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29. Metabolism of Steroids

PERIPHERAL OESTROGEN METABOLISM IN POSTMENOPAUSAL WOMEN WITH OR WITHOUT BREAST CANCER: THE ROLE OF DIETARY LIPIDS AND GROWTH FACTORS

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Summary—Studies we have carried out have revealed significant differences in oestrogen production and metabolism between normal women and postmenopausal women with breast cancer. The free, biologically available fraction of oestradiol is elevated in plasma from women with breast cancer and we have found that metabolic clearance rates and production rates of oestradiol are also increased.

In vitro studies have suggested that lipids can influence the distribution of sex steroids in plasma and we have therefore examined the effect of dietary lipids on the distribution of sex steroids in plasma *in vivo*. Consumption of a meal with a high saturated fat content or the oral or i.v. administration of "Intralipid", a stabilised emulsion of soya bean oil that is high in unsaturated free fatty acids, had little effect on the available fractions of oestradiol in plasma. However, results from a preliminary study suggest that long-term changes in dietary fat intake can alter the distribution of steroids in plasma.

It is concluded that dietary lipids may influence the availability of sex steroids to tissues. Such a mechanism could account for the significant correlation that has been found between dietary fat consumption and the incidence of breast cancer on a world-wide basis.

INTRODUCTION

The failure to find a consistent abnormality in plasma or urinary oestrogen levels of women with breast cancer has stimulated investigations into other mechanisms by which tissue exposure to oestrogens, which have been implicated in the development of breast cancer, may be increased in women with this condition. During the last 5 yr there has been considerable interest in examining whether the free, biologically available, fraction of plasma oestradiol is augmented in women with breast cancer as was originally suggested by Siiteri[1]. In this paper we review some of our recent studies in which we have: (a) examined factors that may influence the distribution of steroid fractions in plasma; (b) measured metabolic clearance rates and production rates of oestradiol and (c) investigated the metabolism of oestrogens by breast tumors.

MATERIALS AND METHODS

The free fraction of oestradiol in plasma was measured by equilibrium dialysis with a Dianorm equilibrium dialysis machine (M.S.E., Crawley, Sussex) using undiluted plasma at 37°C [2]. Non-SHBG bound oestradiol (i.e. mainly bound to albumin) and the binding capacity of sex hormone binding globulin (SHBG) were measured using precipitation techniques [2]. The metabolic clearance rates of oestradiol (MCR-E_2), production rates of oestradiol and the conversion of oestrone to oestradiol were measured using a double isotope infusion technique [3]. The methods used to investigate the *in vitro* metabolism of oestrogens by normal breast and breast tumour tissue and to measure tissue oestrogen concentrations have been previously described [4, 5].

RESULTS AND DISCUSSION

Several studies have now been carried out to measure the free fractions of oestradiol in plasma from women with breast cancer. Most [2, 6–8] but not all [9, 10] investigators have found that the free fraction of plasma oestradiol is increased in women with breast cancer. However, even in studies where a significant increase in the free oestradiol fraction was found the difference in the concentration of free oestradiol was very small and usually less than 1 pg/ml. Although this difference in the free concentration of oestradiol between normal women and women with breast cancer is small it may be significant given the long time-course that has been reported for the development of breast cancer.

While only the free steroid fraction has generally been considered to be available to tissues, recent studies [11] have suggested that albumin-bound steroids may also, by dissociation, be available to some tissues. In a study we have carried out, however, where patients and control subjects were carefully matched for ideal body weight, age and number of years after the menopause, we found no significant difference in the fraction or concentration of oestradiol bound to albumin [12]. Others have reported that the albumin-bound fraction of oestradiol is increased in women with breast cancer [7], although the concentrations of albumin-bound oes-

tradiol were not reported. In our initial study [12] only a single blood sample was taken for estimation of non-SHBG bound oestradiol. In a recent study [13] several blood samples were taken from women with breast cancer over a 24-h period and considerable variations in the non-SHBG bound oestradiol fraction were detected. Some of the changes in the non-SHBG bound oestradiol fraction in these samples could be related to changes in plasma levels of non-esterified free fatty acids (NEFA) and we have therefore investigated the role that lipids may have in regulating the availability of steroids to tissues. In an earlier study [14] it was shown that NEFAs could alter the binding of thyroxine to plasma proteins. There is also good evidence that dietary fat intake is, in an as yet undefined manner, related to the incidence of breast cancer on a geographical basis [15]. If dietary lipids can enhance tissue exposure to oestrogens such a mechanism could account for the relationship between dietary fat intake and breast cancer incidence.

Preliminary *in vitro* studies in which NEFAs were added to plasma confirmed that lipids could influence the binding of oestradiol to plasma proteins. Unsaturated but not saturated lipids increased both the free and non-SHBG oestradiol fractions in plasma [16].

To examine the physiological relevance of these findings we have carried out a number of studies in which normal male volunteers consumed a meal with a high fat content or received an oral or i.v. dose of "Intralipid", a stabilised emulsion of soya bean oil used for parenteral nutrition which is high in unsaturated free fatty acids.

As shown in Fig. 1, consumption of a meal with a high saturated fat content had little effect on the free or albumin bound fractions of oestradiol in plasma. Similarly, no consistent effects of "Intralipid" on these fractions were detected after subjects received an oral (Fig. 2) or i.v. dose (Fig. 3).

To examine the possibility that longer-term changes in dietary fat intake might influence the available fractions of steroids in plasma, volunteer male subjects changed from their normal diet to a high fat diet (>100 g fat/day) for 2 weeks after which a low fat diet (<10 g fat/day) was consumed for a further 2 weeks. As shown in Figs 4(a) and 4(b), in two subjects a high fat diet had a different effect on plasma levels of NEFAs and the free testosterone fraction, although the results are consistent with the hypothesis that lipids may influence the available fraction of steroids in plasma and thus tissue exposure to steroids.

In addition to measuring the distribution of oestradiol in plasma from women with breast cancer, we have also used infusions of isotopically labelled oestrogens to measure the metabolic clearance rates of oestradiol (MCR-E_2), the production rate of oestradiol (PR-E_2) and the conversion of oestrone to oestradiol ($[\rho]_B^{E_1E_2}$) in normal postmenopausal women and women with breast cancer. The results from these investigations have revealed that there are significant differences in the metabolism of oestrogens by normal women and women with breast cancer [17]. The MCR-E_2 was increased in women with breast cancer compared with that in a group of carefully matched control subjects. While this finding is consistent with the increased free fraction of oestradiol reported by some groups for women with breast cancer, it is also possible that increased tissue metabolism may contribute to the increased clearance rate found in women with breast cancer. Patients and control subjects in this study were matched for ideal body weight, age and time elapsed since the menopause. The plasma concentration of oestradiol was significantly higher in cancer patients

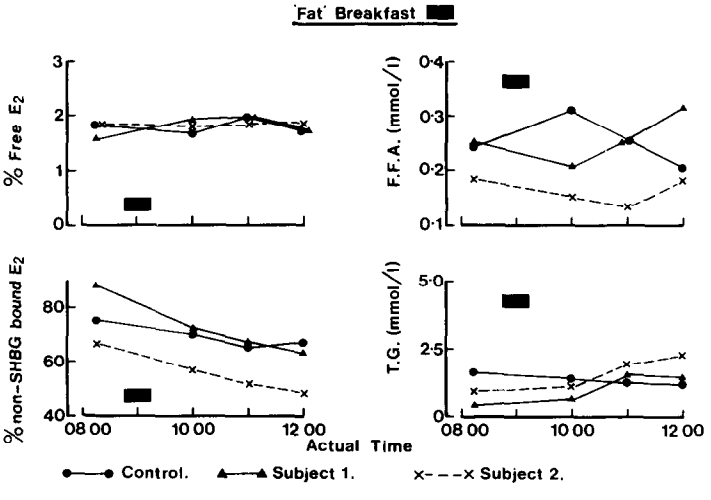

Fig. 1. Percentage of free oestradiol (E_2), non-SHBG bound oestradiol, non-esterified free fatty acids (F.F.A.) and triacylglycerides (T.G.) in plasma from a control fasting male subject, and 2 male subjects before and after a meal with a high saturated fat content.

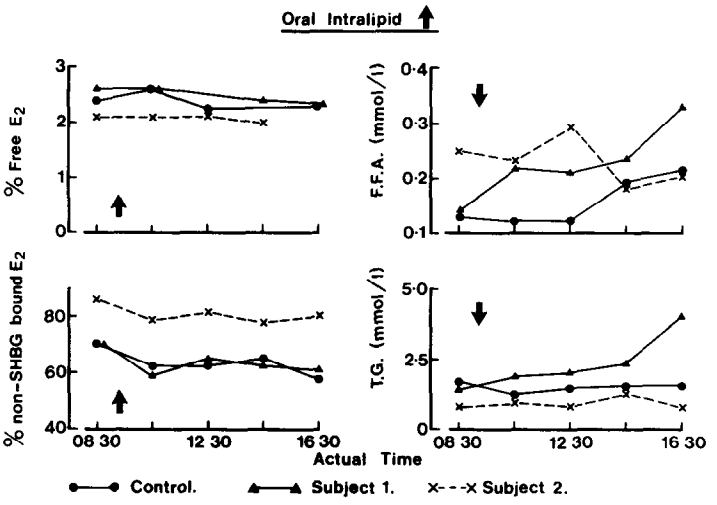

Fig. 2. Percentage of free oestradiol (E_2), non-SHBG bound oestradiol, non-esterified free fatty acids (F.F.A.) and triacylglycerides (T.G.) in plasma from a control fasting male subject and 2 male subjects before and after the oral ingestation of "Intralipid" a stabilised emulsion of soya bean oil which is high in unsaturated fatty acids. Subjects drank 500 ml of 20% "Intralipid" over a 10–15-min period.

and this, together with the increased clearance rate, resulted in a significant increase in the PR-E_2 by women with breast cancer. Although the PR-E_2 was increased in women with breast cancer the conversion of oestrone to oestradiol was similar in women with and without breast cancer [17].

We have therefore obtained evidence for significant differences in oestrogen metabolism in women with breast cancer compared with metabolism by normal women. However, the differences in the available fraction of oestradiol between cancer and normal subjects are relatively small, and it is likely that production of oestrogens by tumours or at a site close to the tumour may be an important source of oestrogen to enhance tumour development. We have therefore measured concentrations of oestrogens in normal breast and breast tumour tissue and examined the activity of oestradiol 17β-hydroxysteroid dehydrogenase (E_2DH), the enzyme responsible for the interconversion of oestrone and oestradiol, and factors which may regulate its activity, in normal breast and breast tumour tissue.

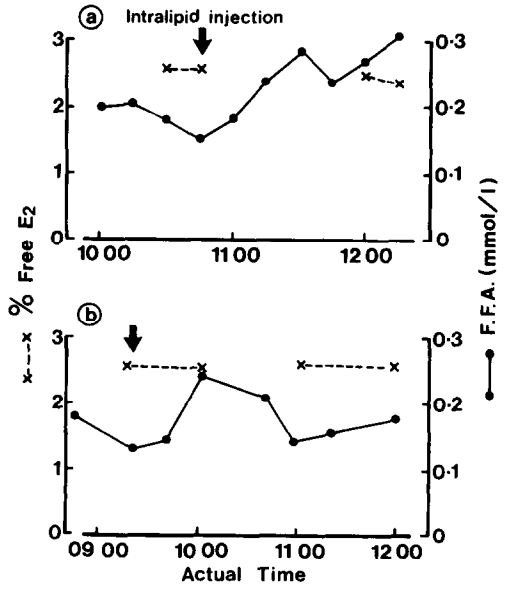

Fig. 3. Percentage of free oestradiol (E_2) and non-esterified free fatty acids (F.F.A.) in plasma from 2 male subjects who received a bolus injection of 60 ml of 20% "Intralipid" over a 2-min period.

The concentration of oestradiol was significantly higher in breast tumour tissue compared with normal breast tissue [5] and this finding has recently been confirmed by others [18]. Infusions of isotopically labelled oestrogens for a sufficient length of time (up to 12 h) to achieve a steady state before the removal of breast tissue at surgery has enabled us to examine the *in vivo* metabolism of oestrogens by normal breast and breast tumour tissue [19].

After infusion of [^{3}H]oestradiol, little metabolism of oestradiol occurred in breast tumour tissue whereas a significantly greater proportion of oestradiol was metabolised to oestrone by normal breast tissue. In contrast, after similar infusions of [^{3}H]oestrone, a greater proportion of oestrone was converted to oestradiol by tumour tissue compared with normal breast tissue. These results suggest that conversion of oestrone to oestradiol is enhanced in breast tumours and this could account for the higher concentrations of oestradiol that we and others have found in breast tumours when compared with levels in normal breast tissue.

We are currently examining two possible mechanisms which might account for the enhanced conversion of oestrone to oestradiol in breast tumour tissue. It is possible that there are differences in

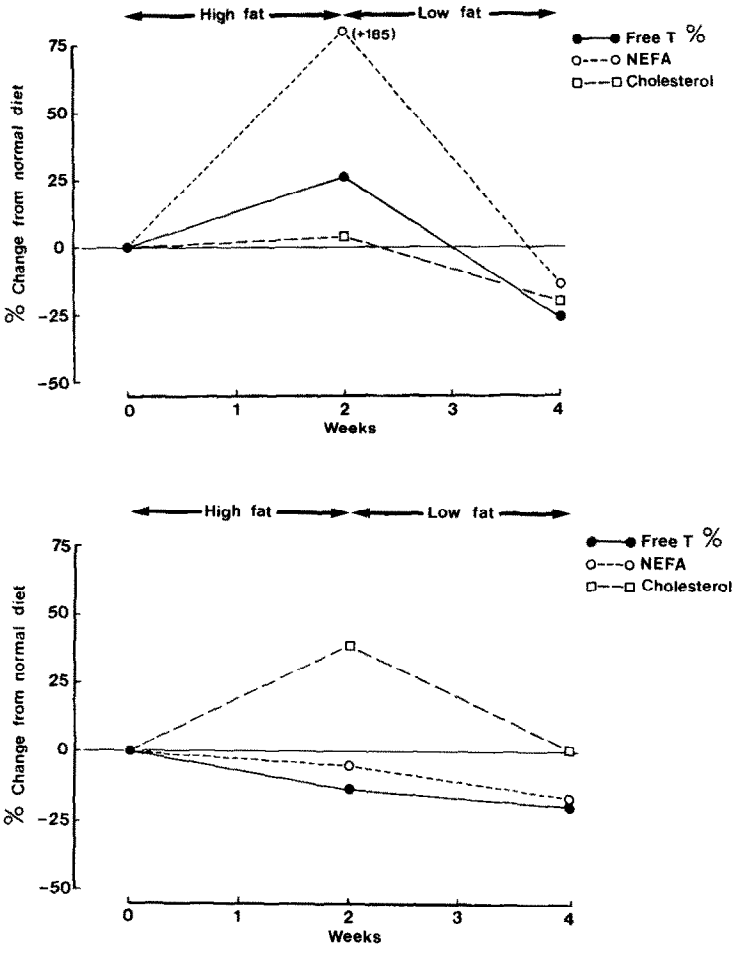

Fig. 4a and b. The free testosterone fraction (T), non-esterified free fatty acids (F.F.A.) and cholesterol in plasma from male subjects who changed from their normal diet to a high fat diet (>100 g fat/day) and then to a low fat diet (<10 g fat/day). Changes are expressed as a percentage of baseline values.

co-factor concentrations between normal and breast tumour tissue and the results from preliminary measurements of co-factor concentrations support this suggestion.

The other possibility that we are actively investigating is that a factor(s) produced by the tumour may influence oestrogen metabolism not only within the tumour but also in tissues adjacent to the tumour. In previous studies we found a significant relationship between E_2DH activity in tissue adjacent to tumours and breast tumour size [20] and also between E_2DH activity in breast tumours and tissue adjacent to the tumours [21]. These findings suggested that a factor(s) produced by the tumour might influence E_2DH activity. To investigate this possibility we have developed an *in vitro* system (Fig. 5) to examine the effect of tumour homogenates on E_2DH activity in cultured adipose tissue.

Using this system we have shown that some, but not all, breast tumour homogenates can stimulate the activity of E_2DH when added to the adipose culture system. A similar stimulation of E_2DH activity can be achieved by the addition of transforming growth factor α to the culture system [4]. The results from these preliminary studies suggest that some breast tumours contain a factor(s) that can modulate E_2DH activity and this may be a mechanism by which oestradiol synthesis within or close to the tumour is increased, thus making more oestradiol available to promote tumour growth.

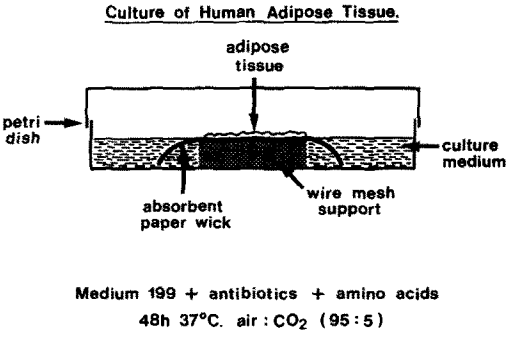

Fig. 5. *In vitro* system used to culture adipose tissue for up to 48 h.

It is apparent from this review that there is now good evidence for differences in oestrogen metabolism by women with breast cancer as compared with normal women. We and others have found that the free oestradiol fraction in plasma is increased in women with breast cancer and our group has shown that both the MCR-E_2 and PR-E_2 are higher in women with breast cancer. Some groups, however, have failed to find any difference in the free oestradiol fraction in plasma from women with breast cancer and even where such a difference has been found the increase in free oestradiol concentration is very small. It is therefore possible that production of oestradiol by the tumour or by tissues close to the tumour has a greater role in supplying oestrogen to the tumour to promote growth than the small fraction of oestradiol which is available to tissues from plasma. Studies we have carried out have shown that a factor(s) produced by some breast tumours can modulate oestradiol synthesis. We are currently isolating and examining the nature of such factors present in breast tumours. Understanding how such factors regulate oestrogen synthesis within the tumour may lead to the development of effective therapeutic agents to block oestrogen formation by tumours and hopefully inhibit tumour growth.

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