Intensive insulin therapy increases sex hormone-binding globulin in newly diagnosed type 2 diabetic patients

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Abstract

Objective: Many studies have shown that low sex hormone-binding globulin (SHBG) is associated with insulin resistance, but only few studies have examined how serum SHBG is regulated by insulin in humans. This interventional study aimed to investigate the effect of insulin therapy (IT) on serum SHBG levels in newly diagnosed type 2 diabetic patients.

Methods: A total of 80 newly diagnosed type 2 diabetic subjects were enrolled and randomly grouped into a 2-week intensive IT with/without metformin. Serum SHBG, total testosterone, glucose, liver enzymes, lipids, insulin, and C-peptide levels were measured before and after IT.

Results: Before IT, serum SHBG levels were negatively correlated with BMI, waist circumference (WC), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ -GT), triglyceride (TG), fasting insulin, and C-peptide, and homeostatic model assessment of insulin resistance (HOMA-IR), and positively with HDL-C (all P for trend <0.05), after adjustment for age and sex. IT increased serum SHBG levels from 26.5 ± 14.5 to 33.2 ± 15.0 nmol/l ($P < 0.001$), increased by 25.2% (95% CI, 20.3 to 30.9%, $P < 0.001$). In a multiple linear regression model adjusting for age, sex, BMI, and WC, the decreases in Δ ALT (standardized regression coefficient $\beta = -0.374$, $P = 0.012$) and Δ TG ($\beta = -0.380$, $P = 0.020$) were independent contributors to the increase in Δ SHBG.

Conclusions: IT increases serum SHBG likely through improving insulin resistance and liver function.

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Introduction

Sex hormone-binding globulin (SHBG) is a glycosylated homodimeric plasma transport protein and is predominantly produced in the liver (1). Traditionally, SHBG is thought to bind and transport sex steroids and thereby to regulate their biological actions and signaling to target tissues (2). Recently, many epidemiological studies have shown that low serum SHBG levels are associated with insulin resistance and hyperinsulinemia (3, 4), and

therefore, SHBG has emerged as a new risk factor and predictor for the incidence of type 2 diabetes (T2D) (5, 6). However, to make SHBG a clinically meaningful risk marker, its regulation in physiological and pathological conditions has to be better understood.

Interestingly, serum SHBG levels are increased by regimens which improve insulin sensitivity. For example, weight loss through calorie restriction has been reported

to increase serum SHBG as well as insulin sensitivity (7, 8). Metformin treatment for patients with insulin resistance often results in an increase in serum SHBG (9, 10). As the improvement of insulin resistance usually concurs with the decrease in pancreas insulin production, insulin has been widely considered to be repressive to hepatic production of SHBG. This concept is supported by an *in vitro* experiment in which human HepG2 hepatoblastoma cells produce less SHBG when treated with insulin (11, 12). However, a few of the subsequent studies have found no direct association between SHBG and insulin or insulin resistance (13, 14, 15). A recent study by Selva *et al.* (16) reports that SHBG is not regulated by insulin but rather repressed by monosaccharide-induced lipogenesis in human HepG2 cells. Thus, the question of whether serum SHBG in human subjects with insulin resistance or T2D is modulated by the insulin sensitivity, insulin *per se* or other factors remains unanswered in prior clinical studies (4, 14, 16). Practically, it is indeed difficult to address the question by conventional therapeutic approach as circulating insulin level and insulin sensitivity are inter-related and usually change in the same direction before overt diabetes develops.

We and others have demonstrated that short-term insulin intensive therapy (IT) is effective to reduce hyperglycemic-induced insulin resistance (17, 18). As insulin resistance would be reduced whereas circulating insulin level is not decreased or even increased during IT, we hypothesized that IT would be an optimal regimen to determine whether it is the reduction of insulin resistance or circulating insulin *per se* that is responsible for serum SHBG elevation. We tested the hypothesis by investigating the effect of short-term IT on serum SHBG levels and metabolic parameters in newly diagnosed type 2 diabetic patients.

Subjects and methods

Subjects and research design

This study was an observational follow-up and expansion of the previously published intensive IT (17) conducted at the Nanjing Drum Tower Hospital between September 2009 and August 2012. Based on World Health Organization 1999 diagnostic criteria, 80 (67 men and 13 postmenopausal women) newly diagnosed type 2 diabetic subjects with hyperglycemia (HbA1c $\geq 8\%$) participated in the study. All patients were non-ketonurine and negative for islet cell antibodies, and had not received antihyperglycemic therapy. We excluded patients who

had autoimmune or viral hepatitis, cholestatic or metabolic/genetic liver disease. And patients with acute or chronic diabetes complications, pregnancy, hypo/hyperthyroidism, renal dysfunction, acute or chronic infections, or history of taking corticosteroids were also excluded. Neither lipid-lowering nor hepatoprotective drugs had been used in all the participants. All women participants were postmenopausal and were not receiving hormone replacement therapy.

Eighty subjects were randomly grouped and treated with continuous s.c. insulin infusion (CSII) alone (insulin group) or with metformin (insulin plus metformin group). The insulin and insulin plus metformin groups received NovoRapid (Novo Nordisk, Bagsvaerd, Denmark) with an insulin pump. Initial insulin dose was 0.4–0.5 IU/kg per day. Total daily insulin dose was adjusted every day depending on the values from the seven-point glucose profile obtained the day before and was divided into 50% of basal and 50% of bolus injection. Insulin plus metformin group received metformin (Glucophage, Bristol-Myers Squibb, Shanghai, China) in addition to insulin. Metformin was given as a fixed dose of 0.5 g twice a day and during the course of treatment. The glycemic control target was set to fasting blood glucose of <7.0 mmol/l and 2 h post-meal glucose of <8.0 mmol/l. Insulin with/without metformin treatment were discontinued 2 weeks later after the target goal was achieved.

During the course of treatment, patients were recommended to eat a low-caloric, low-fat, high-fiber diet and encouraged a moderate exercise of 30 min walking after meals. All studies were done at Nanjing Drum Tower Hospital. All subjects were given written informed consent and agreed to participate in the study and none of the participants dropped out of the study.

Anthropometric measures

Height and weight were measured with subjects wearing light clothing without shoes using a stadiometer and a calibrated scale. Standing waist circumference (WC) was measured at the level of the umbilicus with a flexible tape.

Biochemical measurements

Blood draw was taken in the morning after a 10-h overnight fast before and after IT and was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (γ -GT), total cholesterol and triglycerides (TG), HDL-C, and LDL-C, fasting plasma glucose (FPG), fasting serum insulin and C-peptide

(F-INS and F-CP), HbA1c, serum total testosterone (TT), and serum SHBG levels. The above analyses except HbA1c were repeated after 2 weeks of IT. FPG was measured by glucose oxidase technique method. Serum insulin and C-peptide were measured by electrochemiluminescence (Roche Diagnostics). Serum TT and SHBG were also measured by immunoluminometric assay and chemiluminescence (Siemens Healthcare Diagnostics Products Limited, Bad Nauheim, Germany). HbA1c was measured by HPLC.

Diagnosis of nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) was diagnosed by liver ultrasound, excluding other causes of chronic liver disease and excessive alcohol consumption. Ultrasound scanning for all the patients was conducted by experienced sonologists (19).

Calculations

BMI was calculated by dividing weight (in kilograms) by height squared (in meters). The degree of insulin resistance was determined by the homeostatic model assessment (HOMA) using the formula: $\text{HOMA-IR} = \text{FPG (mmol/l)} \times \text{fasting insulin (mU/l)} / 22.5$. The mean blood glucose levels (MBG) were obtained according to the seven-point glucose profile. $\text{Change (\%)} = (\Delta \text{ value} / \text{before therapy}) \times 100\%$ and the Δ value was the difference of post-therapy variables subtracting pre-therapy ones.

Statistical analysis

Statistical analyses were performed with SPSS 17.0 software. All data were expressed as mean $\pm$ s.d. or mean $\pm$ s.e.m. All analyses, where applicable, were performed with adjustment for age and sex. ALT, AST, TG, γ -GT, F-INS, and F-CP were skewed and log-transformed before analysis. We used the general linear model to analyze the means of SHBG with increased trisections of BMI, WC, FPG, HbA1c, ALT, AST, γ -GT, TG, TC, HDL-C, LDL-C, and HOMA-IR. Nonparametric paired Wilcoxon test is used for comparison before and after IT. Nonparametric Mann-Whitney *U* test is used for comparison between insulin group and insulin plus metformin group. Spearman correlation analysis was conducted to examine the relationship between changes in SHBG and other metabolic variables and multiple linear regression analysis to identify independent metabolic determinants in relation to changes in serum SHBG. $P < 0.05$ was considered statistically significant.

Results

Relationship between serum SHBG and metabolic parameters

Characteristics of the 80 newly diagnosed type 2 diabetic subjects (63 men and 17 women) are presented in Table 1. The average age, BMI, and WC of all the subjects were 52.7 ± 11.8 (mean $\pm$ s.d.) years of age (range, 28.0–76.0 years), 25.9 ± 3.1 kg/m² (range, 20.0–35.5 kg/m²), and 92.9 ± 9.7 cm (range, 71.0–119.0 cm) respectively.

To examine the possible correlation of serum SHBG with metabolic parameters, we divided BMI, WC, FPG, HbA1c, F-INS, F-CP, HOMA-IR, ALT, AST, γ -GT, TG, TC, HDL-C, and LDL-C into tertiles (Fig. 1). After adjustment for age and sex, serum SHBG levels decreased with the increase in BMI, WC, F-INS, F-CP, HOMA-IR, ALT, γ -GT, and TG, and increased with the rise in HDL-C (all *P* for trend < 0.05). However, serum SHBG levels showed no trends with the increase in HbA1c or FPG (both *P* for trend > 0.05) or with the increase in TC and LDL-C (data not shown).

Partial correlation analyses were conducted to further examine the association of serum SHBG with anthropometric and metabolic variables. As shown in Table 2, after adjustment for age and sex, serum SHBG levels were inversely correlated with BMI ($r = -0.355$, $P = 0.001$), WC ($r = -0.385$, $P = 0.001$), ALT ($r = -0.231$, $P = 0.042$), γ -GT ($r = -0.417$, $P < 0.001$), TG ($r = -0.501$,

Table 1 Baseline clinical characteristics of 80 newly diagnosed type 2 diabetic subjects. Data are presented as mean $\pm$ s.d.

Characteristic	Range	Mean $\pm$ s.d.
Age (years)	28.0–76.0	52.7 $\pm$ 11.8
BMI (kg/m ²)	20.0–35.5	25.9 $\pm$ 3.1
Waist circumference (cm)	71.0–119.0	92.9 $\pm$ 9.7
Alanine aminotransferase (U/l)	5.8–121.8	33.8 $\pm$ 23.4
Aspartate aminotransferase (U/l)	9.0–86.0	22.3 $\pm$ 11.7
γ -glutamyl transpeptidase (U/l)	7.6–232.3	49.2 $\pm$ 49.0
Triglycerides (mmol/l)	0.5–13.5	2.6 $\pm$ 2.1
Total cholesterol (mmol/l)	2.3–11.7	4.9 $\pm$ 1.6
HDL-C (mmol/l)	0.5–3.7	1.0 $\pm$ 0.3
LDL-C (mmol/l)	0.7–6.5	2.5 $\pm$ 1.1
Fasting plasma glucose (mmol/l)	6.2–20.1	10.7 $\pm$ 3.3
HbA1c (%) (mmol/mol)	8.0–16.4 (63.9–155.7)	10.7 $\pm$ 1.8 (93.4)
Fasting insulin (mU/l)	5.1–46.0	10.1 $\pm$ 9.1
Fasting C-peptide (pmol/l)	337.9–2363.0	798.0 $\pm$ 450.3
HOMA-IR	1.7–12.6	4.6 $\pm$ 2.1
SHBG (nmol/l)	6.2–61.2	26.5 $\pm$ 14.5
Female (<i>n</i> = 13)	11.2–61.2	27.5 $\pm$ 17.7
Male (<i>n</i> = 67)	6.2–59.2	26.3 $\pm$ 14.0

Figure 1

Correlation of SHBG levels with BMI, WC, FPG, HbA1c, F-INS, F-CP, HOMA-IR, ALT, AST, γ-GT, TG, and HDL-C (divided into tertiles). Black circles, mean level of SHBG. Data are mean ± S.E.M. Trend *P* analyzed after adjustment for age and sex.

$P < 0.001$), F-INS ($r = -0.391$, $P = 0.001$), F-CP ($r = -0.556$, $P < 0.001$), and HOMA-IR ($r = -0.451$, $P < 0.001$). Serum SHBG levels were positively associated with HDL-C ($r = 0.313$, $P = 0.005$). But no associations were found between serum SHBG and AST, TC, LDL-C, FPG, or HbA1c (all $P > 0.05$).

Intensive IT increases serum SHBG levels

To determine the effect of IT on serum SHBG levels, we conducted the short-term therapy of intensive insulin administration with or without metformin in newly diagnosed type 2 diabetic subjects.

Forty subjects of the insulin group (33 men and seven women) were 51.8 ± 12.0 (mean ± S.D.) years of age, with BMI of 25.8 ± 3.6 kg/m², FPG of 10.6 ± 3.3 mmol/l, and

HbA1c of $10.8 \pm 1.8\%$ (94.5 mmol/mol). Another 40 subjects of the insulin plus metformin group (34 men and six women) were 53.6 ± 11.8 years of age, with BMI of 26.0 ± 2.8 kg/m², FPG of 10.7 ± 3.4 mmol/l, and HbA1c of $10.7 \pm 1.8\%$ (93.4 mmol/mol). The difference in age, BMI, FPG, and HbA1c was not statistically significant between the two groups ($P > 0.05$). There are 26 NAFLD patients diagnosed by liver ultrasonography in the insulin group and 27 NAFLD patients in the insulin plus metformin group ($P > 0.05$).

There was no significant difference in the mean daily insulin dose between the insulin group (40.3 ± 12.7 U/day) and the insulin plus metformin group (38.2 ± 10.3 U/day) ($P > 0.05$) required to achieve the targeted fasting glucose level of 7.1 ± 1.8 mmol/l and MBG of 8.5 ± 1.8 mmol/l.

Table 2 Relationship between SHBG and metabolic variables.

	Total (n=80)	
	r	P value
BMI	−0.355	0.001
Waist circumference	−0.385	0.001
Alanine aminotransferase ^a	−0.231	0.042
Aspartate aminotransferase ^a	−0.120	0.296
γ-glutamyl transpeptidase ^a	−0.417	<0.001
Triglycerides ^a	−0.501	<0.001
Total cholesterol	0.019	0.869
HDL-C	0.313	0.005
LDL-C	−0.060	0.609
Fasting plasma glucose	−0.043	0.709
HbA1c	0.189	0.097
Fasting insulin ^a	−0.391	0.001
Fasting C-peptide ^a	−0.556	<0.001
HOMA-IR	−0.451	<0.001

^aLog-transformed for analysis. Partial correlation analyses were performed after adjustment for age and sex.

As shown in Table 3, lipids profiles, liver enzymes, MBG, HOMA-IR, F-INS, and F-CP levels were similar at baseline between the two groups ($P>0.05$) and short-term intensive hypoglycemia therapy did not change BMI in both groups. Both groups showed significant decrease in fasting and mean glucose levels, lipids profiles (TC, TG, LDL-C), and liver enzymes (ALT, AST, γ-GT) compared with those before therapy (all $P<0.05$). Meanwhile, HOMA-IR was significantly lowered (both $P<0.001$), despite that the elevation in F-INS and F-CP were not statistically significant in the two groups ($P>0.05$).

Remarkably, as shown in Fig. 2, serum SHBG levels were significantly increased in both groups. In the insulin group, serum SHBG levels were elevated from 27.4 ± 15.5 to 34.0 ± 14.9 nmol/l ($P<0.001$), an increase by 24.1% (95% CI, 16.8 to 31.6%, $P<0.001$) after therapy. In the insulin plus metformin group, serum SHBG levels were elevated from 25.6 ± 13.6 to 32.5 ± 15.2 nmol/l ($P<0.001$), an increase of 26.9% (95% CI, 18.4 to 35.1%, $P<0.001$). No statistical difference was noted in the SHBG increase between the two groups ($P>0.05$) and between sexes ($P>0.05$). Of the total 80 subjects, serum SHBG levels were elevated in 65 (81.2%), not changed in nine (11.3%), and slightly decreased in six (7.5%) individuals. Overall, intensive hypoglycemia therapy raised serum SHBG levels from 26.5 ± 14.5 to 33.2 ± 15.0 nmol/l ($P<0.001$), an increase of 25.2% (95% CI, 20.3 to 30.9%, $P<0.001$).

Likewise, mean levels of serum TT in both groups were also significantly increased ($P<0.01$, Table 3).

Decrease in ALT and TG accounts for the majority increase in SHBG

We next examined possible association of changes in SHBG (Δ -value) with those of metabolic parameters after intensive IT. Δ SHBG was significantly correlated with Δ TT ($r=0.341$, $P=0.018$), Δ ALT ($r=-0.423$, $P=0.001$), Δ TG ($r=-0.396$, $P=0.001$), and Δ F-CP ($r=0.269$, $P=0.023$), but did not correlate with Δ FPG, Δ MBG, Δ AST, Δ γ-GT, Δ TC, Δ HDL-C, Δ LDL-C, Δ F-INS, and Δ HOMA-IR (all $P>0.05$).

To determine the independent effect of metabolic variables on SHBG, we performed a multiple linear regression analysis with Δ SHBG as the dependent variable, and sex, age, BMI, WC, Δ ALT, Δ TG, Δ TT, and Δ F-CP as the independent variables. As a result, we found that Δ ALT (standardized regression coefficient $\beta=-0.374$, $P=0.012$) and Δ TG ($\beta=-0.380$, $P=0.020$) were independent contributors to the variation in Δ SHBG levels, accounting for 40.6% of the SHBG increase ($R^2=0.406$, $P=0.004$) in combination by IT.

Discussion

This is the first observational clinical study that investigated the effect of IT on serum SHBG levels in newly diagnosed type 2 diabetic patients, to our knowledge.

Epidemiologic and genetic studies have indicated for an expanded role of SHBG in the pathogenesis of insulin resistance, metabolic syndrome, and T2D, rather than a simple transporter of sex hormones in serum (3, 20, 21). Low levels of serum SHBG are frequently observed in states of insulin resistance-related conditions and have emerged as a predictor for the incidence of T2D and metabolic syndrome (6, 22, 23).

In accordance with previous reports (24, 25), our study observed negative associations of serum SHBG with BMI, WC, TG, F-INS, and F-CP, and HOMA-IR in patients with T2D. However, a few studies have reported that serum SHBG concentrations are also inversely correlated with HbA1c and FPG in both men and women without diabetes (13, 15, 26), suggesting a relationship between SHBG and glucose homeostasis before the development of diabetes. However, we did not find such a relationship in patients with T2D in this study, which is consistent with the finding of no correlation between blood glucose and HbA1c with serum SHBG in type 1 diabetes (T1D) patients (27). One possible explanation is that in nondiabetic subjects, HbA1c and FPG may be proportionate to the

Table 3 Changes in clinical characteristics before and after insulin intensive therapy. Data are expressed as mean $\pm$ s.d. Comparison before and after therapy is performed by non-parametric paired Wilcoxon test with *P* value detailed in the table. Comparison between the insulin and insulin plus metformin groups is performed by nonparametric Mann–Whitney *U* test.

	Total (n = 80)			Insulin group (n = 40)			Insulin + metformin group (n = 40)					
	Before IT	After IT	Change (%)	P value	Before IT	After IT	Change (%)	P value	Before IT	After IT	Change (%)	P value
Male/female	67/13				33/7				34/6			
Age (years)	52.7 ± 11.8				51.8 ± 12.0				53.6 ± 11.8			
NAFLD (%)	53 (66.2)				26 (65.0)				27 (67.5)			
HbA1c (%)	10.7 ± 1.8 (93.4)				10.8 ± 1.8 (94.5)				10.7 ± 1.8 (93.4)			
BMI (kg/m ²)	25.9 ± 3.1	25.7 ± 3.1	−0.8	0.855	25.8 ± 3.6	25.5 ± 3.3	−1.1	0.767	26.0 ± 2.8	25.5 ± 2.9	−1.9	0.194
SHBG (nmol/l)	26.5 ± 14.5	33.2 ± 15.0	25.2	<0.001	27.4 ± 15.5	34.0 ± 14.9	24.1	<0.001	25.6 ± 13.6	32.5 ± 15.2	26.9	<0.001
TT (nmol/l)	10.4 ± 4.5	13.7 ± 5.9	31.7	<0.001	11.0 ± 4.9	15.3 ± 7.0	39.1	<0.001	9.8 ± 4.0	12.0 ± 4.1	22.4	0.001
FPG (mmol/l)	10.7 ± 3.3	7.1 ± 1.8	−33.6	<0.001	10.6 ± 3.3	7.0 ± 1.6	−33.9	<0.001	10.7 ± 3.4	7.2 ± 1.9	−32.7	<0.001
MBG (mmol/l)	13.6 ± 3.1	8.5 ± 1.8	−37.5	<0.001	13.4 ± 3.5	8.2 ± 1.7	−38.8	<0.001	13.7 ± 2.8	8.8 ± 1.8	−35.7	<0.001
F-INS (mU/l)	10.1 ± 9.1	10.9 ± 12.7	7.9	0.319	8.6 ± 7.0	10.1 ± 15.1	17.4	0.521	11.6 ± 10.7	11.8 ± 9.8	1.7	0.492
F-CP (pmol/l)	798.0 ± 450.3	830.5 ± 319.9	4.1	0.107	786.8 ± 407.4	824.5 ± 313.5	4.8	0.242	808.2 ± 491.7	836.1 ± 329.9	3.4	0.323
HOMA-IR	4.6 ± 2.1	3.6 ± 0.9	−21.7	<0.001	4.5 ± 1.5	3.4 ± 0.8	−24.4	<0.001	4.7 ± 2.5	3.7 ± 1.1	−21.3	0.001
ALT (U/l)	33.8 ± 23.4	28.3 ± 17.5	−16.3	0.010	36.5 ± 27.5	29.0 ± 18.9	−20.5	0.045	30.9 ± 18.1	26.5 ± 12.6	−14.2	0.025
AST (U/l)	22.3 ± 11.7	18.6 ± 8.2	−16.6	0.005	30.1 ± 21.1	23.3 ± 13.9	−22.6	0.037	21.3 ± 9.3	18.0 ± 8.1	−15.5	0.045
γ-GT (U/l)	49.2 ± 49.0	40.6 ± 46.1	−17.4	<0.001	48.1 ± 48.3	40.1 ± 48.1	−14.5	0.014	50.2 ± 50.5	41.3 ± 44.9	−17.7	<0.001
TG (mmol/l)	2.6 ± 2.1	1.7 ± 1.3	−34.6	<0.001	2.2 ± 1.4	1.5 ± 0.8	−31.8	0.008	3.1 ± 2.6	2.1 ± 1.6	−32.2	<0.001
TC (mmol/l)	4.9 ± 1.6	3.7 ± 0.7	−24.5	<0.001	4.8 ± 1.2	3.9 ± 0.7	−18.7	<0.001	4.9 ± 2.0	3.5 ± 0.8	−22.4	<0.001
HDL-C (mmol/l)	1.0 ± 0.3	0.9 ± 0.2	−4.0	0.169	1.0 ± 0.3	1.0 ± 0.2	−2.9	0.616	1.0 ± 0.2	0.9 ± 0.2	−6.2	0.127
LDL-C (mmol/l)	2.5 ± 1.1	1.7 ± 0.5	−32.0	<0.001	2.5 ± 0.7	1.8 ± 0.5	−28.0	<0.001	2.4 ± 1.4	1.6 ± 0.5	−33.3	0.002

Figure 2

Insulin therapy (IT) increases SHBG levels. Forty diabetes patients received insulin-only treatment and 40 patients received insulin plus metformin treatment. Serum SHBG levels were measured before and after IT. Data are mean $\pm$ S.E.M. *** $P < 0.001$ vs before therapy.

index of insulin resistance whereas the relationship is lost in the state of diabetes.

As SHBG is produced in the liver, we analyzed the relationship of serum SHBG with liver enzymes and have found that lower levels of serum SHBG were associated with higher levels of ALT, AST, and γ -GT, replicating the finding reported by Shin *et al.* (24) in patients with T2D.

Hyperinsulinemia and insulin resistance is associated with low SHBG levels (3, 28), suggesting that insulin level and/or insulin resistance are suppressive to SHBG production. In supporting this notion, Yki-Järvinen *et al.* (29) reported that serum SHBG is increased in T1D patients lacking portal/endogenous insulin, compared with the normal controls. However, a recent *in vitro* study has shown that SHBG is not regulated by insulin but rather repressed by monosaccharide-induced lipogenesis in human HepG2 cells (16), suggesting that fatty liver or liver dysfunction which is usually associated with insulin resistance and diabetes might be a mechanism for the lower SHBG level. In this study, we have demonstrated that IT for patients with T2D significantly reduces glycemia and attenuates insulin resistance, whilst serum SHBG levels are elevated. It is important to note that both insulin and insulin C-peptide levels remained nearly the same or slightly increased after IT (Table 3),

indicating that endogenous insulin production is not reduced and, hence, essentially ruling out the likelihood that the reduction in endogenous insulin production is a cause of increased SHBG level found in this study. Thus, the SHBG increase resulting from IT in T2D patients is likely through a mechanism different from that in T1D patients (29). Furthermore, insulin-only treatment has raised SHBG by 24.1% as effectively as the insulin plus metformin group does by 26.9%, demonstrating that insulin *per se* does not directly suppress SHBG *in vivo* but rather, the improvement of insulin resistance elevates SHBG. Several studies show that metformin treatment for 3–6 months in combination with lifestyle changes attenuates insulin resistance and raises SHBG levels (9, 10). The possible effects of lifestyle change on SHBG is difficult to assess because there is no control group of lifestyle change in the study. However, as the recommended moderate lifestyle change was modest and will take time to effect, its influence on SHBG is likely small, if any. Metformin administration for a short-term with no significant change of BMI after IT and lifestyle modifications seems to exert no additional effect. It is important to note that the changes leading to elevated levels of SHBG are negatively correlated with those of the reduction in ALT and TGs, which, in combination, accounts for ~40% of the serum SHBG increase by the IT, suggesting that improvement of liver function and/or lipid load by IT may be responsible for the elevation of serum SHBG.

The exact mechanism in serum SHBG elevation after intensive insulin hypoglycemic therapy remains to be elucidated. Some *in vitro* and *in vivo* studies have indicated that metabolic disorders reduce production of SHBG via downregulating the expression of HNF4 α in HepG2 cells, and exogenous insulin stimulates the production of SHBG via repressing chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) expression in the liver (16, 30, 31). Thus, we speculate that a collective effect of IT on systemic decrease in glucose and lipid levels, in addition to the improved liver function, may be responsible for the drastic and rapid (in 2 weeks) restoration of serum SHBG.

In conclusion, our data found that intensive insulin hypoglycemic therapy significantly raises serum SHBG, likely through improving insulin resistance and liver function, and that exogenous insulin is not suppressive to SHBG in type 2 diabetic patients.

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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