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THE RELATIONSHIP OF FREE FATTY ACIDS WITH THE BINDING OF OESTRADIOL TO SHBG AND TO ALBUMIN IN WOMEN

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Summary—Free fatty acid concentration, sex hormone binding globulin (SHBG) concentration, % free oestradiol and % SHBG-bound oestradiol were measured in fasting and non-fasting serum samples from 48 women. Free fatty acids were 128% higher ($P < 0.001$) and % SHBG-bound oestradiol was 11% lower ($P = 0.001$) in fasting than non-fasting samples, but these changes were not significantly correlated ($r = -0.16$, $P = 0.287$). Mean SHBG concentration and % free oestradiol did not differ significantly between fasting and non-fasting samples. Between subject correlations were calculated for measurements from 30 premenopausal women: % free oestradiol was inversely correlated with SHBG and free fatty acids and positively correlated with Quetelet's Index; % SHBG-bound oestradiol was positively correlated with SHBG but was not consistently or significantly related to free fatty acids or Quetelet's Index. It was concluded that physiological increases in free fatty acid concentrations do not increase % free oestradiol and that free fatty acids are less important than SHBG concentration and perhaps other variables in determining differences between individuals in oestradiol binding to SHBG.

INTRODUCTION

The serum concentration of sex hormone binding globulin (SHBG) is a major determinant of the partition of oestradiol (E2) between the free, albumin-bound and SHBG-bound fractions, but between person variation in the concentration of this protein does not explain all of the variation in the partition of E2, and it has been suggested that this may also be affected by the serum concentration of free fatty acids (FFA) [1–3].

Bruning and Bonfrer[1] and Reed *et al.*[2, 3] studied the *in vitro* and *in vivo* effects of FFA on the binding of E2 to proteins. In broad terms their findings suggested that a physiological increase in serum FFA concentration might cause an increase in % free E2 and a decrease in the fraction of E2 bound to SHBG (% SHBG–E2). To test this hypothesis we measured % free E2 and % SHBG–E2 (as well as total FFA and SHBG concentrations) in fasting and non-fasting serum samples from 48 women.

The data obtained also made possible a "cross-sectional" examination of the relationships of FFA with variations in E2 binding between individuals. The hypothesis tested in this analysis was that, in addition to the established role of SHBG concentration as a determinant of E2 binding, there would be a positive correlation between total FFA concentration and % free E2 and an inverse correlation between total FFA and % SHBG–E2.

The availability of measurements of high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and triglycerides (TG) in the fasting samples further allowed an examination of the relationships of SHBG with these variables.

METHODS

Subjects

The subjects were healthy women recruited from participants in a lipid screening project [4]. Those accepted for the study met the following criteria: no current medication, including oral contraceptives; no previous cancer; no current disease requiring treatment; weight less than 100 kg. The 48 women studied had a mean age of 42 years (range 31–55) and a mean Quetelet's Index (QI) of 23.8 kg/m^2 (range 18.4–37.1).

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Each woman fasted overnight (time of last meal up to 21.00 h), attended a clinic in the morning when a fasting blood sample was taken (08.45–11.15 h), ate normally during the rest of the day, and was finally visited at home on the evening of the same day when a second, non-fasting blood sample was taken (18.00–20.30 h). Height and weight were measured. The times and brief descriptions of all meals, snacks and drinks in the 24 hours preceding the second blood sample were recorded. Blood obtained by venepuncture was allowed to clot at room temperature for up to 4 h, then centrifuged and serum stored at −20°C in 0.5 ml amounts until analysis. The maximum storage time was one year. Forty eight women were studied: 36 were premenopausal, six were naturally postmenopausal, and six had had a hysterectomy before reaching natural menopause.

Laboratory methods

SHBG was measured by immunoradiometric assay [5]. The intra- and inter-assay coefficients of variation were 5.3% and 3.8% respectively at an SHBG concentration of 57.7 nmol/l.

Total FFA were measured by the method of Shimizu *et al.* [6], modified for use on the Multistat III Microcentrifugal Analyzer (Instrumentation Laboratories). The intra- and inter-assay coefficients of variation were both less than 5% in the range of FFA concentrations measured.

Percent free E2 was measured by centrifugal ultrafiltration-dialysis [7], as modified by Dowsett *et al.* [8]. The distribution of E2 between albumin and SHBG was determined from the % free E2 in native serum and in the same serum heated at 60°C for 1 h [9]. The intra- and inter-assay coefficients of variation for % free E2 in native and heat-treated serum were both less than 10%. For the sake of simplicity, only the % free E2 and % SHBG–E2 are reported, because % albumin–E2 is strongly inversely correlated with % SHBG–E2. Factors which are related to the SHBG-bound fraction are invariably related, in the opposite direction, to the albumin-bound fraction.

SHBG, FFA and E2 distribution were measured in both fasting and non-fasting serum samples. The paired (fasting and non-fasting) samples from each individual were measured in the same assay to eliminate inter-assay variation from the within individual comparisons.

HDLC, LDLC and TG were measured in fasting plasma samples collected at the same time as the fasting serum samples used in the other analyses. The methods were as given in Mann *et al.*[4].

Statistical analysis

SHBG, FFA, LDLC and TG were logarithmically transformed (to base e) to achieve a normal distribution. Transformed variables are indicated by the prefix log in the tables but not in the text. Two sided P values are quoted.

RESULTS

Changes in FFA and E2 binding within individuals

The mean FFA concentration and the mean % SHBG–E2 were 128% higher ($P < 0.001$) and 11% lower ($P = 0.001$) in fasting than in non-fasting samples respectively (Table 1). Neither mean SHBG nor mean % free E2 differed significantly between fasting and non-fasting samples.

Although the mean differences in FFA and in % SHBG–E2 were highly statistically significant, the correlation between the changes was very low ($r = -0.16$, $P = 0.287$), and the changes did not occur in the same direction in all subjects. In four women FFA were lower in the fasting than in the non-fasting sample: in two of these four, and in 11 other women, the % SHBG–E2 was higher in the fasting than in the non-fasting sample. These differences were not related to age, weight, menopausal status, stage of cycle, or duration of fasting.

Factors related to differences between individuals in E2 binding

This analysis was restricted to premenopausal women because of the small number of postmenopausal women. Six premenopausal women were excluded, two because they were perimenopausal (age 50+, irregular menstrual cycles) and four because of missing data. This left data on 30 subjects for analysis (Table 2).

There was an inverse correlation between SHBG and % free E2 (fasting, $r = -0.35$, $P = 0.055$; non-fasting, $r = -0.37$, $P = 0.046$) (Table 3). There was also an inverse correlation between FFA and % free

Table 1. Comparison of mean SHBG, FFA, % free E2 and % SHBG-E2 in fasting and non-fasting serum samples[a]

Variable	Fasting	Non-fasting	P[b]
SHBG, nmol/l[c]	67.1 (4.206 ± 0.341)	65.1 (4.175 ± 0.328)	0.077
FFA, mmol/l[c]	0.411 (−0.890 ± 0.294)	0.180 (−1.713 ± 0.535)	<0.001
% Free E2[d]	1.31 (±0.280)	1.35 (±0.270)	0.187
% SHBG–E2[d]	42.6 (±11.83)	47.7 (±10.80)	0.001

[a]Abbreviations: E2 = oestradiol; FFA = free fatty acids; SHBG = sex hormone binding globulin. Number of paires = 48. [b]Two-sided P value for paired t-test. [c]Geometric mean (ln ± SD). [d]Arithmetic mean (±SD).

Table 2. Characteristics of the subjects considered in the between individual comparisons[a]

Variable	Mean	Range
Age, yrs	39.8	31–49
QI, kg/m^2	24.1	18.4–37.1
Menstrual cycle length, days	27.6	22–32
Fasting SHBG, nmol/l	66.9[b]	34.1–114.4
Fasting FFA, mmol/l	0.396[b]	0.202–0.712
Fasting % free E2	1.37	0.86–2.07
Fasting % SHBG–E2	40.8	20.5–66.4
Fasting HDLC, mmol/l	1.6	1.1–2.5
Fasting LDLC, mmol/l	3.5[b]	1.8–6.5
Fasting TG, mmol/l	1.0[b]	0.6–2.8

[a]Abbreviations: E2 = oestradiol; FFA = free fatty acids; HDLC = high density lipoprotein cholesterol; LDLC = low density lipoprotein cholesterol; SHBG = sex hormone binding globulin; TG = triglycerides. Number of subjects = 30. [b]Geometric mean.

E2, which was not reduced by adjusting for SHBG and which was stronger and was statistically significant ($r = -0.42$, $P = 0.022$) in the non-fasting samples, and there was a significant positive correlation between QI and % free E2 which remained quite large after adjustment for SHBG and which was stronger in the non-fasting (partial $r = 0.43$, $P = 0.020$) than in the fasting samples (partial $r = -0.26$, $P = 0.179$).

Serum SHBG concentration had a consistent strong positive correlation with % SHBG–E2 ($r > 0.6$, $P < 0.001$) (Table 4). FFA were not significantly related to % SHBG–E2. There was a weak inverse simple correlation between QI and % SHBG–E2, but this was not statistically significant and was much reduced by adjusting for SHBG, because of the inverse correlation between SHBG and QI.

Relationships of SHBG with HDLC, LDLC, and TG

SHBG concentration was positively correlated with both HDLC ($r = 0.33$, $P = 0.074$) and LDLC ($r = 0.42$, $P = 0.021$), and non-significantly inversely correlated with TG ($r = -0.25$, $P = 0.174$).

DISCUSSION

Within individual relationships

Contrary to our hypothesis, mean % free E2 was not higher in the fasting samples. Bruning and Bonfrer[1] found that % free E2 was 22% higher ($P < 0.001$) in fasting than in non-fasting samples, but the mean fasting FFA concentration in their study

Table 3. Correlations of % free E2 with log SHBG, log FFA and QI[a]

	Fasting		Non-fasting	
	Simple[b]	Partial[c]	Simple[b]	Partial[c]
log SHBG	−0.35		−0.37	
log FFA	−0.19	−0.19	−0.42	−0.53
QI	0.33	0.26	0.51	0.43

[a]Abbreviations: E2 = oestradiol; FFA = free fatty acids; log = logarithm to base e; QI = Quetelet's Index; SHBG = sex hormone binding globulin. Number of samples = 30. Two-sided significance level for simple correlation coefficients: $r = 0.36$, $P = 0.05$. [b]Simple correlation coefficient. [c]Partial correlation coefficient, adjusted for log SHBG.

Table 4. Correlations of % SHBG-E2 with log SHBG, log FFA and QI[a]

	Fasting		Non-fasting	
	Simple[b]	Partial[c]	Simple[b]	Partial[c]
Log SHBG	0.66		0.62	
Log FFA	0.00	−0.02	−0.19	−0.10
QI	−0.35	−0.23	−0.20	−0.06

[a]Abbreviations: E2 = oestradiol; FFA = free fatty acids; log = logarithm to base e; QI = Quetelet's Index; SHBG = sex hormone binding globulin. Number of samples = 30. Two-sided significance level for simple correlation coefficients: $r = 0.36$, $P = 0.05$. [b]Simple correlation coefficient. [c]Partial correlation coefficient, adjusted for log SHBG.

was 1.24 mmol/l, much higher than that in the current study (0.41 mmol/l); Bruning and Bonfrer suggested that the high value in their study may have been due to stress, since the subjects were studied on the day of surgery for cancer. In an investigation of the effects on % free E2 of intravenous heparin (which causes an acute increase in FFA concentration but has no effect on free E2 *in vitro*), Bruning and Bonfrer[1] found a positive correlation between FFA and % free E2, but this was only clearly seen at FFA concentrations of greater than 1 mmol/l. Furthermore, the previous *in vitro* studies of the effects of the major fatty acids on % free E2 showed an increase in % free E2 only with FFA concentrations of 2 mmol/l and above [1, 2]. The previous studies are therefore compatible with the conclusion that a physiological increase in FFA does not cause an increase in % free E2.

In agreement with our original hypothesis, mean % SHBG–E2 was lower in the fasting samples, but the low correlation between the changes in % SHBG–E2 and in FFA concentration suggests that the decrease in % SHBG–E2 was not caused by the increase in FFA. The mean SHBG concentration was a little higher in the fasting samples, so the change in mean % SHBG–E2 was not due to a change in SHBG. Other proteins were not measured and it is possible that our findings could be due to changes in the concentration of other oestrogen-binding proteins (principally albumin), but this is unlikely because there is no consistent diurnal variation in plasma protein concentrations [10]. It is also possible that the changes in % SHBG–E2 between morning and evening could be at least partly due to changes in serum concentrations of the other steroids which bind to SHBG, but the magnitude of such effects appears to be small; Sodergard *et al.*[11] concluded that the maximum likely variations in the concentrations of three major SHBG steroid ligands would only change the % SHBG–E2 from 45.4% to 46.0%. Further work is needed to confirm and explain the observed change in % SHBG–E2.

Between individual relationships

Percent free E2 had an inverse simple correlation with SHBG in both fasting and non-fasting samples, in agreement with the results of other cross-sectional comparisons [12]. Percent free E2 was also inversely correlated with FFA, particularly in non-fasting

samples: this relationship was not expected, since the within-individual comparisons suggested that changes in FFA within the range encountered in this study do not affect % free E2, and the results of Bruning and Bonfrer [1] suggested that higher FFA concentrations may cause an increase in % free E2. Adlercreutz *et al.*[13] also reported a significant inverse correlation between FFA and % free E2 in 33 premenopausal women. Oral E2 (as oestradiol valerate) causes a decrease in serum FFA concentration [14], so, if % free E2 is correlated with the concentration of free E2, it is possible that the unexpected inverse correlation between FFA and % free E2 could be due to an effect of E2 on FFA.

The positive simple correlations of QI with % free E2 were only slightly reduced by adjusting for SHBG. These results were unexpected and cannot be readily explained.

For % SHBG–E2, the cross-sectional analysis showed that SHBG was the major determinant of this variable, and that neither FFA nor QI showed any important independent correlation with % SHBG–E2. These results for SHBG and QI were as expected, and the absence of any relationship between FFA and % SHBG–E2 gives further support to the tentative conclusion drawn above, that the lower % SHBG–E2 in fasting than in non-fasting samples was not caused by the changes in FFA.

SHBG was positively correlated with both HDLC and LDLC, and weakly inversely correlated with TG. The relationship with HDLC is similar to that observed in some studies of men [15, 16], and confirms the findings in women of Armstrong and colleagues [16, 17]. The relationship with LDLC was not expected and must therefore be interpreted cautiously.

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