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Synthesis and regulation of sex hormone-binding globulin in obesity

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Sex hormone-binding globulin (SHBG) is a plasma glycoprotein with high binding affinity for testosterone and dihydrotestosterone and lower affinity for estradiol. SHBG is synthesized in the liver, and its plasma level is important in the regulation of plasma free and albumin-bound androgens and estrogens. Obesity and particularly excess visceral fat, known risk factors for cardiovascular and metabolic diseases, are associated with decreased testosterone levels in males and SHBG levels in both sexes. SHBG is usually positively correlated with high-density lipoprotein cholesterol and negatively correlated with triglyceride and insulin concentrations. A positive association between SHBG and various measures of insulin sensitivity has been demonstrated in both sexes, suggesting that decreased SHBG levels may be one of the components of the metabolic syndrome. We have examined pituitary–adrenocortical function, glucose tolerance, and lipoprotein and hormone levels in a large cohort of Finnish males. Abdominal obesity appears to be associated with slight hypocortisolemia and increased sensitivity to exogenous adrenocorticotropin stimulation, which may contribute to the hyperinsulinemia and related metabolic changes including decreased SHBG levels in males.
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Introduction

Sex hormone-binding globulin (SHBG) is a major carrier of androgens and estrogens in human plasma. It is a glycoprotein with high binding affinity for testosterone (T) and dihydrotestosterone (DHT) and lower affinity for estradiol (E2). SHBG is produced in the liver, and its plasma levels are important in the regulation of plasma free and albumin-bound androgens and estrogens.[1,2]

Clustering of cardiovascular risk factors, demonstrated in several epidemiological studies, has been designated the metabolic or insulin resistance syndrome, elements of which include visceral obesity, hypertension, insulin resistance and hyperinsulinemia, dyslipidemia, and impaired fibrinolysis.[3–5] Obesity in general, and particularly visceral obesity, is also associated with alterations in the pituitary–adrenal and pituitary–gonadal functions.[6] Visceral fat accumulation decreases testosterone levels in males, and reduces SHBG concentrations in both sexes.[7–9]

SHBG is also positively correlated with HDL-cholesterol and negatively correlated with triglyceride and insulin levels.[10–15] A positive association between SHBG and various measures of insulin sensitivity has been demonstrated in both sexes,[16–18] suggesting that decreased SHBG levels may be one feature of the metabolic syndrome.

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Visceral obesity is a central part of the metabolic syndrome, but it remains to be established whether hormonal changes in obesity contribute to the clustering of other components of the syndrome. We have previously examined the hypothetical possibility of a primary dysfunction of the hypothalamus–pituitary–adrenal (HPA)-axis being one reason for visceral obesity.[19] Basal and functional pituitary–adrenocortical activity and glucose tolerance were studied in a cohort of Finnish males. Based on steroid and other data, tentative mathematical models were established by means of structural covariance (path) analyses, which demonstrated that several alterations in the pituitary–adrenocortical function, suggestive of decreased 21-hydroxylase activity, mild cortisol deficiency and increased adrenal sensitivity to exogenous ACTH stimulation, were associated with visceral obesity which, in turn, was an important prelude to insulin resistance and dyslipidemia.[20,21] Here we have used multivariate and factor analyses to examine how alterations in the pituitary–adrenocortical function associated with visceral obesity contribute to the clustering of various elements of the metabolic syndrome with special reference to SHBG levels.

Subjects and methods

Subjects, protocol and methods

A Finnish population sample of 93 males was examined, of which, 69 were healthy, 22 had borderline hypertension (140/90–160/95), and two definite hypertension (more than 160/95). The diagnosis was

based on history and/or blood pressure measured during the physical examination. They were receiving no medication and had no history or clinical evidence of other diseases, as determined by clinical examination and laboratory analyses.

The subjects were studied as outpatients on three consecutive days at the Helsinki University Central Hospital. On day 1, at 07.30 h, after an overnight fast, blood samples were drawn to determine blood cell counts, serum chemistry profiles, and SHBG and testosterone levels. Starting at 18.00 h, 12 h urine samples were collected to determine free cortisol excretion, which were available from 80 subjects. On day 2, at 07.30 h, an oral glucose tolerance test was carried out. An in-dwelling cannula was inserted into an antecubital vein. Thirty minutes later, a standard dose of 75 g glucose was given. Blood samples were drawn at 0, 60 and 120 min after glucose for the determination of insulin, C-peptide, glucose, dehydroepiandrosterone (DHEA), androstenedione, 17-hydroxyprogesterone (17-OHP), cortisol and ACTH concentrations.

On day 2, each subject received 1 mg dexamethasone orally at 23.00 h. On day 3, at 07.30 h, an antecubital vein was cannulated. Thirty minutes later, ACTH (S-Cortrophin, Organon, Oss, Holland) was injected as an intravenous bolus at a dose of 10 μg/m^2. Blood samples were drawn 30 min before, and at times 0, 30 and 60 min after the administration of ACTH for the determination of DHEA, androstenedione, 17-OHP, 11-deoxycortisol and cortisol concentrations.

Waist-to-hip ratio (WHR), and blood pressure were measured, body mass index (BMI) was calculated, and alcohol consumption, physical activity and smoking were estimated as described previously.[22,24]

Methods for the determination of lipids, lipoproteins, peptide hormones and steroids have been previously described.[22–24]

Statistical analysis

Pearson correlation coefficients were computed, and multiple linear regression and factor analyses were carried out with Systat® statistical program package. In OGTT, the mean of three measured values (0, 60 and 120 min) for ACTH, 17-OHP, cortisol, DHEA and androstenedione was used as an indicator of hormone secretion. The net steroid increment (Δ) was defined as ACTH-stimulated steroid response at 30 min minus DXM-suppressed steroid value. Variables were log transformed for calculations.

Table 1 Anthropometric, life-style, and metabolic characteristics of the study subjects

Characteristic	*Mean ± s.d.*
Age (y)	45.1 (4.9)
Body mass index (kg/m^2)	26.4 (4.0)
Waist-to-hip ratio	0.94 (0.07)
Alcohol (g/week)	181 (129)
Physical activity index	2.1 (0.8)
Smokers (yes/no)	35/58
Systolic blood pressure (mmHg)	129 (14)
Diastolic blood pressure (mmHg)	82 (10)
Cholesterol (mmol/l)	5.7 (1.1)
HDL cholesterol (mmol/l)	1.23 (0.34)
Triglycerides (mmol/l)	1.57 (1.06)
Insulin, fasting (mU/l)	9.09 (7.3)
Glucose, fasting (mmol/l)	3.33 (0.40)
C-peptide, fasting (nmol/l)	0.76 (0.38)

Results

Basic characteristics of the subjects

Anthropometric and lifestyle characteristics, blood pressures and metabolic and hormonal characteristics of the study subjects are presented in Tables 1 and 2, respectively.

Relationship of SHBG to basal and functional activity of pituitary–adrenal axis

Table 3 shows the correlations between SHBG and various steroid determinations. SHBG was positively correlated with mean basal 17-OHP levels (mean of three 17-OHP determinations during the OGTT) and urinary cortisol excretion. In addition, SHBG was positively correlated with mean basal DHEA ($r = 0.217$; $P < 0.05$), and DXM suppressed 17-OHP ($r = 0.393$; $P < 0.001$) and 11-deoxycortisol ($r = 0.246$; $P = 0.018$) levels. The correlations between mean basal cortisol ($r = 0.160$) and androstenedione ($r = 0.174$) and SHBG tended also to be positive, but did not reach statistical significance. In contrast, SHBG was highly significantly negatively related to the ACTH responses of 17-OHP, 11-deoxycortisol, and cortisol. Because urinary cortisol excretion was negatively correlated with 17-OHP, 11-deoxycortisol and cortisol responses to ACTH stimulation, it is more likely that the responsiveness of cortisol, and its immediate biosynthetic precursors

Table 2 Hormonal characteristics of the study subjects

Characteristic	*Mean ± s.d.*
Mean basal ACTH (ng/l)	13.8 (5.1)
17-hydroxyprogesterone (nmol/l)	
Mean basal	7.1 (2.7)
After DXM	4.7 (2.4)
After corticotropin (30 min)	16.1 (3.9)
Δ (0–30 min)	11.4 (4.5)
11-deoxycortisol (nmol/l)	
After DXM	2.4 (0.9)
After corticotropin (30 min)	7.1 (1.8)
Δ (0–30)	4.7 (1.6)
Cortisol (nmol/l)	
Mean basal	349 (100)
After DXM	46 (57)
After corticotropin (30 min)	534 (111)
Δ (0–30 min)	488 (107)
Urinary cortisol (nmol/12 h)	254 (71)
Sex hormone-binding globulin (nmol/l)	38.1 (16.0)
Testosterone (nmol/l)	17.2 (5.3)

Table 3 Pearson's correlations between SHBG and other hormone determinations in the study group

Variable	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1. SHBG	1.00							
2. ACTH, mean basal	0.09	1.00						
3. 17-OHP, mean basal	0.31[b]	0.03	1.00					
4. Cortisol, mean basal	0.16	0.29[b]	0.43[c]	1.00				
5. 17-OHP, net increment	0.40[c]	0.39[c]	0.14	0.21[a]	1.00			
6. 11-deoxycortisol, net increment	0.39[c]	0.36[c]	0.16	0.18	0.65[c]	1.00		
7. Cortisol, net increment	0.40[c]	0.38[c]	0.12	0.05	0.67[c]	0.70[c]	1.00	
8. Urinary cortisol	0.28[a]	0.07	0.05	0.02	0.32[b]	0.32[b]	0.38[c]	1.00

$n = 93$ (for 12 h urinary cortisol $n = 80$).
[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.
Mean basal indicates the mean of three hormone determinations during the oral glucose tolerance test. Net increment is the steroid response to ACTH stimulation from 0 (DXM-suppressed level) to 30 min.
Variables were log transformed for calculations.

reflects increased sensitivity to ACTH rather than increased cortisol reserves of the adrenals.

Correlations between SHBG levels and anthropometric and metabolic variables

Table 4 shows the correlations between SHBG, BMI, WHR, mean blood pressure, fasting insulin, C-peptide, glucose, TG, HDL-cholesterol and testosterone concentrations. SHBG was negatively correlated with all those parameters, except HDL-cholesterol and testosterone, to which it was positively related. As expected, BMI, WHR and metabolic parameters were highly intercorrelated. These results are consistent with several other reports showing similar correlations.[15]

Multiple regression analyses to predict SHBG levels

To get a more precise idea which of the anthropometric, hormonal and metabolic variables were most likely contribute to the variation of serum SHBG levels, stepwise multiple linear regression analyses were carried out with SHBG as the dependent variable. Table 5 shows the model, in which SHBG levels were predictable from fasting insulin and mean basal 17-OHP concentrations, from cortisol response to ACTH stimulation, and from age. Squared multiple r statistics showed that 35.7% of the total variation in SHBG levels could be accounted for by these variables. BMI, WHR, TG and HDL-cholesterol did not enter into the models.

Factor analysis to examine clustering of the components of the metabolic syndrome

A factor analysis has been previously used to demonstrate the clustering of various elements of the insulin resistance syndrome.[25] A similar method (principal components analysis with varimax rotation) was applied to examine the possible clustering of SHBG with the components of the metabolic syndrome and with various determinations of the pituitary–adrenocortical activity. Initial extraction and rotation produced three factors with eigenvalues > 1. As shown in Table 6, fasting insulin, fasting C-peptide, BMI, WHR, TG and cortisol response to ACTH stimulation (positive relationship), and HDL-cholesterol and SHBG (negative relationship) had the highest loadings in factor 1. Factor 2 was characterized by negative loadings for SHBG, urinary cortisol and mean basal 17-OHP, and by positive loading for cortisol response to ACTH stimulation. Factor 3 was characterized by positive loadings for HDL-cholesterol, cortisol response to ACTH stimulation and mean basal 17-OHP, and by negative loading for mean basal ACTH. Obtained factors explained 63% of the total variance of the data: factor 1 accounted for 35.4% of the

Table 4 Pearson's correlations between SHBG, anthropometric and metabolic variables in the study group

Variable	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
1. SHBG	1.00								
2. Body mass index	0.40[c]	1.00							
3. Waist-to-hip ratio	0.40[c]	0.76[c]	1.00						
4. Blood pressure, mean	0.37[c]	0.42[c]	0.40[c]	1.00					
5. Insulin, fasting	0.45[c]	0.73[c]	0.65[c]	0.41[c]	1.00				
6. C-peptide, fasting	0.38[c]	0.66[c]	0.58[c]	0.38[c]	0.79[c]	1.00			
7. Glucose, fasting	0.33[b]	0.36[c]	0.27[b]	0.31[b]	0.39[c]	0.39[c]	1.00		
8. Triglycerides	0.38[c]	0.46[c]	0.50[c]	0.34[c]	0.54[c]	0.54[c]	0.15	1.00	
9. HDL cholesterol	0.27[b]	0.29[b]	0.37[c]	0.07	0.46[c]	0.36[c]	0.15	0.57[c]	1.00
10. Testosterone	0.60[c]	0.32[b]	0.24[a]	0.30[b]	0.29[b]	0.19	0.41[c]	0.17	0.18

$n = 93$.
[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.
The mean blood pressure was defined as (systolic BP + 2×diastolic BP)/3.
Variables were log transformed for calculations.

Table 5 A summary of multiple linear regression analyses for the prediction SHBG levels in the study group

	SHBG		
Explanatory factor or parameter of the model	*b*	*β*	*P*
Fasting insulin	0.216 ± 0.051	0.380	< 0.001
Mean basal 17-hydroxyprogesterone	0.259 ± 0.082	0.275	0.002
Cortisol response to ACTH	0.378 ± 0.167	0.206	0.026
Age	0.013 ± 0.007	0.158	0.074
Waist-to-hip ratio	—		
Triglycerides	—		
HDL cholesterol	—		
Constant	5.2		
r^2	0.357		

Multiple regression coefficients (b) ± s.e.m. and standardized regression coefficients (β) are given for the effects of the factors selected into each model.
(—) The factor did not enter into the model.
r^2 = the square of the multiple regression coefficient (the predictive power of the model).

Table 6 Factor analysis of components of the metabolic syndrome

	Factor		
Variable	*1*	*2*	*3*
Fasting insulin	0.876	0.089	0.141
Fasting C-peptide	0.839	0.104	0.197
Body mass index	0.828	0.077	0.070
Waist-to-hip ratio	0.805	0.091	0.071
Triglycerides	0.713	0.207	0.107
HDL cholesterol	0.580	0.034	0.309
Sex hormone-binding globulin	0.393	0.670	0.138
Urinary cortisol	0.012	0.726	0.159
Cortisol response (Δ 0–30 min)	0.295	0.606	0.542
17-OHP (mean basal)	0.007	0.515	0.455
ACTH (mean basal)	0.047	0.055	0.788
Percentage total variance	35.4	15.4	12.2
Percentage cumulative variance	35.4	50.8	63.0

variance, factor 2 for 15.4%, and factor 3 for 12.2% of the remaining variance.

Discussion

Visceral obesity, insulin resistance and dyslipidemia are central elements of the metabolic syndrome. This and several previous studies have shown a positive association between SHBG and HDL-cholesterol, and negative associations between SHBG and BMI, WHR, insulin and triglycerides, suggesting that decreased SHBG levels may be one feature of the metabolic syndrome.[10–18] Statistical analyses of this study support that view and provide evidence that hyperinsulinemia and decreased cortisol secretion associated with abdominal obesity contribute to the decreased SHBG concentrations, at least in males.

SHBG is synthesized in the liver, and its plasma concentration is regulated by several hormones and growth factors. Clinical studies have shown that SHBG levels decrease in puberty, obesity, hypothyroidism and during androgen treatment, and increase during ageing, short-term fasting, estrogen treatment, in thyrotoxicosis and in various liver diseases. Greatly elevated values occur during pregnancy.[1] Genetic factors and diet also contribute to the variation in serum SHBG levels.[26,27] Cultured human hepatoma cell line (HepG2) has been used to investigate the control of SHBG production *in vitro*. Insulin, prolactin, insulin-like growth factor-I and epidermal growth factor suppress, whereas dexamethasone, cortisol, estradiol, thyroxin, triiodothyronine, estradiol and testosterone stimulate SHBG synthesis.[28–33] Consistent with previous studies, we found that insulin was negatively related to the SHBG levels. In view of the *in vitro* data, it seems possible that insulin acts directly on the liver to inhibit SHBG synthesis.

Cortisol is synthesized from cholesterol in the adrenal zona fasciculata under the regulation of ACTH by five successive enzymatic conversions, of which the last two require hydroxylations of 17-OHP by 21-hydroxylase to yield 11-deoxycortisol, and by 11-hydroxylase to yield cortisol.[34] Mean basal cortisol ($r = 0.311$; $P = 0.002$) and androstenedione ($r = 0.297$; $P = 0.004$) during the OGTT as well as DXM suppressed 17-OHP ($r = 0.328$; $P = 0.001$) were all negatively correlated with WHR suggesting that visceral obesity is associated with decreased production of cortisol and androstenedione. Positive correlations between WHR and cortisol ($r = 0.221$; $P = 0.031$) and 17-OHP ($r = 0.388$; $P < 0.001$) net increments, and between BMI and cortisol ($r = 0.272$; $P = 0.008$) and 17-OHP ($r = 0.389$; $P < 0.001$) net increments suggest increased adrenal sensitivity to ACTH stimulation.

It might be safe to assume that the set point of plasma cortisol feedback inhibition on CRF release and ACTH secretion is 'correct' in normotensive normal weight subjects with good physical fitness. The set point may change in obesity so that the ratio of mean basal ACTH to mean basal cortisol is significantly higher, and the ratio of BMI to cortisol is significantly lower when the lowest tertile of WHR is compared to the highest tertile, as previously reported.[20] Because urinary cortisol excretion was negatively correlated to the ACTH-stimulated 17-

OHP, 11-deoxycortisol and cortisol responses, this relationship appears to reflect appropriately increased adrenocortical sensitivity to slightly decreased basal cortisol secretion.

The results of multiple linear regression analyses, as well as those of factor analyses, suggest that SHBG levels are regulated at least by two mechanisms, one consisting of an inhibitory effect on SHBG synthesis by insulin; the other mechanism appears to be a stimulatory effect of cortisol on SHBG production. Correct regulation of the HPA axis seems also to help in maintaining normal SHBG levels. This notion is supported by the positive correlations of mean basal 17-OHP, and DXM-suppressed 17-OHP and 11-deoxycortisol, as well as of urinary cortisol excretion with SHBG.

The etiology of the metabolic syndrome and insulin resistance remains to be established: the defects responsible for the development of the syndrome have been sought from many directions, from the altered activity of the sympathetic nervous system or the HPA axis to intracellular mechanisms[3–5,19] We have previously shown in normotensive males in this study that decreased basal cortisol levels during OGTT and increased cortisol responses to ACTH stimulation were related to increased WHR which, in turn, was closely associated with hyperinsulinemia and dyslipidemia.[21] Circadian variations of insulin sensitivity and cortisol secretion are inversely related, presumably as a result from the inhibitory effect of cortisol on insulin secretion.[35] Decreased cortisol secretion in visceral obesity may thus lead to higher fasting insulin levels and higher insulin responses during the OGTT, ie to insulin resistance. In healthy subjects, fasting insulin levels are relatively well correlated to the insulin resistance measured by the glucose clamp-techniques.[36] Thus, some features of the metabolic syndrome can probably be explained at least partly by the altered HPA function in visceral obesity.

What accounts for the altered HPA function in obesity? Several clinical observations suggest a relationship between obesity and the HPA function; overproduction of cortisol in Cushing's disease is associated with increased, and in Addison's disease, characterized by cortisol deficiency, with decreased adipose tissue mass. By analogy, stress-modulated neuroendocrine responses via hypersensitive HPA axis have been proposed to play a role in the development of abdominal obesity.[19]

Consistent with that hypothesis, but in contrast to the present data, previous studies have demonstrated increased urinary cortisol excretion and greater cortisol responses to exogenous ACTH stimulation, and greater ACTH and cortisol responses to exogenous CRH stimulation in women with abdominal obesity compared with those with peripheral obesity.[37,38] These results were explained as suggesting that women with abdominal obesity have hyper-responsiveness of the HPA-axis either of central origin or as a result of cortisol resistance. Whether there exists gender difference in obesity-related cortisol secretion remains to be elucidated.

Recent discovery of leptin has greatly advanced the understanding of the physiology of obesity. Leptin is a peptide hormone produced exclusively in adipose tissue.[39] The plasma levels of leptin rise with feeding and drop with fasting,[40,41] and are closely correlated with body fat mass.[42] It has been suggested that secreted leptin signals nutritional status to the central nervous system and modulates feeding behavior and energy expenditure.

Women have higher leptin levels than men and a diurnal rhythm with nocturnal rise has been shown in both sexes.[40,43] In addition to its possible function in the regulation of tissue metabolism, leptin has modulating effects on the hypothalamic–pituitary–adrenal[44] and–gonadal functions.[45] Diurnal levels of leptin correlate inversely with ACTH and cortisol levels, and an inhibitory effect of leptin on the HPA axis has been demonstrated both in mice[46] and men.[47–49] This phenomenon might offer one explanation for the results presented in this paper.

In conclusion, obesity and visceral fat distribution is associated with slight hypocortisolemia and increased sensitivity to exogenous ACTH stimulation, suggesting mild inhibition of the HPA function in males. This may contribute to hyperinsulinemia and related metabolic and hormonal changes, including decreased SHBG levels.

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