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Effects of intraperitoneal insulin versus subcutaneous insulin administration on sex hormone-binding globulin concentrations in patients with type 1 diabetes mellitus

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Abstract

Aims: Elevated sex hormone-binding globulin (SHBG) concentrations have been described in patients with type 1 diabetes mellitus (T1DM), probably due to low portal insulin concentrations. We aimed to investigate whether the route of insulin administration, continuous intraperitoneal insulin infusion (CIPII), or subcutaneous (SC), influences SHBG concentrations among T1DM patients.

Methods: *Post hoc* analysis of SHBG in samples derived from a randomized, open-labeled crossover trial was carried out in 20 T1DM patients: 50% males, mean age 43 (±13) years, diabetes duration 23 (±11) years, and hemoglobin A1c (HbA1c) 8.7 (±1.1) (72 (±12) mmol/mol). As secondary outcomes, testosterone, 17-β-estradiol, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were analyzed.

Results: Estimated mean change in SHBG was −10.3 nmol/L (95% CI: −17.4, −3.2) during CIPII and 3.7 nmol/L (95% CI: −12.0, 4.6) during SC insulin treatment. Taking the effect of treatment order into account, the difference in SHBG between therapies was −6.6 nmol/L (95% CI: −17.5, 4.3); −12.7 nmol/L (95% CI: −25.1, −0.4) for males and −1.7 nmol/L (95% CI: −24.6, 21.1) for females, respectively. Among males, SHBG and testosterone concentrations changed significantly during CIPII; −15.8 nmol/L (95% CI: −24.2, −7.5) and −8.3 nmol/L (95% CI: −14.4, −2.2), respectively. The difference between CIPII and SC insulin treatment was also significant for change in FSH 1.2 U/L (95% CI: 0.1, 2.2) among males.

Conclusions: SHBG concentrations decreased significantly during CIPII treatment. Moreover, the difference in change between CIPII and SC insulin therapy was significant for SHBG and FSH among males. These findings support the hypothesis that portal insulin administration influences circulating SHBG and sex steroids.

Introduction

Among type 1 diabetes mellitus (T1DM) patients, subcutaneous (SC) insulin administration is associated with low portal insulin concentrations and a consequent hepatic underinsulinization (1). Hepatic underinsulinization has been suggested to influence several extra-glycemic, metabolic, and endocrinological parameters, such as the sex hormone-binding globulin (SHBG). SHBG is a glycoprotein produced in the liver, which regulates the bioavailability of sex steroids for target tissues and cells in the plasma (2). SHBG tends to be elevated among adult T1DM patients when compared with control subjects, possibly leading to changes in the bioavailability of gonadotropins and sex steroids (1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13).

Previous research has demonstrated that SHBG concentrations are inversely associated with (fasting) insulin concentrations *in vivo* (1, 6, 9, 14, 15, 16). Furthermore, *in vitro*, insulin has an inhibitory effect on the basal and stimulated SHBG production by HepG2 cells of the liver (17). Yki-Järvinen and coworkers previously suggested that portal insulin concentrations, and not insulin sensitivity, determines SHBG concentrations in T1DM patients (1). With continuous intraperitoneal insulin infusion (CIPII), insulin is infused in the intraperitoneal space and absorbed to a large extent in the portal vein catchment area (18, 19, 20). Hence, CIPII will result in higher portal insulin concentrations and lower peripheral plasma insulin concentrations, creating a more physiological situation as compared with SC insulin administration (19, 20, 21, 22).

We hypothesized that treatment with CIPII would result in lower SHBG concentrations as compared with SC insulin treatment. Therefore, the aim of this study was to analyze the effects of the route of insulin administration, CIPII versus SC, on SHBG concentrations in T1DM patients. As alterations in SHBG concentrations may result in changes of gonadotropins and sex steroids, concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and 17-β-estradiol were also assessed.

Subjects, materials and methods

Study design

This study is a *post hoc* analysis of a randomized, open-label crossover trial that was carried out in a single center (Isala, Zwolle, The Netherlands) (23). The aim of this crossover trial was to investigate the influence of CIPII versus SC insulin treatment on glycemic control and hypoglycemic events among T1DM patients. Full design and outcomes have been published previously (23).

Study procedures

The crossover trial was divided into four phases: the qualification phase, the first treatment phase, the crossover phase, and the second treatment phase. The qualification phase had a duration of 3 months. During this period, it was attempted to achieve optimization of the patients' glycemic control on the current SC insulin treatment, for example, multiple daily injections (MDI) or (mostly) continuous subcutaneous insulin infusion (CSII). After the qualification phase, patients were randomized into the first treatment phase to continue with SC insulin administration or start with CIPII using an implantable insulin pump. Both treatment phases were of 6 months duration with a crossover phase of 4 weeks in between, in which patients received SC insulin, to minimize possible carry-over effects of CIPII.

At the start of the CIPII phase, the insulin pump (MIP 2007C; Medtronic/Minimed, Northridge, CA, USA) was implanted under general anesthesia in all subjects. Insulin (U400 semi-synthetic human insulin of porcine origin; Sanofi-Aventis, Frankfurt, Germany) was administered through the implanted pump. For patients who received SC insulin treatment in the second treatment phase, the CIPII pump remained *in situ*, but was filled with an inert fluid at the end of the first treatment phase. During the SC treatment phase, patients used their own mode of SC insulin treatment consisting of rapid-acting insulin analogs (for CSII) combined with a long-acting analog (for MDI).

Study population

Subjects with T1DM with fasting C-peptide concentrations <0.2 nmol/L and intermediate or poor glycemic control, defined as HbA1c ≥7.5% (58 mmol/mol) and/or ≥5 incidents of confirmed hypoglycemia (<4.0 mmol/L) per week, were eligible for participation in the study. A total of 24 patients were included and randomly allocated into one of the two treatment sequences. One patient who was allocated to start with CIPII treatment withdrew informed consent shortly after implantation of the insulin pump. As a result, 23 patients completed the follow-up period (23).

For the current analysis, patients with a known history of polycystic ovary syndrome (PCOS), hirsutism,

Measurements

The following data were recorded at baseline: smoking, alcohol habits, height, weight, any comorbidity, year of diagnosis of diabetes, C-peptide levels, medication, and presence of microvascular and macrovascular complications.

HbA1c was measured at baseline, at the end of the qualification phase, and at the start, halfway, and at the end of both treatment phases using a Primus Ultra 2 with high-performance liquid chromatography (reference value 4.0–6.0% (20–42 mmol/mol)). Measurements of SHBG, testosterone, 17-β-estradiol, LH, and FSH were performed in 1.5 cc serum samples that were collected at baseline and at the start, halfway, and at the end of both treatment phases. All samples were collected at non-fasting moments and stored at −80°C until analysis. Measurements were performed using a Cobas e601 immunoassay analyzer (Roche Diagnostics). The inter-assay coefficients of variation (CV) were <6% for SHBG, <8% for testosterone if >1.6 nmol/L or <20% if <1.6 nmol/L, <10% for 17-β-estradiol, <6% for LH, and <6% for FSH. Measurements performed at the start, halfway, and at the end of both treatment phases were used for analysis.

Primary and secondary outcomes

The primary outcome was the difference in SHBG concentrations between the CIPII and SC treatment phase. Secondary outcomes included the course of SHBG concentrations during both treatment phases. Because of known gender differences and because collection of samples took place irrespective of phase of the menstrual cycle, the results of SHBG and testosterone were presented for males and females separately and the results of 17-β-estradiol, LH, and FSH were only presented for males.

Statistical analysis

To calculate the estimated mean difference, with a 95% confidence interval (CI), between the two therapies, the linear mixed models analysist that takes treatment order into account was used according to the Hills–Armitage principle. This accounts for any period effect. To test

whether variables had a normal distribution, Q-Q plots were used. SHBG, testosterone, 17-β-estradiol, LH, and FSH had a skewed distribution and were presented as median and interquartile ranges. Both observed and estimated outcomes were reported. Comparisons between outcomes during both treatment modalities were made using the Wilcoxon signed-rank test for non-parametric data. Data were presented as total number (% of total group), mean (s.d.), or median with interquartile range [IQR]. A two-sided *P*-value of <0.05 was considered to be significant. All analyses were performed using SPSS version 22 software.

The study was carried out in accordance with the Declaration of Helsinki and the protocol was approved by the Medical Ethics Committee of Isala, Zwolle. Informed consent from all patients was obtained.

Results

Study population

A total number of 23 patients completed the original crossover trial. For the current analysis, three patients were excluded due to the use of oral contraceptive drugs ($n=1$) and hypothyroidism ($n=2$). At baseline, there were no significant differences between patients who started CIPII or SC insulin treatment in the first phase regarding clinical and biochemical characteristics (Table 1).

Primary outcome: SHBG concentrations

The observed concentrations of SHBG at the start, halfway, and end of the CIPII and SC treatment phases are presented in Table 2. The estimated mean change in SHBG concentrations during the CIPII phase was −10.3 nmol/L (95% CI: −17.4, −3.2) and −3.7 nmol/L (95% CI: −12.0, 4.6) during the SC phase. When taking the effect of treatment order into account, the estimated mean difference between the CIPII and SC treatment phases was −6.6 nmol/L (95% CI: −17.5, 4.3). No carry-over effect was observed ($P=0.226$).

Secondary outcome: SHBG, testosterone, 17-β-estradiol, LH, and FSH concentrations

Among males, SHBG decreased significantly −15.8 nmol/L (95% CI: −24.2, −7.5) during CIPII treatment, while there was no significant change during SC treatment (Table 2). When taking the effect of treatment order into account, the estimated mean change between the

Table 1 Baseline characteristics of all patients.

| | All patients | Treatment mode in first phase | |
		CIPII	SC insulin treatment
N	20	10	10
Age (years)	43.1 (12.7)	42.5 (13.0)	43.6 (13.1)
Gender (male)	10 (50)	5 (50)	5 (50)
Weight (kg)	82.5 (16.4)	81.3 (18.7)	83.7 (14.5)
BMI (kg/m²)	26.6 (5.1)	26.2 (5.8)	26.9 (4.6)
Diabetes duration (years)	23.4 (11.0)	20.5 (10.6)	26.4 (11.2)
HbA1c (%)	8.7 (1.1)	8.7 (1.1)	8.7 (1.2)
HbA1c (mmol/mol)	71.5 (12.3)	71.4 (11.7)	71.7 (13.5)
Macrovascular complications	2 (10)	1 (10)	1 (10)
Microvascular complications	9 (45)	5 (50)	4 (40)
Neuropathy	6 (30)	3 (30)	3 (30)
Nephropathy	1 (5)	0 (0.0)	1 (10)
Retinopathy	6 (30)	2 (20)	4 (40)
Total daily insulin dose	50.0 [40.0, 77.5]	50.0 [40.0, 59.3]	48.5 [34.0, 77.5]
SHBG concentrations (nmol/L)	67.0 [42.5, 79.2]	61.7 [46.9, 73.1]	75.5 [38.6, 83.9]

Data are presented as: a number (% of total group), mean (s.d.), or median [IQR]. Numbers may not add up due to rounding. Macrovascular complications: PCI ($n=1$) and angina pectoris ($n=1$).

CIPII treatment phase and the SC treatment phase was −12.7 nmol/L (95% CI: −25.1, −0.4). Among males, only the testosterone concentrations decreased significantly during CIPII treatment with −8.3 nmol/L (95% CI: −14.4, −2.2) and FSH concentrations increased significantly with CIPII treatment when compared with SC insulin therapy with 1.1 U/L (95% CI: 0.1, 2.2) (Table 3). Among females, there were no changes in SHBG and testosterone within and between both treatment modalities.

Discussion

Treatment with CIPII resulted in a significant decrease of SHBG concentrations, while concentrations remained stable during treatment with SC insulin. The difference between both treatment modalities in SHBG concentrations was significant among men. These findings provide support for the hypothesis that enhancing portal insulin levels, through treatment with CIPII, influences circulating SHBG concentrations.

Although the exact mechanism remains unknown, an inhibitory effect of insulin on the synthesis of SHBG seems a valid explanation, as direct inhibition of SHBG synthesis by insulin has been observed in HepG2 cells in

the liver, both in vitro and in vivo (1, 6, 9, 14, 15, 16). Since there is (almost) no endogenous insulin production in T1DM patients, an increase of portal insulin concentration with CIPII and the subsequent increased hepatic insulinization may cause a more pronounced suppression of SHBG production. Apart from portal insulin concentrations, other factors such as glycemic control, insulin dose, insulin resistance, and the presence of microvascular complications have been suggested to influence SHBG concentrations (1, 4, 14, 17) among T1DM patiets.

In a previous study, Lassmann-Vague and coworkers measured SHBG concentrations before and after initiation of CIPII among 11 T1DM patients (5 males and 6 females) and found a decrease of SHBG concentrations after 3 months of CIPII therapy as compared with prior SC insulin treatment: 41±4 to 33±2 nM/L for males and 84±6 to 63±8 nM/L for females (9). The current study confirms these results and adds by describing an increase in FSH and a decrease in testosterone concentrations during CIPII treatment among males. These changes may be accounted to as a refractory response to altered SHBG levels. Although speculative, a direct effect of insulin on testosterone by selectively inhibiting adrenal androgen production by suppressing 17,20-lyase activity in females may be an alternative explanation (24).

The nonsignificant change of SHBG concentrations in the total group between both routes of insulin administration may be explained by the small sample size ($n=20$) and/or the duration of the study. Nevertheless, among males, the difference in change between both routes of insulin administration was significant for SHBG and FSH. Although hypothetically, these gender differences may be due to differences in SHBG function, in particular a lower testosterone binding degree of SHBG in female, different (testosterone related) gonadotropin feedback on SHBG synthesis, and cycle variation (25, 26, 27, 28).

Alterations found in SHBG and testosterone concentrations might be associated with disorders with an increased incidence among T1DM patients such as reproductive disorders, PCOS, increased risk of osteoporotic fractures in females, and a tendency to hypogonadism in males (29, 30, 31, 32, 33, 34, 35, 36, 37). Moreover, low testosterone concentrations were recently associated with the development of microvascular and macrovascular complications (38). However, the clinical consequences of the current study are unclear at present.

When interpreting the results of this study, several limitations should be taken into account. First and foremost, the original study was not designed to detect differences in SHBG concentrations. Consequently, the sample

Table 2 Observed and estimated SHBG changes during and between CIPII and SC insulin treatment.

	All patients (n = 20)		Male (n = 10)		Female (n = 10)	
	CIPII	SC insulin	CIPII	SC insulin	CIPII	SC insulin
0 months	58.0 [40.2, 75.6]	74.4 [40.4, 80.3]	57.1 [40.2, 70.4]	64.5 [39.9, 79.8]	62.1 [39.8, 98.5]	74.4 [40.7, 117.0]
3 months	43.1 [35.0, 76.1]	60.4 [42.0, 74.6]	38.7 [29.4, 62.3]	59.2 [42.7, 70.0]	66.9 [40.8, 100.7]	60.4 [40.1, 111.9]
6 months	47.7 [33.8, 73.3]	57.0 [40.4, 73.9]	38.3 [28.9, 64.5]	57.0 [40.4, 73.9]	64.4 [42.2, 87.6]	70.3 [38.0, 123.1]
Difference during treatment	−10.3 (−17.4, −3.2)*	−3.7 (−12.0, 4.6)	−15.8 (−24.2, −7.5)*	−3.1 (−12.2, 6.1)	−6.0 (−20.0, 8.0)	−4.3 (−22.4, 13.8)
Difference between treatment groups	−6.6 (95% CI: −17.5, 4.3)		−12.7 (−25.1, −0.4)*		−1.7 (−24.6, 21.1)	

Data are shown as median [IQR] and estimated mean changes (95% CI) in nmol/L. Number of samples available for treatment mode: CIPII: 0 months (n = 18), 3 months (n = 19), 6 months (n = 19). SC: 0 months (n = 19), 3 months (n = 19), 6 months (n = 16).
*P < 0.05.

Table 3 Observed testosterone, 17-β-estradiol, LH, and FSH concentrations and estimated changes during CIPII and SC insulin treatment.

	Testosterone (nmol/L)		17-β-estradiol (pmol/L)		LH (U/L)		FSH (U/L)	
	CIPII	SC insulin	CIPII	SC insulin	CIPII	SC insulin	CIPII	SC insulin
Males (n = 10)								
0 months	22.9 [16.1, 27.7]	23.4 [17.5, 26.4]	79.0 [71.7, 148.7]	80.2 [57.5, 125.6]	5.9 [3.1, 6.6]	5.3 [3.6, 7.7]	3.8 [2.5, 6.6]	4.8 [3.3, 8.3]
3 months	16.5 [16.0, 19.0]	23.5 [17.7, 27.5]	60.2 [48.0, 111.4]	74.6 [73.4, 123.6]	3.5 [2.5, 6.1]	6.6 [3.8, 7.2]	3.2 [2.1, 6.6]	4.6 [2.7, 8.0]
6 months	20.8 [13.5, 24.2]	19.8 [18.1, 24.9]	73.3 [51.8, 132.0]	74.2 [60.6, 132.5]	7.0 [4.2, 8.6]	5.1 [4.6, 6.7]	6.9 [3.0, 10.3]	4.1 [2.5, 6.7]
Difference during treatment	−8.3 (−14.4, −2.2)*	−2.3 (−8.7, 4.0)	−14.2 (−41.0, 12.7)	9.7 (−17.5, 36.9)	2.2 (−0.8, 5.2)	−0.1 (−2.9, 2.6)	0.4 (−9.1, 9.9)	−0.5 (−1.2, 0.2)
Difference between treatment groups	−6.0 (−14.7, 2.8)		−23.9 (−61.9, 14.2)		2.3 (−1.8, 6.4)		1.2 (0.1, 2.2)*	
Females (n = 10)								
0 months	1.1 [0.5, 1.7]	0.9 [0.4, 1.0]						
3 months	0.4 [0.2, 1.2]	0.6 [0.3, 1.2]						
6 months	0.6 [0.2, 1.2]	0.8 [0.3, 1.1]						
Difference during treatment	−0.1 (−0.4, 0.1)	−0.1 (−0.5, 0.3)						
Difference between treatment groups	−0.1 (−0.5, 0.4)							

Data are shown as median [IQR] and estimated mean changes (95% CI) in nmol/L for SHBG and testosterone, in pmol/L for 17-β-estradiol and in U/L for LH and FSH.
*P < 0.05.

size was small, blood samples were taken at random, non-standardized moments and at non-fasting moments and the effect of other factors suggested to influence SHBG and sex hormone synthesis, that is, microvascular complications, insulin-like growth factors, menstrual cycle, monosaccharides, and oxative stress levels in the liver remain unknown (39, 40, 41). Due to these limitations, we also did not measure free testosterone as these measurements are challenging (42). Adding them could have led to (additional) limitations to our data and would increase the chance to make a type I error. Because of low testosterone concentrations among females, the coefficient of the assays (estimated to be >20% in the range of 0.15–0.50 nmol/L) may also have influenced these results. The use of porcine insulin in the CIPII group, used for CIPII until 2010 due to delayed progress in the development of new insulin, may also have influenced the results. Finally, there was a lack of a non-T1DM reference population.

Nevertheless, this study is the first to describe the effects of different routes of insulin administration on SHBG, sexual steroids, and gonadotropins among T1DM patients. Furthermore, this study provides proof-of-principle and supports the hypothesis that portal insulin administration has an effect on circulating SHBG concentrations and on sexual steroids, in particular in male T1DM patients. The effects of various routes of insulin administration on SHBG, steroids, and associated pathology merit further study.

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Endocrine Connections