

RESEARCH

Human sex hormone-binding globulin does not provide metabolic protection against diet-induced obesity and dysglycemia in mice

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Abstract

Background: Sex hormone-binding globulin (SHBG) is the main transporter of sex hormones in most vertebrates. Low SHBG levels have been linked to increased risk for diabetes and metabolic syndrome. Polymorphisms of the *SHBG* gene linked to low SHBG protein levels also strongly predicted increased risk of type 2 diabetes, thus raising the possibility that SHBG may play a role in the pathogenesis of insulin resistance and diabetes.

Aim: To examine whether expression of human *SHBG* in mice may ameliorate the development of diabetes and metabolic syndrome in response to a high-fat diet (HFD).

Methods: Transgene mice expressing a human *SHBG* transgene (*SHBG+*) ($N=10/11$; males/females) and their wild type littermates ($N=12/8$; males/females) were fed HFD for 4.5 months.

Results: HFD induced comparable obesity in control and *SHBG+* mice. Male transgenes had higher muscle mass after 2–3.5 months HFD (0.43 ± 0.028 ($n=4$) vs 0.38 ± 0.053 g ($n=7$), $P=0.05$). Fasting blood glucose, as well as insulin or HOMA-IR, was not different in transgenic vs wild-type males after 4–5 months HFD. Female transgenes had higher fasting glucose (152 ± 29 ($n=7$) vs 115 ± 27 mg/dL, $P=0.01$ ($n=8$)), but mean insulin and HOMA-IR were not different. Likewise, insulin tolerance test and intra-peritoneal glucose tolerance test (GTT) were not different. Finally, *SHBG+* mice were not different from controls in terms of liver enzymes, serum triglyceride levels and blood pressure.

Conclusion: In mice with diet-induced obesity, human *SHBG* did not protect against development of obesity or dysglycemia.

Key Words

- ▶ SHBG
- ▶ diabetes
- ▶ metabolic syndrome

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Introduction

SHBG is a homodimeric plasma glycoprotein produced by the liver that acts as the main transporter of active estrogens and androgens in almost all vertebrates (1). The protein has high affinity and selectivity to these sex steroids, and it has been traditionally postulated that the role of SHBG is limited to the transport of sex steroids into

the target cells (2, 3). However, it has also been reported that SHBG interacts with a trans-membrane receptor that activates cellular cascades after binding sex steroids (4, 5).

The relation between low SHBG levels and diabetes now appears well established, both in men and women (6, 7, 8) and remains significant even after adjustment

for BMI (9, 10, 11) and waist circumference (12). A meta-analysis of more than ten prospective studies has demonstrated that women with SHBG levels higher than 60 nmol/L had 80% lower risk of type 2 diabetes (7). Men with SHBG higher than 28.3 nmol/L had a 52% smaller risk of developing diabetes (7). High SHBG levels are apparently also associated with lower incidence of insulin resistance in men (10). Furthermore, cross-sectional studies in non-diabetic men and women have shown an inverse relationship between SHBG levels and HbA1c (11, 13). Hypoandrogenism in men and hyperandrogenism in women (e.g., the polycystic ovary syndrome) have been linked to insulin resistance and the metabolic syndrome (13, 14). In one report, SHBG levels predicted the development of gestational diabetes as early as 11–13 weeks of gestation (15). Of note, several studies have shown that the relationship between SHBG levels and diabetes is independent of sex hormones, in both female and male subjects (16, 17, 18). In a few studies, SHBG has also been linked to the metabolic syndrome in both adolescents (19, 20) and adults (21). Other metabolic components such as liver fat have also been strongly and inversely correlated with SHBG levels (22). Favorable lifestyle changes caused a parallel regression of fatty liver and increase in plasma levels of SHBG (23). A few studies have suggested that elevated insulin levels suppress SHBG production (24, 25), and insulin-sensitizing drugs, such as metformin and thiazolidinediones, have been shown to increase SHBG levels in men and women (26, 27). Other studies have shown that dietary monosaccharides can decrease *SHBG* expression (28).

There is evidence that SHBG levels may be influenced by specific polymorphisms in the *SHBG* gene *per se* and also by an array of loci in genes involved in biologic networks such as liver function, lipid metabolism, glucose metabolism, androgen and estrogen receptor function and epigenetic effects (29). Recent studies have also shown that specific genetic polymorphisms of *SHBG* were not only predictive of SHBG protein levels, but also of the apparently consequent risk of type 2 diabetes in men and women (12, 30). Also, one study suggested an association between a polymorphism in the *SHBG* promoter and polycystic ovary syndrome (31). Collectively, these findings are consistent with the hypothesis that the SHBG protein may not only comprise a 'reverse' biomarker of insulin resistance and diabetes, but could curb their evolution (32). To directly address this question, we examined whether or not mice expressing human *SHBG* have attenuated tendency to develop diabetes and other characteristics of the metabolic syndrome.

Materials and methods

High-fat diet-fed mice

Animal care and experiments were approved by the Institutional Animal Care and Use Committee of the Tel Aviv University. Twenty C57Bl/6J WT and twenty transgene littermates (non-congenic) expressing a 4 kb human *SHBG* transgene under the control of its own promoter sequence (33) were separated by sex at age 3 weeks and at age 6 weeks were placed on a high-fat diet (HFD, Research Diets, Inc, New Brunswick, NJ, USA). The diet was composed of 58% fat from lard, 25.6% carbohydrate and 16.4% protein (total caloric value, 23.4 kJ/g). Weight of mice was checked weekly. Identification of transgenic mice was done using PCR for human *SHBG*, as described previously (32). DNA was extracted from mice tails using REDExtract-N-Amp Tissue PCR Kit. Thereafter, the DNA was subjected to PCR amplification (1 cycle of 95°C for 2 min, 40 cycles of 94°C for 30 s, 64°C for 30 s and 72°C for 30 s) using specific forward (5'-GATCCCCAGAGGGGTGATAGC-3') and reverse (5'-GGGTAAAGGAAACAGGGGCAC-3') primers designed to amplify a 153-bp region in the human *SHBG* promoter (34).

Further validation of the model was done by measuring serum SHBG (Siemens 06603393 Immulite SHBG Kit). After 20 weeks of the HFD, blood samples were collected for the measurement of fasting glucose, insulin, lipids, liver enzymes and testosterone levels. A glucose tolerance test (GTT) was done after an overnight fast with an intra-peritoneal injection of glucose (2 mg/kg). Glucose was measured at the following time points: 0, 15, 30, 60, 90 and 120 min after the injection of glucose. Similarly, an insulin tolerance test (ITT) was carried out with an intra-peritoneal injection of insulin (Humalog, 0.75 U/kg; Eli Lilly) and subsequent measurement of glucose at same time points. Blood pressure was measured in awake mice by a non-invasive approach using a three-channel computerized tail-cuff method (35, 36). The recording system consisted of an animal restrainer, which had a sensor block containing a photoelectric sensor built inside an occlusion cuff, an inflation bulb, a sphygmomanometer, an amplifier (model 3M229 BP, attached to the 31BP software package, IITC, Inc. Woodland Hills, CA, USA) and a chart recorder.

Technical procedures

Assays

Serum insulin was determined using a radioimmunoassay Insulin-CT (MP Biomedicals, Orangeburg, NY) with

a limit of detection of 3 μIU/mL at 95% confidence interval, measuring range from 5 to 25 μIU/mL; intra-assay CV being 7.6% and inter-assay CV BEING 8.9%. Testosterone was measured with the Siemens ADVIA Centaur Testosterone system with a limit of detection of 0.025 ng/mL; measuring range 0.025–15.0 ng/mL; intra-assay CV of 6% and inter-assay CV of 9%. SHBG was measured with the two-site chemiluminescent immunometric assay (Siemens IMMULITE 2000), with a detection limit of 0.8 nmol/L, detection range reaching 100 nmol/L and intra/inter-assay CV <5.5% and 6.6%, respectively. Muscle mass was measured using PIXImus II Densitometer (L4unar, Madison, WI, USA), which utilizes dual-energy X-ray absorptiometry (DEXA) technology.

Statistical analysis

Statistical calculations were carried out using ‘Statistica 12’ software (Statsoft, Inc). Statistical analyses were performed using 2-tailed Student’s *t* test, Mann–Whitney *U* test and Kolmogorov–Smirnov test. A *P* value less than 0.05 was considered significant. Data are presented as mean ± s.d. Calculation of the area under the curve for measured responses has been described elsewhere (37).

Results

Validation of the model

Because mice and rats do not postnatally express *Shbg* in the liver, which is the site of production of circulating SHBG in other mammals such as humans, circulation testosterone in these rodents are generally low and tend to exhibit large fluctuations, presumably owing to the lack of the buffering effect afforded by SHBG (32). Expectedly, on the background of the previously described large variability in circulating testosterone in wild-type mice, serum total testosterone levels were much higher in female *SHBG* transgenic mice as compared to wild-type mice. Indeed, testosterone levels were undetectable in

5 of 6 wild-type females, with the exception of one female mouse showing a value of 0.82 ng/mL. In contrast, in the 10 female *SHBG* transgenic mice, the mean testosterone level was 1.47 ± 1.57 ng/mL. Male *SHBG* transgenes had non-significantly higher testosterone levels than the wild-type mice (5.77 ± 6.09 ng/mL; (*n*=12) vs 2.31 ± 4.31 ng/mL (*n*=11)), owing to the large spread of testosterone levels in both strains. There was a 100% concordance between the presence of a human *SHBG* gene as detected by PCR and the presence of measurable serum SHBG levels (data not shown). Concordantly, SHBG levels were undetectable in wild-type mice.

Effect of SHBG expression on weight gain and body composition on HFD

There was no significant difference in the initial weight of *SHBG*-expressing mice as compared to wild-type littermates. Further, weight gain on HFD and weight were similar throughout the experiment (data not shown). In male mice, median peak weight (4 months) was 48.3 ± 2.87 g and 50.2 ± 5.72 g for WT and *SHBG*+, respectively, and the corresponding attained mean weights for female mice were 45.5 ± 7.4 g and 40.0 ± 8.7 g (*P*=ns, for both comparisons).

Due to the known association between total testosterone and muscle (38), we compared muscle and fat mass of the gastrocnemius muscle, using dual-energy X-ray absorptiometry (DEXA). Male transgenes had significantly higher muscle mass after 3.5-month HFD, 0.68 ± 0.19 (median 0.67) g/cm², as compared to 0.43 ± 0.1 (median 0.38) g/cm², respectively (*P*=0.032), but this difference was reversed after 5 months (Table 1). Bone mineral density and % fat were not different between transgenes and controls. In HFD female mice, % fat and muscle mass was not related to the SHBG status: WT mean muscle mass 0.3125 ± 0.07 g/cm² (median 0.295) (*n*=4) as compared with *Shbg*+ mean muscle mass 0.413 ± 0.1 g/cm² (median 0.37) (*n*=3). WT mean % fat is 33.12 ± 4.08

Table 1 Muscle mass (g/cm²) and % fat of gastrocnemius in male wild-type (WT) and SHBG+ mice after HFD.

Duration	2 months		3.5 months		5 months	
	Muscle mass mean (median) (g/cm ²)	%FAT mean (median)	Muscle mass mean (median) (g/cm ²)	%FAT mean (median)	Muscle mass mean (median) (g/cm ²)	%FAT mean (median)
WT	0.39 ± 0.05 (0.38) <i>n</i> =7	21.89 ± 10 (16.23) <i>n</i> =7	0.43 ± 0.1 (0.38) <i>n</i> =3	24.76 ± 3.4 (27.04) <i>n</i> =3	0.48 ± 0.02 (0.49) <i>n</i> =3	25.94 ± 2.55 (27.13) <i>n</i> =3
<i>SHBG</i> +	0.43 ± 0.03 (0.43) <i>n</i> =4	27.58 ± 9.48 (32.36) <i>n</i> =4	0.68 ± 0.19 (0.67) <i>n</i> =6	34.42 ± 11.3 (32.21) <i>n</i> =6	0.39 ± 0.09 (0.37) <i>n</i> =8	21.12 ± 7.7 (23.79) <i>n</i> =8
<i>P</i>	NS	NS	0.032	0.065	0.02	0.097

Table 2 Glucose metabolism in female mice 4.5 months on HFD.

	Weight (g) mean (median)	Fasting glucose (mg/dL) mean (median)	Insulin (IU/L) mean (median)	HOMA-IR mean (median)
WT (n=8)	35.66±4.43 (34.99)	115±27 (102)	15±4* (15)	4.28±1.43* (4.85)
SHBG+ (n=7)	42.3±3.41 (101.5)	152±29 (152)	48**±32 (44)	16.89±8.80** (17.03)
P	0.0082	0.014	0.13	0.007

*n=6; **n=4.

(median 32.52) as compared with 32.3±5.43 (median 34.42); differences were not statistically significant.

Effect of SHBG on glucose metabolism

Fasting blood glucose was not different in WT and SHBG+ mice ~6 months on HFD (143±36 vs 166±33 mg/dL, respectively; $P=0.18$), and likewise, no statistically significant differences were seen in insulin (51±30 vs 59±29 IU/L, $P=0.25$ or HOMA-IR 18.94±14.61 vs 25.67±15.58, $P=0.13$) in male wild-type mice compared to transgenic mice. Four to five months into the HFD, female transgenes had significantly higher fasting glucose than their WT controls (152.30±27.12 mg/dL vs 114.50±28.73 mg/dL, $P=0.01$), but there were no differences in mean insulin or HOMA-IR (Table 2). Similarly, there was no discernible difference between transgenic wild-type mice in the liver enzymes (GOT and GPT) and serum triglycerides and HDL. GTT and ITT were similar in WT and transgene male and female mice (data not shown).

Discussion

The mechanisms through which SHBG, a binding protein for sex hormones with high affinity to active androgens and estrogens, is linked to protection from diabetes or insulin resistance (39) remain enigmatic. Prospective and cross-sectional studies have already established that such putative protection was not mediated through the prevention of weight gain or the presence/degree of obesity (10, 11, 12, 13). Interestingly, SHBG-related protection from diabetes was seen in both women and men (12) and is independent of sex hormone levels *per se*. Still, it is apparently linked to insulin resistance, as several studies noted a strong reverse correlation between SHBG levels and metabolic syndrome and insulin resistance (40).

In the present study, SHBG+ mice were not protected from high fat diet-induced obesity. Further, diabetes, as evidenced by high glucose levels, was evident in mice fed on high-fat chow regardless of the expression of SHBG, in both intact male and female mice.

This outcome has some similarities and major differences compared to the human data. First, the presence of SHBG did not prevent weight gain on HFD in our experiments. In humans, higher SHBG levels appear to confer protection from diabetes after adjustment for the level of obesity (12). Phrased alternatively, in humans, SHBG is linked to lesser risk for diabetes even in the obese state (12, 31). Second, in contrast to what might have been expected based on the human data, high circulating SHBG levels did not protect mice fed on a HFD from diabetes. Unlike humans, SHBG does not circulate in adult rodents (39). Despite early interest in a putative cellular receptor for SHBG (41), evidence for its physiological role is lacking. Therefore, the possibility that SHBG's putative protective metabolic effect can be exerted by some direct interaction with cellular target sites/receptors to enhance insulin sensitivity remains entirely speculative.

In a recent report (42), crossbreeding of the same SHBG+ transgenic mouse used in our study with the db/db transgenic mice lacking the leptin receptor, attenuated the massive weight gain seen in the latter mouse model, but did not affect the metabolic anomalies seen in this setting, i.e., high glucose and increased serum lipid levels. The latter is in general agreement with our results, in that neither baseline nor post-HFD glucose and triglycerides differed between SHBG+ and wild-type mice in the obese state.

Whether the linkage of SHBG to dysglycemia and diabetes in humans is species specific or mediated by some mechanism that is not operative in the HFD mouse model cannot be determined based on our experiments. Our data are limited to a particular animal model under a particular experimental set of metabolic conditions (HFD). Hence, we are unable to extrapolate our results to the much more complex clinical setting in humans, where genetics, nutrition, environment, life style and aging all interact in the pathogenesis of diabetes. Nevertheless, our results do not support the concept that SHBG expression alone modifies the abnormalities in glucose metabolism, which are linked to HFD and the associated obese state.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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