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PATHOPHYSIOLOGY OF SEX HORMONE BINDING GLOBULIN (SHBG): RELATION TO INSULIN

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Summary—In humans, the plasma level of sex hormone binding globulin (SHBG) is regulated by several hormones. We have now accumulated evidence that SHBG is also intimately related to nutritional state. However, we do not yet know what specific signal, if any, may be the regulator of SHBG.

There is a strong and negative correlation between fasting insulin level and SHBG in obese as in hyperandrogenic women. Under such circumstances, a high fasting insulin level, normal glycemia and a low SHBG level suggest insulin resistance in terms of glucose disposal but not in terms of SHBG inhibition. This is a rather complex situation.

It is too early to judge the importance of IGF-I in the regulation of SHBG. But it may turn out that IGF-I is the main regulator of SHBG and that, by interaction with the IGF-I receptors, insulin carries on its inhibitory activity on SHBG.

INTRODUCTION

Sex hormone binding globulin (SHBG), also referred to as sex steroid binding protein (SBP) [1] or testosterone estradiol binding globulin [2], is an extracellular protein which transports sex steroid hormones with high specificity. In humans, the hepatocytes are the main, if not the only, cells producing SHBG, albeit in rats the Sertoli cells produce an androgen binding protein (ABP) that is closely related to SHBG [3].

Several hormones which influence the plasma concentration of SHBG have been identified. Thyroid hormone administration increases SHBG levels, and this effect explains the high levels of SHBG in hyperthyroid patients [4]. Oral administration of estrogens also increases SHBG levels in women, although the percutaneous route has little, if any, influence on SHBG [5]. Androgens are generally believed to reduce SHBG levels, but their effects are still a matter of controversy.

In spite of the amount of data documenting the changes in SHBG concentrations under hormonal influence [6], there is no study which has measured the metabolic clearance rate and the production rate of SHBG in humans, which would help the understanding of the mechanism(s) involved in changes in levels of SHBG.

Recently, several groups have undertaken research on the regulation of SHBG by using culture hepatoma cell lines [7]. Khan *et al.* [8] were the first to show that the Hep G2 cell produced SHBG, as well as corticosteroid binding globulin (CBG). On this cell line, thyroid hormones increased the production of SHBG. A stimulatory activity of estradiol was found by Lee *et al.* [9] but not by Rosner *et al.* [10]. The effect of androgens is disputed. In one study, testosterone was shown to increase SHBG secretion [9], although no activity of androgens on SHBG was reported by two other groups [10, 11].

It was generally believed that the fall in SHBG concentration, occuring during puberty in boys, was to be attributed to rising androgen concentrations [12]. This has been questioned however, since a fall in SHBG level has been also shown in two 46 XY siblings with complete androgen insensitivity [13]. This interesting observation suggests that unidentified modulators of SHBG may contribute to the fall in SHBG levels which occurs simultaneously with sexual maturation.

O'Dea *et al.* [14] reported that, in obese women, the SHBG level was low, and that a low-calorie diet increased the SHBG level while

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inducing weight loss. A clear relationship between low SHBG and increased body fat levels has not been established, but so far remains unexplained [15].

Recently, the effect of insulin on SHBG has been questioned. Insulin is a pancreatic hormone involved in the regulation of carbohydrate and protein metabolism. Fasting insulin levels are increased in obese patients [16]. There is also an increase in basal and stimulated serum insulin levels at the end of the first decade of life, the same time that serum SHBG declines to adult levels [17]. With this in mind, Plymate *et al.* [18] investigated the effect of insulin on the secretion of SHBG by the Hep G2 cells. Their study showed that insulin was a potent inhibitor of basal production of SHBG by cultured cells, and blocked the stimulatory effect of 17β-estradiol and thyroxine [18].

As pointed out by Rosner [19], "there was no reasonable hypothesis available to explain the vanishingly small levels of SHBG in massive obesity" before this important result was found.

The aim of the present review will be to present the data arguing for a role for insulin in the regulation of SHBG in humans. The more recent findings concerning the possible influence of insulin growth factor-I (IGF-I) on SHBG will be also discussed.

THE RELATIONSHIP OF SHBG TO ADIPOSITY

Nutrition and reproductive function

There is accumulating evidence that nutrition, eating behavior and adiposity exercise an influence on ovarian functions. Simple dieting to less than 10% below ideal body weight, fad diets and vegetarian diets are often associated with altered menstrual cyclicity [20].

In males with protein-calorie malnutrition, total and protein-unbound testosterone levels are low, and increase during refeeding with a high-calorie, high-protein diet [21]. Patients who acquired gynecomastia during refeeding showed an increase in unbound estradiol levels [21]. In male anorexics, normal values of SHBG have been reported, which were not significantly reduced during weight gain [22].

Conversely, in women with anorexia nervosa, we have shown that SHBG levels are high, and that calorie infusion [23] or gentle refeeding [24] decreases the levels of SHBG.

Obesity

High incidence of obesity in women with menstrual disturbance has been reported [25], and obesity, hirsutism and sterility are the most frequent symptoms of polycystic ovarian (PCO) disease [26]. Although the physiopathology of PCO is uncertain, obesity has been suspected to play a "central role" [27–29].

The relationship between androgens and the various indices of obesity is variable. Obese women do not always show ovarian dysfunction, and many show no clinical evidence of hyperandrogenism [30] although increased production rates and increased metabolic clearance rates for androgens have been reported in obese non-hirsute women [31, 32]. Low levels of SHBG have been found in obese females [33, 34], and also in obese males [35]. In the latter, mild hypogonadotropic hypogonadism with decreased levels of free testosterone have been shown [36].

In humans, SHBG shows a rough negative correlation with adiposity [37]. The relationship of SHBG levels to BMI in obese women is illustrated in Fig. 1.

The San Antonio Heart Study is a very careful examination of the relationship of SHBG to overall adiposity and body fat distribution in two populations of Mexican American and of non-Hispanic white premenopausal women [38, 39]. The body mass index (BMI), was calculated as weight (in kilograms) divided by height (in meters) squared, as well as the ratio of waist-to-hip circumference (WHR), an index of upper body adiposity, and the ratio of subscapular-to-triceps skinfolds, an index of central adiposity. In this report, premenopausal Mexican American women showed greater overall and upper body adiposity and lower SHBG levels than premenopausal non-Hispanic

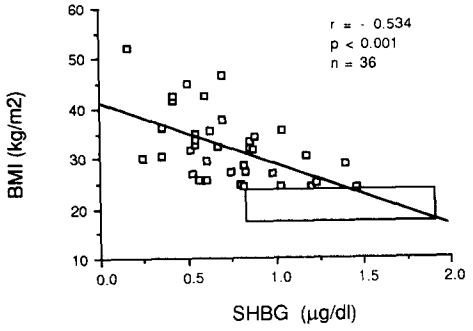

Fig. 1. Inverse relationship of body mass index (BMI) and SHBG in obese women. The large rectangle indicates the normal range for BMI and SHBG in normal women.

white women. In both populations, SHBG correlated negatively with BMI, WHR and the centrality index [39]. In premenopausal women, Evans *et al.* [15] reported that increasing androgenicity, as reflected by a decrease in SHBG and an increase in free testosterone levels, was accompanied by an increase in the waist-to-hip ratio (WHR). This relationship was independent of and additional to that of obesity. Conversely, Grenman *et al.* [40] failed to find a significant correlation of SHBG to WHR in massively obese premenopausal women, but Peiris *et al.* [41] found a strong correlation of SHBG with fat mass measured by hydrostatic weighing.

RELATIONSHIP OF SHBG WITH SEX STEROIDS, ADIPOSITY AND LIPID METABOLISM

Normal women

In women in the premenopause [15, 41] as well as in the postmenopause [42], it is now established that the level of SHBG is partly related to adiposity and to the degree of physical fitness [43, 44]. However, no significant correlation of SHBG with androgens levels has been reported in the normal female population.

Although in postmenopausal women SHBG levels are slightly lower than during reproductive life [45], SHBG levels does not decrease significantly in the perimenopause [46]. It has been shown that SHBG levels were significantly higher in women with type I osteoporotic fracture (vertebral and forearm fractures) as compared to controls, and body mass index was especially correlated to levels of estrone, estradiol and SHBG. The authors of this last study state that SHBG is a better predictor of osteoporisis in post menopausal women than are endogenous oestrogens [47]. In agreement with this study, Wild *et al.* [48] reported a negative relationship between SHBG level and vertebral bone density.

It has been shown that SHBG was significantly related to HDL-cholesterol and apoprotein A1 in women [46]. A fascinating study made in a population of 253 Swedish postmenopausal women has shown that a low SHBG level represents as a significant risk factor for 12-yr overall mortality [49].

Normal men

In men, SHBG is negatively correlated to percentage body fat, and strongly associated with the waist to hip girth ratio (WHR) [50, 51]. There is a tendency for SHBG levels to increase and for SHBG-unbound testosterone levels to decrease with age in men [45, 52–54].

The interrelationships between endogenous sex hormones and lipid metabolism in males have been investigated by several groups [55]. It has been suggested that the androgen–lipoprotein relationship might not be causal but mainly determined by the association between SHBG, lipoproteins and liver function. In a study by Hämäläinen *et al.* [55], it was found that serum levels of SHBG were positively associated to HDL-cholesterol and apoprotein-A1 in normal healthy middle-aged Finnish males.

By increasing the amount of bioavailable testosterone, low levels of SHBG in relation to body weight distribution might have some influence on the physiopathology of arteriosclerosis, and be associated with the risk of coronary heart disease.

Hirsutism

In hirsute women, a variable reduction of SHBG levels has been shown, these levels correlating to body weight rather than to androgens levels [56, 57]. In agreement with this finding, we reported, in patients with moderate hirsutism, no significant correlation between SHBG and androgen levels, but a significant correlation between SHBG level and the body mass index [58, submitted]. Ruutiainen *et al.* [59], in a study of two hundred hirsute women, reported that the SHBG level and the ratio of testosterone to SHBG (an index of free testosterone) were significantly correlated with BMI, independently of the effect of age.

Polycystic ovarian disease

High levels of androgens with increased secretion of luteinizing hormone (LH) are associated in most patients with PCO [27]. The increased levels of estrogens have been incriminated in the physiopathology of the abnormal secretion of LH, the amplitude and the frequency of which is increased [29].

In patients with PCO, the levels of SHBG are much lower in obese than in lean women [60]. An increased level of free testosterone is one of the most common hormonal features in this syndrome [61]. The decrease in SHBG level has been suggested to be the consequence of the increased production rate of androgens from ovaries, adrenals or both.

THE EFFECTS OF DIET, EXERCISE AND WEIGHT LOST ON SHBG

Normal men

A few studies have emphasized the importance of diet on the plasma levels of SHBG [62]. In normal men given a high carbohydrate diet, both testosterone and SHBG levels increase while cortisol and transcortin levels decrease, as compared to the effect of a high-protein diet equivalent in total calories and fat [63]. During a two-week high-fat diet cholesterol levels increase while SHBG levels decrease significantly in normal men [64]. By contrast, when given a low-fat diet containing less than 20 g of fat per day, for two weeks, all subjects increase their SHBG levels [64]. A high-fiber diet is associated with an increased level of SHBG [65].

Studies of the effects of exercise have shown that, during training for endurance running and/or jogging, SHBG levels were normal, except in women with severe menstrual disorders [66, 67]. The combined effect of exercise and food intake on hormonal profile has been investigated in male volunteers given a marginally negative (less than 15%) or an equilibrated energy balance [68]. When the energy expenditure increased by 15% with 2 h of exercise per day, food intake increased so as to maintain the energy balance. In this condition, a significant rise in SHBG concentration was observed.

Normal women

The effect of a low-calorie diet has been investigated in normal women [69]. It was shown that after two weeks of dieting, SHBG levels increase by two-fold.

Anorexia nervosa

In anorexia nervosa, a disease involving amenorrhea, denutrition and dramatic weight loss, Estour *et al.* [23] have reported high levels of SHBG that decreased significantly during a week of a 1200 kcal per day infusion. In these patients, SHBG levels decreased down to the normal range during gentle refeeding and weight gain. The kinetics of this decrease and its relationship to the quality of the diet is unknown [24].

Obese women

The effects of diet on SHBG levels of obese patients was originally documented by O'dea *et al.* [14]. Several reports have confirmed that a low-calorie diet increases SHBG in obese women, this effect being significantly related to weight lost [40, 24, 70–72].

However, it has been pointed out that weight loss can restimulate ovulatory menses in amenorrheic obese patients [70], and that although the SHBG level rose following weight loss, it continued to be markedly low, because the patients were still obese [71].

Polycystic ovarian disease

The effect of diet on the SHBG level of patients with PCO has been studied by various groups. Recently, Kiddy *et al.* [69] showed that, by giving a 330 kcal per day diet to six obese patients with PCO, a two-fold increase in SHBG concentrations was observed, this increase being sustained over two weeks; the rise in SHBG was accompanied by a fall in free testosterone.

Although there is no data available on the mechanism of SHBG level reduction in obese patients, one might suggest either an increased metabolic clearance rate of SHBG, or a reduced secretion by the liver. The increase in SHBG levels during diet-induced weight loss in obese patients suggests that an unknown factor or factors, responsible for and/or associated with adiposity, might regulate the circulating level of SHBG.

EFFECTS OF INSULIN ON SHGB *IN VITRO*

After the initial demonstration by Khan *et al.* [8] of the secretion of SHBG by a human hepatoma cell line (Hep G2), important new information on the substances that can directly inhibit or stimulate SHBG secretion have become available. Plymate *et al.* [18] reported that the basal secretion of SHBG by Hep G2 cells was greatly reduced by the physiological concentration of insulin. This inhibitory effect of insulin has been further supported by the demonstration that insulin blocks the stimulatory activity of thyroxine and estradiol on SHBG [18]. During this meeting, Mercier-Bodard *et al.* [73], reported that, using a human cDNA probe, a 1.6 kb SHBG mRNA was detected in Hep G2/H5A cells. The SHBG mRNA was up-regulated by estrogens and T3, and down-regulated by insulin at μmolar concentrations.

These important findings lead to the hypothesis that *in vivo* insulin might be significant in the regulation of circulating SHBG. There is now indirect evidence for this hypothesis.

THE RELATIONSHIP OF SHBG TO INSULIN *IN VIVO*

Normal women

In healthy subjects, it has been known, since the findings of Vague *et al.* [16], that upper body fat distributions, as estimated by measuring the ratio of waist-to-hip circumference, is associated with increased insulin levels. This finding has been brought to light again recently [74].

In pre- and postmenopausal women, negative correlations between fasting insulin and SHBG levels are now well established [75, 76]. In the original study by Peiris *et al.* [41], fat mass, estimated by hydrostatic weighing, was found to be related to the insulin response to the oral glucose tolerance test, and inversely related to SHBG levels. The relationship between SHBG level, and the cumulative insulin response remained significant after adjusting for fat mass and testosterone levels. It was concluded that the relationship of insulin to SHBG is independent of the degree of adiposity and of androgenic activity in premenopausal women. Several groups have reported similar findings [15, 39].

Polycystic ovarian disease

In PCO, concentrations of androgens are significantly correlated with fasting insulin levels [61, 77]. This correlation may be explained by the stimulatory effects of insulin on ovarian androgen production [78]. During low-calorie diet in obese patients with PCO, parallel changes in serum insulin concentrations, which decreased during the diet period, and SHBG levels, which increased, have been reported [61].

However, one cannot conclude from these results that a causal relationship between insulin and SHBG levels exists as such: that is to say, that too much insulin is causally responsible for the low SHBG levels found in overweight women. In this regard, we measured the concentration of C-peptide, the connecting segment in the proinsulin molecule, secreted in equimolar concentration to insulin, in a group of hirsute women, and studied its relationship to SHBG. In this population, as in other studies of hirsute women, the SHBG level was found to be inversely related to the body mass index and to fasting insulin levels. As shown in Fig. 2, there was a very strong inverse relationship of fasting levels of C-peptide and SHBG. Since C-peptide is devoid of any known significant biological effect, it would be hazardous to conclude that C-peptide exercises a direct inhibitory effect on SHBG secretion, unless there were some evidence of such an effect from *in vitro* studies.

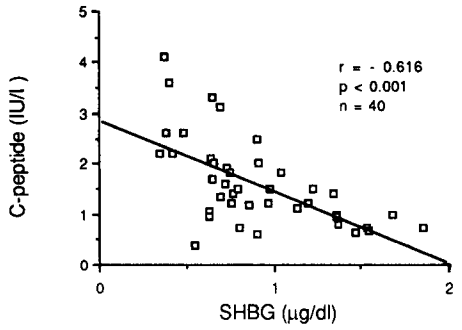

Fig. 2. Inverse relationship of the plasma concentrations of C-peptide and SHBG in hirsute women.

However, there are a few arguments as there are in favor of a insulin effect on SHBG *in vivo*.

SHBG and diabetes

It has been reported that SHBG and estrogen levels were significantly increased in a group of insulin-treated diabetic postmenopausal women, compared to a group of healthy women of comparable age and percentage of ideal body weight. It was not clear whether an insulin defect or estrogen excess was responsible for the increased SHBG levels [79].

SHBG and insulin infusion

The hypothesis that hyperinsulinemia may cause the polycystic ovary syndrome (PCO) by directly stimulating gonadal steroidogenesis [78] and/or gonadotropin secretion was explored by giving an infusion of insulin to 10 insulin-resistant women with PCO and to five age- and weight-matched normally ovulating women. The results of this study, by Dunaif and Graf [80], show that insulin acutely increased mean delta levels of androstenedione and estradiol, and reduced total and non-SHBG-bound testosterone levels in the PCO patients, but did not change the SHBG concentration in either PCO or normal women.

SHBG and diazoxide

To test the hypothesis that insulin plays a role in the hyperandrogenism of obese women with PCO syndrome, the androgen profile of five obese women was assessed before and after 10 days of oral administration of diazoxide (300 mg per day), an inhibitor of endogenous insulin secretion [81]. Diazoxide administration resulted in lower serum insulin levels, and in a 28% decrease in non-SHBG-bound testosterone. There was some increase in SHBG

concentration but this was not significant. The same group reported that in PCO patients given a long-acting GnRH agonist to reduced androgen levels plus diazoxide to suppress insulin secretion, SHBG levels increased significantly by 32% [82].

To indicate the general drift of all these studies, one would say that although the correlations between insulin, body fat distribution and SHBG are well established, there is no conclusive data supporting the statement that an excess of insulin is the mechanism of reduced levels of SHBG in obese women.

THE RELATIONSHIP OF SHBG TO INSULIN-LIKE GROWTH FACTOR-I

Most studies have extensively investigated the role of obesity in the physiopathology of polycystic ovarian disease. In anorexia nervosa, we found that the increased levels of SHBG were inversely related to the decreased BMI. In these patients, the concentrations of fasting insulin and C-peptide were low (Pugeat M. *et al.*, submitted). However, there was no significant relationship between SHBG and either insulin or C-peptide levels. This suggested that another factor or factors than insulin, but related to denutrition, may also regulate the level of SHBG.

Levels of insulin-like growth factor-I (IGF-I) or somatomedin-C, like insulin, have been shown to fall during fasting [83, 84] and to be reduced in undernutrition. This effect of diet on IGF-I has been recently investigated during diet-induced weight loss [69]. In both normal women and PCO patients, there were parallel changes in serum insulin and IGF-I concentrations which decreased during the diet period. SHBG levels, which increased during diet, were negatively correlated to insulin and IGF-I [69]. In addition, the serum concentration of IGF-I-BP, an insulin-dependent, small molecular weight (34 kDa) binding protein for IGF-I [85, 86], increased significantly during diet. IGF-I-BP levels were negatively correlated with insulin and positively with SHBG [69].

Following this study, the possibility that IGF-I may be an additional regulator of SHBG, was investigated using human hepatoma cells, Hep G2 [87]. IGF-I, at a concentration of 100 nmol/l, and insulin, at a concentration of 10 μmol/l, was shown to inhibit SHBG secretion by about 40%. Additionally, insulin, but not IGF-I, was also found to inhibit the secretion of IGFI-BP, which is also secreted by Hep G2 cells [87].

In extreme obese, menstruating women, reduced IGF-I-BP levels, with a strong positive correlation to SHBG levels were shown [88].

In patients with anorexia nervosa, although we found a very large range of plasma concentrations of IGF-I, SHBG was inversely related to IGF-I level. Moreover, during refeeding, the IGF-I level increased in each patient, this increase being correlated to the decrease in the SHBG level (Pugeat *et al.*, submitted).

CONCLUSIONS

We have now accumulated evidence that SHBG in humans is intimately related to nutritional state. However, we do not yet know what specific signal, if any, may be the regulator of SHBG.

While a strong correlation between insulin and SHBG is indeed generally observed, this correlation could perfectly well be fortuitous. In obese [89] as in hyperandrogenic patients [90, 91], there is data supporting the concept that high fasting insulin levels indicate some degree of insulin resistance. Under such circumstances, an individual might have insulin resistance in terms of glucose transport yet remain sensitive to the action of insulin on SHBG. This is a rather complex situation.

It is too early to judge the importance of IGF-I in the regulation of SHBG. But it may turn out that IGF-I is the main regulator of SHBG during puberty, a period in which the fall in SHBG levels does not appear to be uniquely under the influence of rising androgens [92]. The role of IGF-I in adults merits further investigations [93].

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