Relationships of Circulating Sex Hormone–Binding Globulin With Metabolic Traits in Humans

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OBJECTIVE—Recent data suggested that sex hormone–binding globulin (SHBG) levels decrease when fat accumulates in the liver and that circulating SHBG may be causally involved in the pathogenesis of type 2 diabetes in humans. In the present study, we investigated mechanisms by which high SHBG may prevent development to diabetes.

RESEARCH DESIGN AND METHODS—Before and during a 9-month lifestyle intervention, total body and visceral fat were precisely measured by magnetic resonance (MR) tomography and liver fat was measured by ^{1}H-MR spectroscopy in 225 subjects. Insulin sensitivity was estimated from a 75-g oral glucose tolerance test (IS_{OGTT}) and measured by a euglycemic hyperinsulinemic clamp (IS_{clamp}, $n = 172$). Insulin secretion was measured during the OGTT and an ivGTT ($n = 172$).

RESULTS—SHBG levels correlated positively with insulin sensitivity (IS_{OGTT}, $P = 0.037$; IS_{clamp}, $P = 0.057$), independently of age, sex, and total body fat. In a multivariate model, these relationships were also significant after additional adjustment for levels of the adipokine adiponectin and the hepatokine fetuin-A (IS_{OGTT}, $P = 0.0096$; IS_{clamp}, $P = 0.029$). Adjustment of circulating SHBG for liver fat abolished the relationships of SHBG with insulin sensitivity. In contrast, circulating SHBG correlated negatively with fasting glycemia, before ($r = -0.17$, $P = 0.009$) and after ($r = -0.14$, $P = 0.04$) adjustment for liver fat. No correlation of circulating SHBG with adjusted insulin secretion was observed (OGTT, $P = 0.16$; ivGTT, $P = 0.35$). The SNP rs1799941 in *SHBG* was associated with circulating SHBG ($P \leq 0.025$) but not with metabolic characteristics (all $P > 0.18$).

CONCLUSIONS—Possible mechanisms by which high circulating SHBG prevents the development of type 2 diabetes involve regulation of fasting glycemia but not alteration of insulin secretory function. ***Diabetes*** **59:3167–3173, 2010**

Recently, two studies applying the Mendelian randomization approach, an elegant tool to study causal relationships between plasma parameters and traits in humans (1), suggested that circulating sex hormone–binding globulin (SHBG) may be involved in the pathogenesis of type 2 diabetes in men and women (2,3). Because circulating SHBG regulates biological action and signaling of sex hormones (4), these data strengthen the notion that sex hormones play an important role in the pathogenesis of the disease. So far, however, results from several studies revealed that this field of research is very complex. Whereas high testosterone levels were found to be associated with insulin resistance, glucose intolerance, and increased risk of type 2 diabetes in women, in men high testosterone levels appear to protect from insulin resistance and diabetes. In contrast, in both sexes, although not consistent among all studies, high estradiol levels were associated with elevated insulin resistance and increased risk of type 2 diabetes (5,6).

For relationships of circulating SHBG with insulin sensitivity and risk of type 2 diabetes, the data were more consistent among sexes. Low plasma levels of SHBG were found to be similarly strongly associated with insulin resistance and increased risk of type 2 diabetes, in men and in women in most (7–11), but not in all, studies (12–15).

The question now is, besides genetic variability in *SHBG*, which parameters involved in the natural history of type 2 diabetes regulate plasma levels of SHBG? Studies indicated that high insulin levels decrease the release of SHBG from hepatocytes (16,17), which represent the major source of SHBG biosynthesis (18). However, Selva et al. provided convincing evidence that expression of SHBG in hepatocytes could be suppressed by glucose or fructose, strong inducers of hepatic lipogenesis (19), but not by insulin (20). This is in agreement with human studies showing that low circulating SHBG was not associated with elevated insulin levels (21) but was associated with obesity and dyslipidemia, conditions that are strongly associated with fatty liver in humans (22–29). On the basis of the data from Selva et al. (20), we hypothesized that specifically fatty liver may be associated with low circulating SHBG in humans, and we could recently support this in a small study (30).

Because of the unexpected finding of no significant relationships between genetic variants in *SHBG* and metabolic traits in the study by Perry et al. (3), in the present study we investigated mechanisms behind the association between low SHBG levels and increased risk of type 2 diabetes in our precisely phenotyped subjects. Therefore, we additionally genotyped them for a variant that was found to be strongly associated with plasma SHBG levels and the risk of type 2 diabetes (3).

RESEARCH DESIGN AND METHODS

Caucasians from the southern part of Germany participated in the ongoing Tübingen Lifestyle Intervention Program (TULIP) (31–33). Individuals were included in the study when they fulfilled at least one of the following criteria: a family history of type 2 diabetes, a BMI > 27 kg/m^2, or previous diagnosis of impaired glucose tolerance or gestational diabetes. In a small pilot study, we

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Received 4 February 2010 and accepted 3 September 2010. Published ahead of print at http://diabetes.diabetesjournals.org on 14 September 2010. DOI: 10.2337/db10-0179.

previously investigated relationships of circulating SHBG with total body fat, visceral fat, and liver fat in a total of 114 subjects (30). One hundred seven subjects from that study had these measurements and blood samples, both at baseline and after 9 months of lifestyle intervention, and were also included in the present study. We subsequently included another set of 118 subjects, who met the aforementioned requirements, resulting in a total of 225 subjects in the present study.

Subjects were considered healthy according to a physical examination and routine laboratory tests. As assessed by means of a standard questionnaire, the participants had no history of liver disease such as hepatitis and did not consume more than two alcoholic drinks per day. Informed written consent was obtained from all participants, and the local medical ethics committee approved the protocol.

Lifestyle intervention. After the baseline measurements, individuals underwent dietary counseling and had up to ten sessions with a dietitian. Counseling was aimed to reduce body weight, intake of calories, and particularly intake of calories from fat, as well as to increase intake of fibers. No specific recommendations regarding protein intake were given. Individuals were asked to perform at least 3 h of moderate sports per week. Aerobic endurance exercise (e.g., walking or swimming) with an only moderate increase in the heart rate was encouraged. Participants were seen by the staff on a regular basis to ensure that these recommendations were accomplished. Furthermore, all subjects completed a standardized and validated questionnaire to estimate physical activity, and a habitual physical activity score was calculated.

Body fat distribution and liver fat. Waist circumference was measured at the midpoint between the lateral iliac crest and the lowest rib. Furthermore, we measured total and visceral fat with an axial T1-weighed fast spin echo technique with a 1.5 T whole-body imager (Magnetom Sonata, Siemens Healthcare) (34). Volunteers were in prone position, and images were recorded from fingers to toes with a slice thickness of 10 mm and a gap between slices of 10 mm. Segmentation of images was performed by semiautomatic thresholding based on a Matlab routine (Mathworks, Inc.). Visceral fat was quantified in 16–21 slices (depending on the size of the volunteer) between femoral heads and diaphragm, not differentiating between intra- and retroperitoneal fat, by manual delineation of the visceral compartment. Fat volume from images was calculated by multiplying the in-plane pixel dimensions with the slice thickness and the number of pixels classified as fat. Volumes between contiguous slices are calculated by simply doubling the volume of the adjacent slice. Liver fat was measured by localized ^{1}HMR spectroscopy as previously described (34).

Maximal aerobic capacity. Individuals underwent a continuous, incremental exercise test to volitional exhaustion using a cycle ergometer. The cycle ergometer test was performed on an electromagnetically braked cycle ergometer (Ergometrics 800 S; Ergoline, Bitz, Germany). Oxygen consumption was measured using a spiroergometer (MedGraphics System Breese Ex 3.02 A; MedGraphics). Maximal aerobic capacity (VO_{2max}) is expressed as VO_2 (ml/min) per kg body weight ($ml \cdot min^{-1} \cdot kg^{-1}$).

Oral glucose tolerance test. All individuals underwent a 75 g oral glucose tolerance test (OGTT). We obtained venous plasma samples at 0, 30, 60, 90, and 120 min for determination of plasma glucose and insulin. Glucose tolerance was determined according to the 1997 World Health Organization diagnostic criteria (35).

Euglycemic hyperinsulinemic clamp. Insulin sensitivity was determined with a primed insulin infusion at a rate of 40 $mU \cdot m^{-2} \cdot min^{-1}$ for 2 h as previously described (34).

Analytical procedures. Blood glucose was determined using a bedside glucose analyzer (glucose-oxidase method; YSI, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and C-peptide were determined by microparticle enzyme immunoassays (ADVIA Centaur, Siemens Healthcare Diagnostics, Eschborn, Germany). Fasting plasma levels of adiponectin were determined with enzyme-linked immunosorbent assays (ELISA, Linco Research, Inc., St Charles, MO). Fasting plasma levels of fetuin-A were measured by an immunoturbidimetic method (BioVendor Laboratory Medicine, Modreci, Czech Republic). Serum SHBG levels were measured using the fully automated solid-phase, chemiluminescent immunoassay IMMULITE 2,500 (Siemens Healthcare Diagnostics, Eschborn, Germany).

Calculations. The insulin sensitivity index measured during the clamp (in $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pM^{-1}$) was calculated as the mean infusion rate of glucose (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$) necessary to maintain euglycemia during the last 40 min of the euglycemic hyperinsulinemic clamp divided by the steady-state plasma insulin concentration. The latter was the mean insulin concentration at 100, 110, and 120 min of the clamp. Insulin sensitivity from the OGTT was estimated as proposed by Matsuda and DeFronzo (36). The insulinogenic index was assessed from the OGTT as follows: (insulin at 30 min − insulin at 0 min)/(glucose at 30 min − glucose at 0 min). Insulin secretion during the intravenous GTT (IVGTT) was assessed as the sum of C-peptide levels and insulin levels, respectively, during the first 10 min after glucose administration.

Genotyping. For genotyping, DNA was isolated from whole blood using a commercial DNA isolation kit (NucleoSpin, Macherey & Nagel, Düren, Germany). The SNP rs1799941 in *SHBG* (37,38), which was found to be associated with circulating SHBG and type 2 diabetes (3), was genotyped using TaqMan assay (Applied Biosystems, Foster City, CA, USA).The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 7,000 (50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min), and fluorescence was detected on an ABI Prism sequence detector (Applied Biosystems, Foster City, CA, USA). The TaqMan assay was validated by direct sequencing of the SNP in 50 subjects, and both methods gave identical results. The genotyping success rate for the SNP rs1799941 was 99.9%, and rescreening of 3.3% of the subjects with the TaqMan assay gave 100% identical results.

Statistical analyses. Data that were not normally distributed (e.g., liver fat, circulating SHBG, insulin sensitivity, body fat distribution; Shapiro-Wilk W test) were logarithmically transformed. Differences between baseline and follow-up were tested using the matched-pairs t test. Univariate associations between parameters were tested using Pearson correlation analyses. To adjust the effects of covariates and identify independent relationships, multivariate linear regression analyses were used. To test the effect of the genotype on the metabolically relevant parameters, the parameter to be considered was set as the dependent variable. The genotype was included in both analyses as an independent nominal variable. The statistical software package JMP 5.1 (SAS Institute Inc, Cary, NC, USA) was used.

RESULTS

Characteristics of all 225 subjects. Anthropometrics and metabolic characteristics of the 225 subjects at baseline (Table 1) covered a wide range that was particularly large for total body fat, visceral fat, liver fat, insulin sensitivity, and circulating SHBG. A total of 66 subjects had fatty liver (liver fat $>$ 5.56%) (39). Circulating SHBG was lower in males than females (means ± SE; 24.8 ± 1.1 vs. 56.2 ± 4.3 mM, $P < 0.0001$) and did not correlate significantly with age ($r = 0.01$, $P = 0.17$) or VO_{2max} ($r = 0.10$, $P = 0.16$).

During the lifestyle intervention, measures of total and visceral adiposity and liver fat decreased and physical activity and VO_{2max} increased (Table 1). A decrease was also found for fasting and 2-h glycemia and insulinemia. While insulin sensitivity increased, measures of insulin secretion remained unchanged. High-sensitivity C-reactive protein levels decreased, and adiponectinemia increased. Circulating SHBG also increased. Regarding dietary intake, at baseline circulating SHBG correlated negatively with total energy intake ($r = -0.28$, $P < 0.0001$) but not with percentage of carbohydrate, fat, or protein intake. During the intervention, a trend for a larger increase in circulating SHBG with larger decrease in carbohydrate intake was observed ($r = -0.10$, $P = 0.13$).

Relationships of circulating SHBG with total body fat, visceral fat, and liver fat at baseline and during the lifestyle intervention in 118 subjects. First we tested whether we can replicate our previous findings of a strong and independent relationship of circulating SHBG with liver fat in the 118 subjects that were not included in our previous analysis about relationships of circulating SHBG with total body, visceral, and liver fat (30). In the 118 subjects, circulating SHBG adjusted for sex and age correlated negatively with total body, visceral, and liver fat (Table 2). In multivariate linear regression analyses including age, sex, and liver, total body, and visceral fat, liver fat (F-value = 11, $P = 0.0016$) was the strongest determinant of circulating SHBG followed by sex (F-value = 6, $P = 0.017$), while total body (F-value = <1, $P = 0.62$) and visceral fat (F-value = <1, $P = 0.61$) were not independent determinants.

On the basis of the strong correlations between liver fat

TABLE 1
Subject characteristics at baseline and at follow-up in all 225 subjects

Parameter	Baseline	Follow-up	*P* value
Sex (male/female)	90/135	90/135	
Age (years)	47 (19–69)	48 (20–70)	<0.0001
Body wt (kg)	86.2 (52.5–144.7)	84.0 (52.0–142.8)	<0.0001
Waist circumference (cm)	96.5 (63.0–135.0)	92.0 (66.5–131.5)	<0.0001
BMI ($kg \cdot m^{-2}$)	29.0 (19.4–43.5)	27.9 (18.9–42.9)	<0.0001
Total body fat_{MRT} (kg)	24.9 (4.0–61.0)	21.9 (0.2–57.5)	<0.0001
Visceral fat_{MRT} (kg)	2.59 (0.25–9.73)	2.04 (0.15–10.72)	<0.0001
Liver fat_{MRS} (%)	3.10 (0.16–30.88)	2.09 (0.00–23.21)	<0.0001
HPA score	8.13 (3.510–11.00)	8.50 (5.32–11.38)	<0.0001
VO_{2max} ($ml \cdot min^{-1} \cdot kg^{-1}$)*	24.5 (9.5–54.1)	25.0 (2.0–47.1)	0.03
Fasting glucose (mM)	5.17 (4.33–7.42)	5.11 (4.11–7.31)	0.0012
2-h glucose (mM)	6.67 (4.17–11.17)	6.39 (3.50–13.39)	0.004
Fasting insulin (pM)	50 (19–373)	43 (14–175)	<0.0001
2-h insulin (pM)	395 (47–2,132)	310 (37–3,112)	<0.0001
$Insulin\ sens._{OGTT}$ (arb. units)	11.7 (1.6–32.1)	13.2 (2.4–39.2)	<0.0001
$Insulin\ sens._{Clamp}$ ($\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pM^{-1}$)§	0.057 (0.008–0.347)	0.065 (0.021–0.267)	0.047
$Insulin\ secretion_{OGTT}$ (pM/mg/dl)	109 (25–2,346)	110 (16–9,197)	0.28
$Insulin\ secretion_{ivGTT}$ (pM)§	6,937 (1,731–16,162)	7,152 (2,527–26,462)	0.54
hs-CRP (mg/dl)	0.12 (0.01–2.31)	0.08 (0.01–1.78)	0.001
SHBG (nM)	29.8 (7.2–283.0)	33.6 (9.0–287.0)	<0.0001
Adiponectin (μg/ml)	12.0 (3.0–53.0)	14.0 (4.0–117.2)	<0.0001
Fetuin-A (μg/ml)	258 (126–478)	.	.

Data represent unadjusted medians (range). Differences between baseline and follow-up were tested using the matched-pairs *t* test. MRT, magnetic resonance tomography; MRS, magnetic resonance spectroscopy; HPA, habitual physical activity. *Available in 200 subjects at baseline and at follow-up. §At baseline available in 172 subjects, and at follow-up available in 47 subjects.

and circulating SHBG, we then tested whether an increase in circulating SHBG was specifically found in subjects in whom a large decrease in liver fat was observed. Indeed, there was a strong correlation between change in liver fat and change in circulating SHBG ($r = -0.37$, $P < 0.0001$). Negative correlations, albeit weaker in magnitude, were also found between changes in circulating SHBG with total body ($r = -0.28$, $P = 0.0018$) and visceral fat ($r = -0.23$, $P = 0.011$). In a multivariate analysis, change in liver fat correlated with change in circulating SHBG, independently of sex, age, and changes in total body and visceral fat ($r = -0.29$, $P = 0.0023$). In this model, changes in total body and visceral fat did not correlate with changes in circulating SHBG anymore (both $P \geq 0.24$).

Relationship between circulating SHBG and metabolic characteristics in 225 subjects. We next investigated whether circulating SHBG correlated with metabolic traits in 225 subjects. SHBG levels were negatively associated with fasting glycemia ($r = -0.17$, $P = 0.009$) but not with fasting insulinemia ($r = -0.07$, $P = 0.27$), after adjustment for sex, age, and total body fat. Furthermore, no correlations with adjusted 2-h glycemia ($r = -0.04$, $P = 0.55$) or insulinemia ($r = -0.10$, $P = 0.12$) during the OGTT were found. Before testing whether additional adjustment for liver fat modulated the relationships of fasting glycemia with SHBG levels and to avoid overadjustment, we first searched for a parsimonious model. In a stepwise regression model including the parameters sex, total body fat, age, SHBG levels, and additionally liver fat, the latter three were determinants of fasting glycemia. In a multivariate model including these three parameters, the relationship between circulating SHBG and fasting glycemia was significant ($r = -0.14$, $P = 0.04$).

SHBG levels did not correlate with measurements of insulin secretion from the OGTT ($r = 0.09$, $P = 0.16$) or the ivGTT ($r = 0.07$, $P = 0.35$), adjusted for sex, age, total body fat, and insulin sensitivity estimated from the OGTT. In contrast, positive correlations between circulating SHBG with insulin sensitivity estimated from the OGTT ($r = 0.14$, $P = 0.037$) and measured by the clamp ($r = 0.15$, $P = 0.057$), adjusted for sex, age, and total body fat, were found. Prior to the inclusion of liver fat in these models, again stepwise linear regression analyses were performed. In these models, circulating SHBG was not a determinant of insulin sensitivity (OGTT, $P = 0.88$; clamp, $P = 0.63$).

We then investigated whether circulating SHBG impacts on insulin sensitivity independently of adiponectin and fetuin-A levels, parameters that were found to determine insulin sensitivity and incident diabetes (40–44). In multivariate regression models, we first found that both adiponectin and fetuin-A were independent determinants of

TABLE 2
Correlations between circulating SHBG and fat compartments at baseline adjusted for sex and age in the subgroup of 118 subjects

Visceral fat		Liver fat		Circulating SHBG		
r	*P*	*r*	*P*	*r*	*P*	
0.72	<0.0001	0.35	0.0002	−0.22	0.016	Total body fat
		0.54	<0.0001	−0.28	0.0022	Visceral fat
				−0.40	<0.0001	Liver fat

Total body and visceral fat were measured by MR tomography, and liver fat was measured by ^{1}HMR spectroscopy.

TABLE 3
Determinants of insulin sensitivity in multivariate linear regression models

Covariates	Insulin sensitivity$_{OGTT}$ Estimate ± SE	*P*	Insulin sensitivity$_{Clamp}$ Estimate ± SE	*P*
Model 1				
Female sex	0.16 ± 0.04	<0.0001	0.17 ± 0.04	<0.0001
Age	−0.004 ± 0.130	0.98	−0.06 ± 0.13	0.66
Total body fat	−0.58 ± 0.09	<0.0001	−0.60 ± 0.09	<0.0001
Model 2				
Female sex	0.11 ± 0.04	0.01	0.08 ± 0.04	0.06
Age	−0.29 ± 0.14	0.04	−0.27 ± 0.14	0.06
Total body fat	−0.47 ± 0.09	<0.0001	−0.50 ± 0.09	<0.0001
Adiponectin levels	0.25 ± 0.09	0.007	0.38 ± 0.09	<0.0001
Fetuin-A levels	−0.76 ± 0.20	0.0002	−0.47 ± 0.21	0.027
Model 3				
Female sex	0.06 ± 0.04	0.19	0.03 ± 0.05	0.46
Age	−0.30 ± 0.14	0.03	−0.26 ± 0.14	0.07
Total body fat	−0.42 ± 0.09	<0.0001	−0.47 ± 0.09	<0.0001
Adiponectin levels	0.22 ± 0.09	0.01	0.37 ± 0.09	<0.0001
Fetuin-A levels	−0.83 ± 0.20	<0.0001	−0.50 ± 0.21	0.018
SHBG levels	0.16 ± 0.06	0.0096	0.14 ± 0.06	0.029

insulin sensitivity. Interestingly, circulating SHBG also turned out to be an independent determinant of insulin sensitivity, both estimated from the OGTT and measured by the clamp (Table 3).

Relationship between the rs1799941 SNP in *SHBG* with metabolic characteristics. In our population, the SNP rs1799941 in *SHBG* was in Hardy-Weinberg equilibrium (χ^2 test; $P \geq 0.97$) and the allele frequency of the minor A allele was 0.29. In agreement with Perry et al. (3), we found that carriers of the minor A allele had higher circulating SHBG, independently of sex, age, and total body fat ($P = 0.025$). This relationship became stronger after additional adjustment for the important determinant of circulating SHBG, liver fat ($P = 0.007$). Also in agreement with Perry et al. (3), we found no significant relationships of the SNP with fasting glycemia or insulinemia, insulin sensitivity, estimated from the clamp and the OGTT, or measurements of insulin secretion (all $P > 0.18$).

DISCUSSION

Two recently published studies using the Mendelian randomization approach provided strong evidence that circulating SHBG is directly involved in the pathogenesis of type 2 diabetes, both in men and in women (2,3). This may now help to more deeply address the complex, but doubtlessly important, role of sex hormones in the natural history of the disease. However, prior to this, it is critical to understand which mechanisms involved in the pathogenesis of type 2 diabetes regulate circulating SHBG. Certainly genetic variability in *SHBG* is a candidate. Although the studies addressing this issue consistently found the SNPs in *SHBG* to be associated with SHBG levels in humans, the variants explained merely about 2% of the variance in circulating levels of the protein (2,37). Therefore, other mechanisms appear to be more important in the regulation of circulating SHBG.

Selva et al. convincingly showed that glucose- and fructose-induced hepatic lipogenesis, via decrease in cellular hepatocyte nuclear factor-4α, inhibits hepatic SHBG expression in vitro and in mice (20). On the basis of the fact that hepatic lipogenesis is strongly associated with fatty liver in humans (19), we could recently show that elevated liver fat content strongly correlated with circulating SHBG in humans (30). In the present study, we could replicate these findings in an independent group of subjects. In univariate cross-sectional analyses, both, total body and visceral adiposity correlated negatively with circulating SHBG. However, when liver fat was included in these analyses, the relationships became nonsignificant. Using data from our longitudinal lifestyle intervention study, we were then also able to investigate which parameters were associated with a change in circulating SHBG. In agreement with the cross-sectional data, we found that subjects having the largest decrease in total body fat, visceral fat, or liver fat also had the largest increase in SHBG levels during the intervention. Furthermore, in multivariate analyses, also in the longitudinal data, changes in total body or visceral fat were no more associated with a change in circulating SHBG, but a change in liver fat still correlated negatively with a change in SHBG levels. These findings suggest that any correlation observed between measures of total body and visceral adiposity with SHBG levels is most probably driven by liver fat.

What is the novel impact of these findings on future research or interventions? From a pathophysiological aspect, these data support that interventions specifically inhibiting or reducing hepatic lipogenesis may represent the most promising tools to increase SHBG levels in humans. So far, recommendation of diets with a low glycemic index or a low carbohydrate content may be the most efficient and safe intervention. Although it is still not solved whether such diets are superior to other nutritional approaches to reduce body weight (45,46), there is clear evidence that they are very efficient in improving dyslipidemia and parameters of glucose metabolism, even when the effect on body weight was small (45,47). Now it would be very important to study whether a low glycemic index diet is particularly effective in decreasing liver fat and increasing circulating SHBG in humans.

Besides carefully investigating parameters increasing SHBG levels, our aim of the present study was to investigate by which mechanisms SHBG levels impact on type 2 diabetes. First, we were very surprised at learning from

the study by Perry et al. (3) that SNPs, which were clearly associated with both circulating SHBG and the prevalence of type 2 diabetes, were not associated with any measures of type 2 diabetes related traits in population-based studies. It may well be that different assays used to measure insulin and glucose between the studies may have made the data difficult to interpret. Furthermore, it is possible that the effect of the SNPs on circulating SHBG is small. This is supported by the little variance in SHBG levels that can be explained by the SNP rs1799941, which showed the strongest association with SHBG levels (3,37). Consequently, also in our study, this SNP was not associated with insulinemia, glycemia, or measures of insulin sensitivity or insulin secretion.

However, we found a significant negative correlation of circulating SHBG with fasting glycemia. Interestingly, no correlation with fasting insulinemia was observed. These findings argue against the hypothesis that hyperinsulinemia is responsible for the decline in SHBG levels in humans and support that glucose may be involved in the regulation of SHBG production. Alternatively, it is also possible that SHBG may impact on hepatic gluconeogenesis, the most important determinant of fasting glycemia (48) in humans. This hypothesis is supported by the fact that the correlation between circulating SHBG and fasting glycemia remained statistically significant, even after adjustment for fasting insulinemia ($P = 0.002$) or liver fat.

Regarding whole-body insulin sensitivity, we provide novel data that circulating SHBG was a determinant of this parameter, which was estimated from the OGTT and measured by the clamp, independently of established humoral factors, the adipokine adiponectin (40,43,49) or the hepatokine fetuin-A (41,50), that are associated with liver fat and regulate whole-body insulin sensitivity. This finding suggests that SHBG may be involved in the regulation of whole-body insulin sensitivity via mechanisms that do not necessarily involve adiponectin or fetuin-A signaling. Interestingly, circulating SHBG did not correlate with measurements of whole-body insulin sensitivity after further adjustment for liver fat. This is in contrast to the results regarding fasting glycemia. A conservative interpretation of these data would result in the conclusion that circulating SHBG may impact on hepatic glucose production but not on insulin-mediated glucose disposal. In the future studies in humans, applying tracer methods may help to precisely address this question.

Furthermore, no correlation of circulating SHBG with insulin secretion was found. For these analyses, we used two measures of insulin secretion, the insulinogenic index obtained from the OGTT, representing an estimate of glucose- and incretin-induced insulin secretion, and the ivGTT, a powerful tool to measure glucose-induced insulin secretion. These data argue against a major role of circulating SHBG in the pathogenesis of β-cell dysfunction in humans.

Altogether, when summarizing the data in the literature in the field of SHBG research and our present data, the following hypothetical picture emerges regarding causes and metabolic consequences of circulating SHBG in humans (figure 1): First, also in our replication cohort, SHBG levels strongly correlated with liver fat in cross-sectional and in longitudinal analyses, supporting that conditions regulating fat accumulation in the liver affect circulating SHBG. Second, because the SNP rs1799941 of *SHBG* is associated with SHBG levels independently of liver fat, genetic variability in *SHBG* directly appears to regulate the

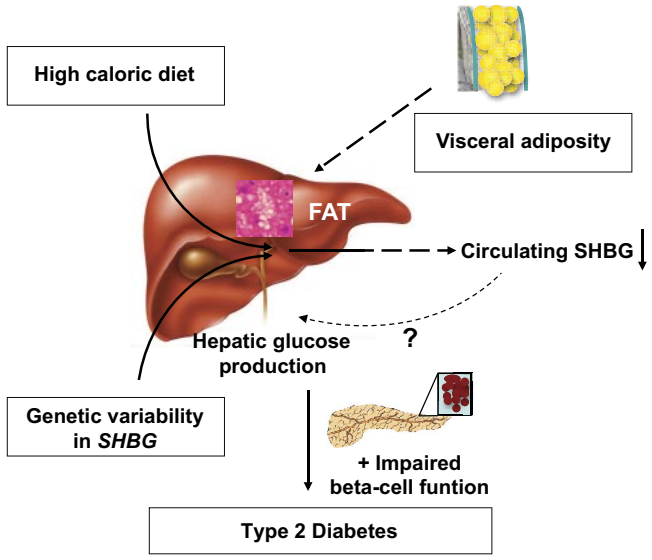

FIG. 1. Hypothetical picture regarding cause and metabolic consequences of circulating SHBG in humans. Conditions regulating fat accumulation in the liver and the SNP rs1799941of *SHBG* are candidates affecting circulating SHBG. In contrast, visceral adiposity does not appear to directly affect circulating SHBG. The relationships of SHBG levels with whole-body insulin sensitivity are most probably explained by liver fat. The significant relationship of SHBG levels with fasting glycemia, which was independent of liver fat, supports that SHBG may have direct effects on hepatic glucose production. Increased hepatic glucose output in concert with a β-cell defect might explain the association of circulating SHBG and *SHBG* genetic variants with type 2 diabetes. (A high-quality color representation of this figure is available in the online issue.)

circulating levels of the protein. Third, the relationships of SHBG levels with whole-body insulin sensitivity are most probably explained by liver fat. Fourth, a significant relationship of SHBG levels with fasting glycemia, which was independent of liver fat, was found. Because fasting glycemia is most strongly affected by hepatic glycogenolysis and/or gluconeogenesis (48), our findings suggest that SHBG may have direct effects on hepatic glucose production. Increased hepatic glucose output in concert with a β-cell defect might explain the association of circulating SHBG and *SHBG* genetic variants with type 2 diabetes.

In conclusion, using cross-sectional and longitudinal data in an independent group of subjects, we can now support our previous findings that high liver fat most strongly associates with low circulating SHBG. Possible mechanisms by which high circulating SHBG prevents the development of type 2 diabetes involve regulation of fasting glycemia but not alteration of insulin secretory function.

ACKNOWLEDGMENTS

The study was supported by grants from the Deutsche Forschungsgemeinschaft (KFO 114 and a Heisenberg Grant to N.S., STE 1096/1-1) and in part by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.). No potential conflicts of interest relevant to this article were reported.

A.P., K.K., and N.S. researched data and wrote the manuscript. J.M., F.S., H.S., F.M., and A.F. researched data and edited the manuscript. E.S. and H.U.H. edited the manuscript and contributed to the discussion.

We thank all the participants for their cooperation and Mareike Walenta, Roman Werner, Alke Guirguis, and the

phenotyping team, all from the Department of Internal Medicine, Division of Endocrinology, Diabetology, Vascular Medicine, Nephrology and Clinical Chemistry, University of Tübingen, Germany, for their help to collect the data.

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