the increase in the conversion of androgen to oestrogen reported to occur after the menopause may be due to the cessation of the inhibition imposed by ovarian progesterone production on peripheral aromatase.

REFERENCES

1. Siiteri P. K. and MacDonald P. C.: Role of extraglandular oestrogen in human endocrinology. In *Handbook of Physiology* (Edited by R. O. Greep and E. B. Astwood). American Physiology Society, Washington D.C., Vol. 2 (1973) pp. 615–629.
2. Grodin M. J., Siiteri P. K. and MacDonald P. C.: Sources of oestrogen production in postmenopausal women. *J. clin. Endocr. Metab.* **36** (1973) 207–214.
3. Edman C. D. and MacDonald P. C.: Effect of obesity on the conversion of plasma androstenedione to oestrone in ovulatory and anovulatory young women. *Am. J. Obstet. Gynec.* **130** (1978) 456–461.
4. 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 oestrone in postmenopausal women with and without endometrial cancer. *Am. J. Obstet. Gynec.* **130** (1978) 448–455.
5. Hemsell D. L., Grodin J. M., Brenner P. F., Siiteri P. K. and MacDonald P. C.: Plasma precursors of oestrogen II. Correlation of the extent of conversion of plasma androstenedione to oestrone with age. *J. clin. Endocr. Metab.* **38** (1974) 476–479.
6. Cleland W. H., Mendelson C. R. and Simpson E. R.: Effects of aging and obesity on aromatase activity of human adipose cells. *J. clin. Endocr. Metab.* **60** (1985) 174–177.
7. Folkerd E. J., Reed M. J. and James V. H. T.: Oestrogen production in adipose tissue from normal women and women with endometrial cancer. *in vitro. J. steroid Biochem.* **16** (1982) 297–302.
8. Allen H. H. and Fraleigh D. M.: Carcinoma of the endometrium. *Am. J. Obstet. Gynec.* **85** (1963) 302–306.
9. Vorherr H. and Messer R. H.: Breast cancer: potentially predisposing factors. *Am. J. Obstet. Gynec.* **130** (1978) 335–358.
10. Dunn L. J. and Bradbury J. T.: Endocrine factors in endometrial carcinoma. *Am. J. Obstet. Gynec.* **97** (1967) 465–471.
11. Knab D. R.: Oestrogen and endometrial carcinoma. *Obstet. Gynec. Surv.* **32** (1977) 267–281.
12. Simpson E. R., Ackerman G. E., Smith M. E. and Mendelson C. R.: Oestrogen formation in stromal cells of adipose tissue of women induced by glucocorticoids. *Proc. natn. Acad. Sci. U.S.A.* **78** (1981) 5690–5694.
13. Mendelson C. R., Cleland W. H., Smith M. E. and Simpson E. R.: Regulation of aromatase activity of stromal cells derived from human adipose tissue. *Endocrinology* **111** (1982) 1077–1085.
14. Folkerd E. J. and James V. H. T.: Aromatisation of steroids in peripheral tissues. *J. steroid Biochem.* **19** (1983) 687–690.
15. Cooper C. L.: Psychological stress and cancer. *Bull. Br. Psychol. Soc.* **35** (1982) 456–459.
16. DeWard F. Breast cancer incidence and nutritional status with particular reference to body weight and height. *Cancer Res.* **35** (1975) 3351–3356.
17. Slavnov V. N. and Epshtein E. V.: Somatotrophic, thyrotrophic and adrenocorticotrophic functions of the anterior pituitary in obesity. *Endocrinologie* **15** (1977) 213–218.
18. Mlynark P., Gillies R. R., Murphy B. and Pattee C. J.: Cortisol production rates and obesity. *J. clin. Endocr. Metab.* **22** (1962) 587–591.
19. Jones M. K., Dyer G. I., Ramsay I. D. and Collins W. P.: Studies on apparent free cortisol and testosterone

in plasma from patients with breast tumours. *Postgrad. Med. J.* **57** (1981) 89–94.
20. Roselli C. E., Ellinwood W. E. and Resko J. A.: Regulation of brain aromatase activity in rats. *Endocrinology* **114** (1984) 192–200.
21. Tseng L., Mazella J., Mann W. J. and Chumas J.: Oestrogen synthesis in normal and malignant human endometrium. *J. clin. Endocr. Metab.* **55** (1982) 1029–1031.
22. Tseng L.: Effect of oestradiol and progesterone on human endometrial aromatase activity in primary cell culture. *Endocrinology* **115** (1984) 833–835.
23. Thompson E. A. and Siiteri P. K.: Utilisation of oxygen and reduced nicotinamide adenine dinucleotide phosphate by human placental microsomes during aromatisation of androstenedione. *J. biol. Chem.* **249** (1974) 5364–5372.
24. Ackerman G. E., Smith M. E., Mendelson C. E., MacDonald P. C. and Simpson E. R.: Aromatisation of androstenedione by human adipose tissue stromal cells in monolayer culture. *J. clin. Endocr. Metab.* **53** (1981) 412–417.
25. Nimrod A. and Ryan K. J.: Aromatisation of androgens by human abdominal and breast fat tissue. *J. clin. Endocr. Metab.* **40** (1975) 367–372.
26. Lowry O. H., Rosenberg N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin-phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
27. Beranek P. A., Folkerd E. J., Ghilchik M. W. and James V. H. T.: 17β-hydroxysteroid dehydrogenase and aromatase activity in breast fat from women with benign and malignant breast tumours. *Clin. Endocr.* **20** (1984) 205–212.
28. Feher T., Brodrogi L., Vallent K. and Ribai Z.: Role of human adipose tissue in the production and metabolism of steroid hormones. *Endocrinologie* **80** (1982) 173–180.
29. Bleau G., Roberts K. D. and Chapdelaine A.: The *in vitro* and *in vivo* uptake and metabolism of steroids in human adipose tissue. *J. clin. Endocr. Metab.* **39** (1974) 236–246.
30. Davis M. E. and Plotz E. J.: Hormones in human reproduction. *Am. J. Obstet. Gynec.* **76** (1958) 939–954.
31. Gray J. M. and Wade G. N.: Cycoplasmic progestin binding in rat adipose tissue. *Endocrinology* **104** (1979) 1377–1382.
32. Feldman D. and Loose D.: Glucocorticoid receptors in adipose tissue. *Endocrinology* **100** (1977) 398–405.
33. Dorrington J. H., Moon Y. S. and Armstrong D. T.: Oestradiol-17β biosynthesis in cultured granulosa cells from hypophysectomised immature rats; stimulation by follicle stimulating hormone. *Endocrinology* **97** (1975) 1328–1331.
34. Dorrington J. H. and Armstrong D. T.: Follicle-stimulating hormone stimulates oestradiol-17 synthesis in cultured sertoli cells. *Proc. natn. Acad. Sci. U.S.A.* **72** (1975) 2677–2681.
35. Folkerd E. J., Jacobs H. S., Van Der Spuy Z. and James V. H. T.: Failure of FSH to influence aromatisation in human adipose tissue. *Clin. Endocr.* **16** (1982) 621–625.
36. James V. H. T., Reed M. J., Berenak P. A., Bonney R. C., Samuel D. L., Newton C. J., Franks S. and Ghilchik M. W.: Androgen-oestrogen metabolism in breast cancer patients. *Ann. N.Y. Acad. Sci.* In press.
37. Schreiber J. R., Nakamura K. and Erikson G. F.: Progestins inhibit FSH-stimulated granulosa oestrogen production at a post c-AMP site. *Molec. cell. Endocr.* **21** (1981) 161–170.
38. Vesely D. L.: Testosterone and its precursors and metabolites enhance guanylate cyclase activity. *Proc. natn. Acad. Sci. U.S.A.* **76** (1979) 3491–3494.
39. Vesely D. L. and Hill D. E.: Oestrogens and progesterone increase fetal and maternal guanylate cyclase activity. *Endocrinology* **107** (1980) 2104–2109.
40. Goldberg N. D., Haddox M. K., Nicol S. E., Glass D. B., Sanford C. H., Kuehl F. A. Jr. and Estensen R.: Biological regulation through opposing influences of cyclic GMP and cyclic AMP: the yin yang hypothesis. *Adv. Cyclic nucleotide Res.* **5** (1975) 307–330.
41. Mendelson C. R., Smith M. E., Cleland W. H. and Simpson E. R.: Regulation of aromatase activity of cultured adipose stromal cells by catecholamines and adrenocorticotropin. *Molec. cell. Endocr.* **37** (1984) 61–72.
42. Longcope C.: The significance of steroid production by peripheral tissue. In *Endocrinology of the Ovary* (Edited by R. Scholler). Sepe, Paris (1978) pp. 23–33.
43. Ling W. Y.: Androgens stimulate oestradiol production in cultured human placental explants from term pregnancy. *J. clin. Endocr. Metab.* **57** (1983) 439–441.
44. Roselli C. E. and Resko J. A.: Androgens regulate brain aromatase activity in adult male rats through a receptor mechanism. *Endocrinology* **114** (1984) 2183–2189.
45. Schreiber J. R., Nakamura K. and Erickson G. F.: Progestins inhibit FSH-stimulated steroidogenesis in cultured rat granulosa cells. *Molec. cell. Endocr.* **19** (1980) 165–173.