PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS

Prostaglandins Leukotrienes and Essential Fatty Acids (1993) 48, 111–116

The Role of Free Fatty Acids in Regulating the Tissue Availability and Synthesis of Sex Steroids

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ABSTRACT. **Sex steroids and dietary fat intake have been implicated in the growth of breast tumours. We have previously shown that the plasma free oestradiol fraction is increased in women with breast cancer and that the addition of free fatty acids (FFA) to plasma can increase the free oestradiol fraction in vitro. In the present study we have examined the distribution of oestradiol and testosterone in serum obtained from European women (EW) and Asian (Gujarati) women (GW) living in north-west London. Fat intake by these women is similar but GW, who are vegetarians, consume a greater proportion of unsaturated fats.**

In serum from perimenopausal GW, the free testosterone concentration was significantly higher than for EW (11.1 ± 3.6 pmol/l vs 8.7 ± 3.4 pmol/l, $p<0.05$). Although a significant correlation was found between the free testosterone and FFA concentrations for GW ($r = 0.49$, $p<0.05$), concentrations of sex-hormone binding globulin (SHBG) were significantly lower in GW than EW. The finding of lower SHBG concentration in GW was confirmed in a second study in postmenopausal women (EW, 60.1 ± 34.1 nmol/l; GW, 37.8 ± 20.5 nmol/l, $p<0.05$). However, no difference in the free oestradiol fraction or concentration was detected for EW and GW and no correlations with total or individual FFA were found. It is concluded from this study that while dietary fats may have an important role in the development of breast tumours, it is unlikely to be mediated by FFA inhibiting the binding of sex steroids to plasma proteins.

Breast cancer remains a major cause of death for women in most Western countries. While many different factors are likely to be involved in initiating the development of breast tumours, it is generally accepted that sex steroids and diet have important roles in the promotion of tumour growth. Marked differences in the incidence and mortality from breast cancer throughout the world has led to the view that fat intake may be linked to an increased risk of breast cancer (1–3). The finding that Japanese women who migrate to America, where fat intake is increased and who have an increased incidence of breast cancer, has been used as evidence to support a role for dietary fat in the development of breast cancer (4). The most convincing evidence used in support of the fat–breast cancer hypothesis is the highly significant correlation between fat intake and breast cancer mortality on a world-wide basis (1). If dietary fat intake is related to an increased risk of breast cancer, it is important to understand mechanisms which might account for such a link. However, in spite of much research, a convincing mechanism linking fat intake to an increased risk of breast cancer has not been found.

Our own studies have focussed on the role that sex steroids (oestradiol and testosterone) may have in promoting the growth of breast tumours, as there is a considerable body of evidence implicating these steroids in the growth of breast tumours (5). Although many investigations have been carried out seeking an abnormality in plasma sex steroid concentrations, it is only recently that evidence has been obtained for a consistent increase in the free, non-protein bound fractions of oestradiol and testosterone in women with breast cancer (6–10). Sex steroids circulate in blood in a free form or bound to albumin or sex-hormone binding globulin (SHBG). Only the free form is available to tissues, and thus biologically active. There is some evidence, however, to suggest that albumin-bound steroids may, by dissociation, also be available to some tissues (11). Several possible mechanisms have been put forward to account for the high incidence of breast cancer that is found in most Western countries where fat intake is high. These include alterations in gut flora (12), secretion of prolactin (3) or increased mass of adipose tissue for peripheral oestrogen formation (13).

Results from recent studies have suggested additional mechanisms by which dietary lipids may increase breast cancer risk, in that the addition of unsaturated free fatty acids (FFA) to plasma in vitro results in an increase in both the free and albumin bound fractions of oestradiol and testosterone (14–18). In addition to the possibility that FFA might interfere with the binding of sex steroids to plasma proteins, it has also been demonstrated that changing from a normal diet to a high fat and then to a low fat diet was associated with a significant increase and decrease respectively in plasma SHBG concentrations (19). Thus dietary lipids may be an additional regulator of SHBG, which is the major determinant of the free sex steroid concentrations in blood.

To examine the physiological relevance of our in vitro findings, a number of investigations were carried out in which normal male volunteers consumed a meal with a high fat content or received an oral or i.v. dose of 'Intralipid'. 'Intralipid' is a stabilized emulsion of soya bean oil used for parenteral nutrition which is high in unsaturated FFA. However, these short-term changes in lipid intake failed, in most cases, to cause any significant change in the free oestradiol or testosterone fractions in blood (20, 21).

Having failed to show an effect of short-term changes in fat intake on the distribution of sex steroids in blood and because of the problems associated with changing dietary lipid intake over a prolonged period of time, the effect of ingesting different dietary lipids on free steroid fractions has been examined in women of different ethnic origins living in north-west London. Subjects investigated were European women (EW) consuming an omniverous diet and Asian, Gujarati women (GW) consuming a mainly vegetarian diet. Proportional mortality ratios for breast cancer for immigrants from the Indian subcontinent and British women are 37 and 90 respectively (22). The lipid intake of EW and GW living in this part of London has previously been investigated (23). These studies have established that although the amount of fat consumed by EW and GW is similar, unsaturated fats (mainly linoleic acid (LA) in ground nut oil) form a much greater proportion of total fat intake in GW.

Insulin and insulin-like growth factor-type I (IGF-I) concentrations were also measured in serum samples obtained from these women as there is evidence that these factors may also have an important role in regulating SHBG production (24).

In addition to these dietary studies, we have also investigated the cellular mechanisms by which lipids might alter steroid synthesis or tissue availability. For this we have examined the effect of FFA on SHBG secretion and aromatase activity using human hepatoma (Hep G2) cells.

SUBJECTS AND METHODS

Subjects

Two studies were carried out to investigate the possible relationship between dietary fat intake and the distribution of sex steroids in blood of perimenopausal EW and GW. Subjects were recruited from general practitioners' lists in north-west London and women on lipid lowering diets or medication were excluded. In the first study fasting blood samples were obtained from 20 EW and 20 GW between 09:00 and 10:00. In this study serum free testosterone concentrations were related to FFA levels. In order to examine the relationship between FFA and the distribution of oestradiol in serum, a second study was carried out in which fasting blood samples were obtained from 32 postmenopausal EW and 24 postmenopausal GW. Clinical details for these subjects are shown in the Table.

Experimental

Blood samples obtained from the subjects in this study were allowed to clot for 1 h after which serum was separated from red blood cells by centrifugation and stored at –70 °C until assayed. Samples were either assayed in the same batch or, where this was not possible, equal numbers from each group were analysed in the same batch.

Hormone and SHBG assays

The serum concentrations of free testosterone was measured by radioimmunoassay (DPC Ltd). We have previously shown that values obtained by this method correlate with those derived from measurements of the free testosterone fraction and total concentration of testosterone (25). The free oestradiol fraction was measured by an ultrafiltration technique (26). Serum testosterone and oestradiol concentrations were measured by radioimmunoassay and SHBG by an immunoradiometric method (27). IGF-I and insulin concentrations were measured by specific radioimmunoassays (28).

Measurement of FFA

The serum concentrations of FFA were measured enzymatically by a routine diagnostic procedure. The composition of FFA present in serum was examined by gas chromatographic analysis. The extraction, thin-layer

Table Clinical details

	EW	GW
Study 1		
n	20	20
Age (years)	49.2 ± 6.2[b]	44.9 ± 6.2
BMI[a]	24.7 ± 2.5	26.5 ± 3.9
Study 2		
n	32	24
Age (years)	65.0 ± 4.5	62.1 ± 5.6
BMI	29.1 ± 4.5	28.3 ± 5.3

[a]BMI = Body Mass Index = Wt/ht^2
[b]Mean ± SD

chromatography, and methylation states were similar to that described by Mulder et al (29) with the exception that the amount of serum used was reduced to 0.2 ml. The internal standard used was margaric acid (20 μl) at a concentration of 370 μmol/l in hexane. A Pye-Unicam 304 gas chromatograph was used with a fused quartz capillary column, 50 m × 0.32 mm (ID) CP-5:1 88 (30). Methyl esters of FFA were identified by comparing their retention times with mixtures of methyl esters of known composition. The area of each peak on the chromatograph was expressed as a percentage of the total area of fatty acids. The concentrations of each FFA in plasma was calculated from the product of the total FFA concentration and the percentage of each FFA in serum.

In vitro studies

The effect of FFA on SHBG secretion and aromatase activity was examined in vitro using techniques similar to those previously described (24).

Statistics

The statistical differences between groups were assessed by Student's t-test, and linear regression was used for correlation analysis.

RESULTS

Free testosterone fraction in perimenopausal women

The first study was carried out to examine the effect that different dietary fat intake might have on the distribution of testosterone in serum from perimenopausal EW and GW. No significant difference in the total FFA concentrations was detected for EW and GW but the concentration of LA was significantly higher in serum from GW (Fig. 1). A representative gas chromatographic profile for serum from a GW and EW are shown in Figure 2.

The free testosterone concentration was significantly higher ($p < 0.05$) (11.1 ± 3.6 pmol/l vs 8.7 + 3.4 pmol/l)

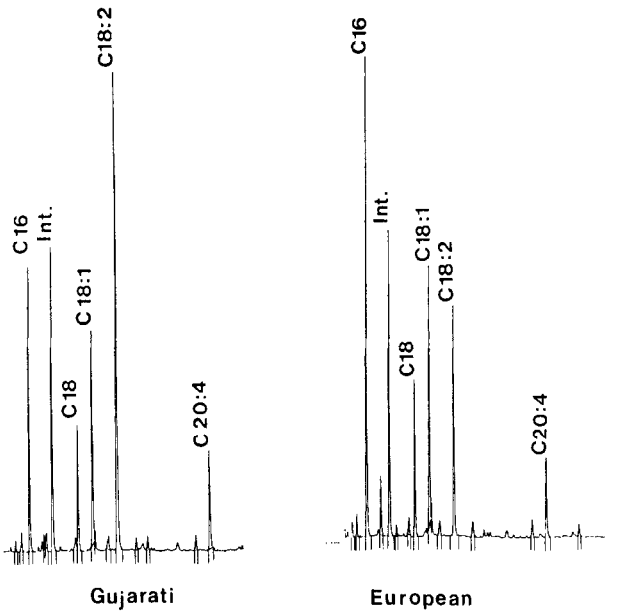

Fig. 2 Gas chromatographic profile of fatty acids in serum from Gujarati or European women.

in GW than EW. In contrast the total testosterone concentration was higher in EW than GW. SHBG levels were significantly lower in GW than for EW. These results are summarized in Figure 3. A significant correlation was detected between the free testosterone and FFA concentration for GW ($r = 0.49$, $p < 0.05$) but not for EW ($r = 0.33$, N.S.) (Fig. 4).

Free oestradiol fraction in postmenopausal women

In a second study we investigated the effect of different dietary fat intake on the distribution of oestradiol in serum from postmenopausal EW and GW. For postmenopausal women the total concentration of FFA in serum was significantly higher for GW than EW and both LA and palmitic acid (PA) levels were significantly increased in GW (Fig. 5). As found in the study in perimenopausal women, the concentration of SHBG was significantly lower for postmenopausal GW than EW,

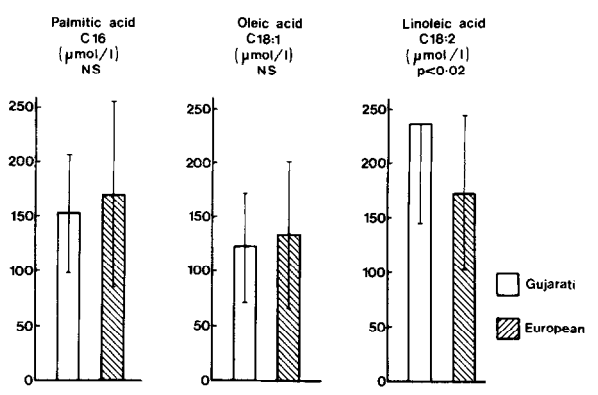

Fig. 1 Concentrations of palmitic, oleic and linoleic acid in serum from perimenopausal Gujarati or European women.

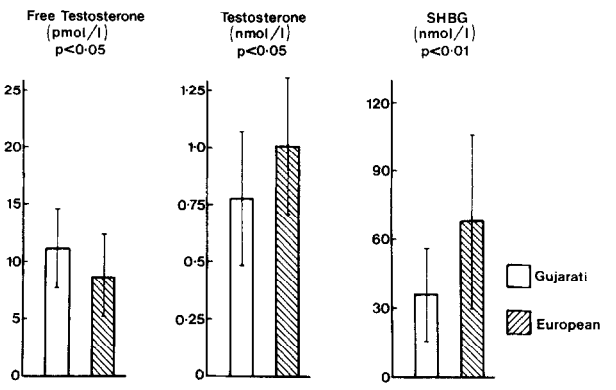

Fig. 3 Concentrations of free testosterone, total testosterone and sex-hormone binding globulin (SHBG) in serum from perimenopausal Gujarati and European women.

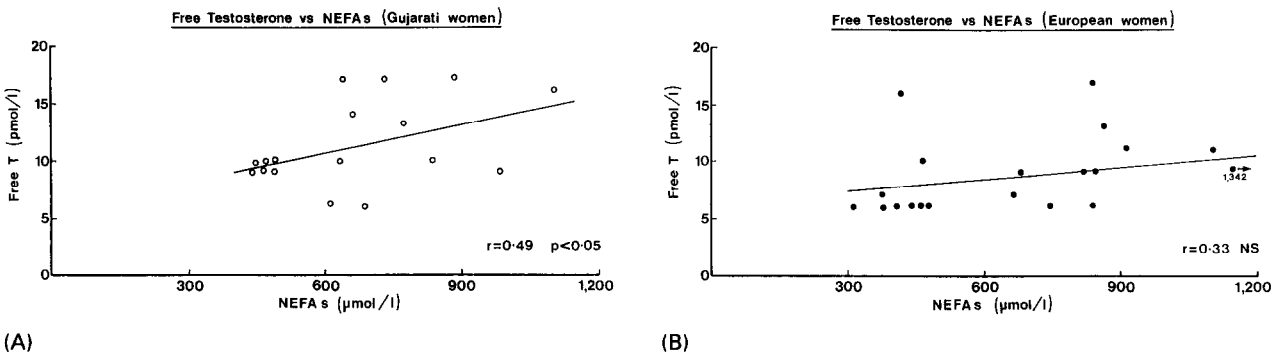

Fig. 4 Correlation between free testosterone and FFA concentrations in serum from: (A) Gujarati women, (B) European women.

but surprisingly no significant difference in the free oestradiol fraction or concentration was detected (Fig. 6). In this study no significant correlations were found between the free oestradiol fraction and concentration of total or individual FFA for either GW or EW. Highly significant correlations were found, however, between the free oestradiol fraction and SHBG concentration for both EW ($r = -0.87$, $p < 0.001$) and GW ($r = -0.67$, $p < 0.01$).

As IGF-I and insulin may have a role in regulating serum SHBG concentrations, we examined the correlation between serum concentration of these factors and SHBG. Although it was not possible to measure IGF-I and insulin in all samples, due to a lack of sufficient serum, a significant negative correlation was found between SHBG and insulin concentrations for both EW and GW (Fig. 7). A significant negative correlation was also found between SHBG and IGF-I concentrations for EW but not for GW (Fig. 8).

In vitro studies

The effect of pretreating Hep G2 cells with palmitic or oleic acid (OA) for 48 h on SHBG secretion by these cells is shown in Figure 9. The higher concentration of OA produced a 21% decrease in SHBG production. Hep G2 cells also possess aromatase activity. Pretreatment of these cells for 48 h with OA or PA significantly increased aromatase activity whereas LA significantly reduced aromatase activity (Fig. 10).

DISCUSSION

Several studies have now confirmed the ability of unsaturated FFA to inhibit the binding of oestradiol or testosterone to plasma proteins (17, 18) or purified SHBG (16). The physiological relevance of such findings has, however, been questioned (31, 32). Although the hypothesis that FFA, by inhibiting the binding of sex steroids to plasma proteins increases the tissue availability of steroids is attractive, results from this and our previous short-term studies (20, 21) lend little support to this hypothesis.

In the first study in perimenopausal women the serum concentration was significantly higher in GW than EW. The free testosterone concentration was also increased in GW in this group but SHBG concentrations were significantly lower than in EW. Although a significant correlation was detected for GW between the free testosterone and total FFA concentrations, the increase in free testosterone in GW most likely results from their lower SHBG concentrations. No correlation was found between the free testosterone and LA concentrations in this group of GW.

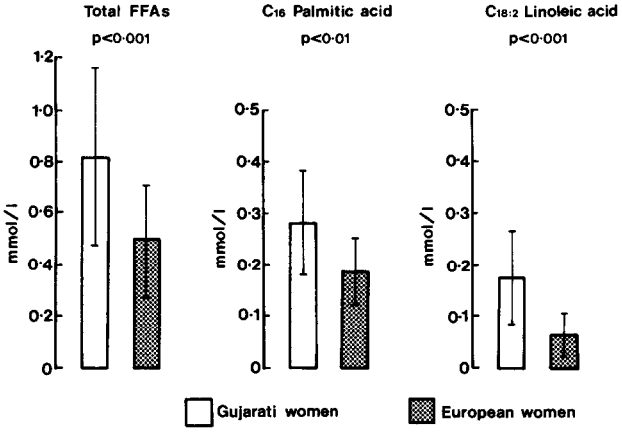

Fig. 5 Concentrations of total FFA, and palmitic and linoleic acids in serum from postmenopausal Gujarati and European women.

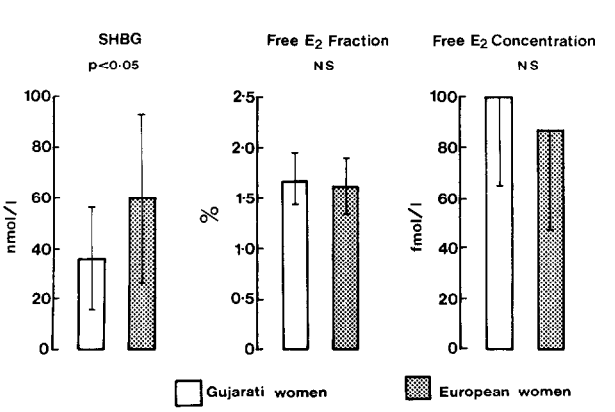

Fig. 6 Concentrations of SHBG, the free oestradiol (E2) fraction and free E2 concentration in serum from postmenopausal Gujarati and European women.

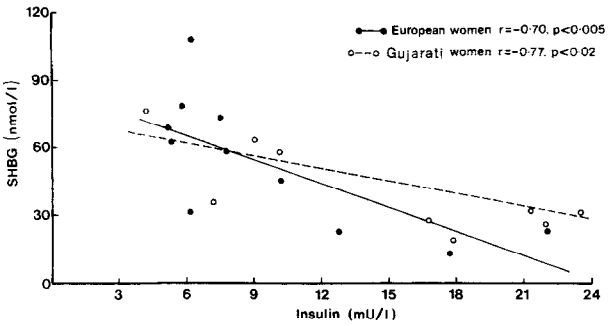

Fig. 7 Correlations between serum concentrations of SHBG and insulin for European and Gujarati women.

SHBG concentrations were also significantly lower in postmenopausal GW compared with EW. However, in spite of the SHBG level being 37% lower in GW than in EW, no difference in the free oestradiol fractions was detected. While it is accepted that SHBG has a major role in the transport of testosterone, its physiological role in binding oestradiol has been questioned (33). SHBG binds oestradiol with a much lower affinity than testosterone and therefore any alterations in SHBG concentrations result in a greater change in the free testosterone fraction compared with the free oestradiol fraction (34). It is possible that the increased levels of free testosterone in GW contributes to the higher prevalence of hirsutism amongst women from the Indian subcontinent.

In an attempt to explain the lower SHBG concentrations in both peri- and postmenopausal GW, we measured serum IGF-I and insulin concentrations. Significant negative correlations were found between SHBG and IGF-I levels for EW and SHBG and insulin levels for both GW and EW. Four of the GW had a medical history of diabetes whereas no EW had this disease. As there is good evidence that both IGF-I and insulin modulate SHBG production (29), it is possible that the reduced SHBG levels in GW relates to the insulin status of these women. Examination of the medical or family histories of the postmenopausal women also revealed a statistically significant difference (Chi-squared test, $p<0.001$) in the cancer history between EW and GW. GW women

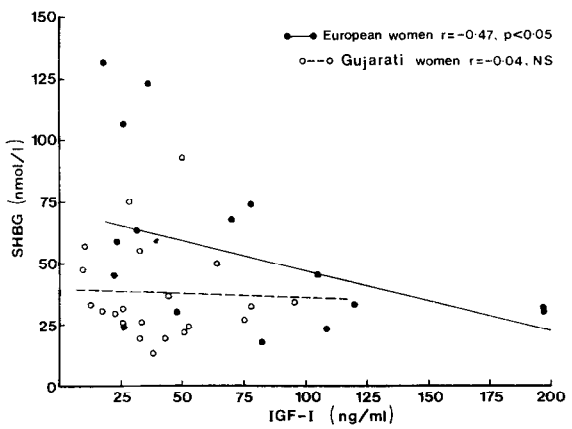

Fig. 8 Correlations between serum concentrations of sex-hormone binding globulin (SHBG) and insulin-like growth factor-type I (IGF-I) for European and Gujarati women.

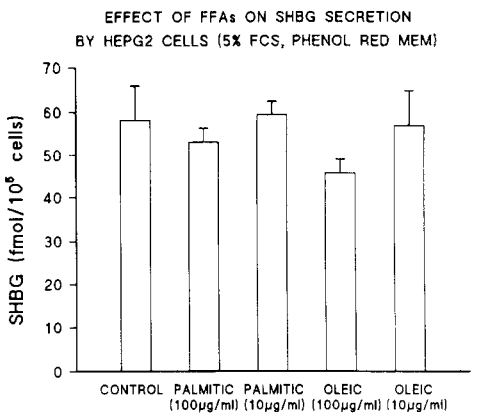

Fig. 9 Effect of fatty acids on SHBG secretion by Hep G2 cells.

had no medical or family history of cancer whereas 28% of EW had such a history.

In the postmenopausal GW both serum LA and PA concentrations were higher in GW than EW. Several animal models have implicated unsaturated FFA, such as LA, in the promotion of mammary tumour growth. It is therefore difficult to reconcile the differences in the medical or family history of cancer in EW with the lower serum levels of unsaturated FFA found in these women.

In order to resolve the mechanisms by which FFA support tumour growth, it will be necessary to develop appropriate in vitro techniques. To this end we have examined the effect of different FFA on SHBG production by Hep G2 cells and shown that OA, but not PA, can reduce SHBG secretion (35).

As there is evidence that some FFA may alter the activity of enzymes involved in oestrogen synthesis, we have also examined the effect of FFA on aromatase activity using Hep G2 cells. Results from this investigation have revealed that, while PA stimulates, LA inhibits aromatase activity. Thus it is possible that the balance of FFA in breast tissues may have a crucial role in regulating aromatase activity and thus oestrogen formation in breast tissues. Increased LA concentration could have a protective effect by reducing the exposure of epithelial cells to oestrogen.

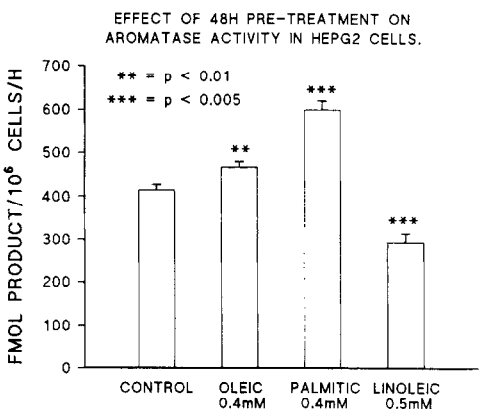

Fig. 10 Effect of fatty acids on aromatase activity in Hep G2 cells.

In conclusion, while there is good evidence implicating dietary fat intake in a number of endocrine dependent cancers, the mechanisms by which a diet with a high fat content favours tumour development remain unresolved. Even so a reduction in the amount or change in the type of fat consumed would appear warranted from the information already available.

Acknowledgement

This work was supported by a grant from the Joint Standing Research Committee at St. Mary's Hospital, London.

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