CLINICAL STUDY

Short-term continuous subcutaneous insulin infusion decreases the plasma vaspin levels in patients with type 2 diabetes mellitus concomitant with improvement in insulin sensitivity

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Abstract

Objective: To investigate the effects of short-term continuous subcutaneous insulin infusion (CSII) on plasma vaspin levels in patients with newly diagnosed type 2 diabetes mellitus (T2DM).

Method: Thirty patients with severe newly diagnosed T2DM, 37 subjects with impaired glucose tolerance (IGT) and 38 gender-, age- and body mass index (BMI)-matched normal GT (NGT) controls participated in the study. The T2DM group was treated with CSII for 2 weeks. Euglycemic-hyperinsulinemic clamps were performed in 16 subjects of the T2DM group. Plasma vaspin concentrations were measured with a commercial ELISA kit. The relationship between plasma vaspin levels and metabolic parameters was also analyzed.

Results: Fasting plasma vaspin levels were higher in the T2DM group than in IGT and NGT groups (1.83 ± 0.55 vs 0.51 ± 0.21 vs 0.53 ± 0.24 $\mu\text{g/l}$, $P < 0.05$), but there was no difference between IGT and NGT groups. In T2DM patients, fasting plasma vaspin concentrations were significantly decreased after CSII treatment for 2 weeks (1.83 ± 0.55 vs 1.19 ± 0.57 $\mu\text{g/l}$, $P < 0.05$), accompanied by significant amelioration of insulin sensitivity and glucose control. The changes in plasma vaspin levels were positively associated with the amelioration of insulin resistance (IR) shown by the changes in homeostasis model assessment of IR.

Conclusion: Plasma vaspin level is associated with IR and is significantly reduced following short-term CSII treatment.

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Introduction

Insulin resistance (IR) plays a primary role in the development of type 2 diabetes mellitus (T2DM) and is a characteristic feature of other health disorders, including obesity, dyslipidemia, hypertension, and cardiovascular disease. Recently, some evidence demonstrate that the increased adiposity is linked to IR (1–3). The role of adipose tissue as both an inflammatory mediator and endocrine organ has raised interest in the academic community (4, 5). Adipokines, adipose-derived factors, have been shown to mediate both inflammatory and insulin signaling pathways.

Recently, visceral adipose tissue-derived serpin (VASPIN or SERPINA12) was identified as a member of the serine protease inhibitor family, which was expressed in visceral adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats (6). Furthermore, VASPIN was proved to have an insulin-sensitizing effect,

which may act through normalizing the altered expression of genes relevant to IR in diet-induced obese mice (6). In human beings, VASPIN mRNA or serum concentration was reported to be associated with blood glucose concentration (7, 8), insulin sensitivity (8, 9), and body mass index (BMI) or body fat percentage (9, 10). In contrast, Seeger *et al.* (11) failed to find this correlation. Youn *et al.* (9) found an association between serum vaspin levels, BMI and insulin sensitivity, but could not confirm this correlation in patients with T2DM. Thus, these conflicting results indicate that the physiological role of vaspin remains uncertain. Transient insulin treatment optimized by continuous subcutaneous insulin infusion (CSII) is effective in rapidly reducing hyperglycemia and glucotoxicity, and leads to the restoration of insulin sensitivity in T2DM (12). Early intensive insulin therapy in patients with newly diagnosed T2DM recovers β -cell function to a certain degree, along with a concurrent

improvement in insulin sensitivity (13, 14). The aim of this study was to examine plasma vaspin levels in newly diagnosed patients with T2DM and to evaluate the effects of CSII on plasma vaspin level in these patients.

Patients and methods

Subjects

Chinese volunteers ($n=105$) were involved in this study and categorized into three groups. The first group comprised of 30 individuals with new-onset T2DM. The second group of 37 subjects were patients with impaired glucose tolerance (IGT, 2 h postprandial blood glucose ≥ 7.8 and <11.1 mmol/l). The diagnostic criteria of T2DM and IGT were based on a 75 g oral glucose tolerance test (OGTT) recommended by World Health Organization criteria (15). These patients had not taken any diabetic medications/diet prior to the present study. The selection criteria of the T2DM group was: i) 40–65 years of age without the presence of major diabetic complications and major organ diseases; ii) fasting blood glucose (FBG) >11.0 mmol/l; and iii) BMI within the range of 20–30 kg/m².

Thirty-eight healthy volunteers, age- and BMI-matched with T2DM and IGT groups, were chosen as the normal GT (NGT) controls. Their non-diabetic status was confirmed with a normal OGTT. This study was carried out in accordance with the recommendations of the Declaration of Helsinki. The study was approved by the Human Research Ethics Committee of Chongqing Medical University. An informed consent was obtained from all participants in this study.

Study design

All T2DM patients were admitted to the hospital, and then treated with intensive insulin therapy by CSII with MiniMed 712E insulin pumps (Medtronic MiniMed, Northridge, CA, USA). The initial daily insulin dose was calculated as follows: total insulin dose daily = (0.4–0.6) unit $\times$ body weight (kg). The basal rate (units/hour) was calculated as 50% of the total insulin dose, and other 50% was administered as preprandial bolus before each of the three meals. The basal and bolus of insulin infusion were adjusted according to the fasting and postprandial (of three meals) capillary blood glucose. Excellent blood glucose control was defined as FBG <6.1 mmol/l and postprandial blood glucose (PBG) <7.8 mmol/l, and was achieved within 3–5 days. After 2 weeks of CSII, insulin treatment was stopped.

Euglycemic-hyperinsulinemic clamp (EHC) was performed in 16 of the 30 T2DM subjects before CSII, and was repeated at least 24 h after insulin cessation. Briefly, after an overnight fast, an intravenous catheter was placed in the antecubital vein to infuse insulin and glucose. Another catheter was placed retrograde in the

dorsal vein of the contralateral hand for blood withdrawal. Regular human insulin (1 mU/kg per min) was infused for 2 h and a variable infusion of 20% glucose was administered to maintain plasma glucose at the fasting level. During the procedure, plasma glucose was measured every 5 min to guide the glucose infusion. The rate of glucose disposal was defined as the glucose infusion rate during the stable period of the clamp and was related to body weight (M value, mg/kg per min) (16). The insulin sensitivity index (M/I) was calculated as the whole body glucose uptake (M value) divided by insulin concentration (I value).

The NGT and the IGT group did not receive any therapy. No anti-diabetic agents, oral contraceptives or anti-hyperlipidemic agents were used during the study. No subject dropped out of the study.

Plasma biochemical parameters and vaspin

Blood samples were taken before CSII treatment and after at least 24 h insulin cessation for the measurements of metabolic parameters and plasma vaspin levels. Typically, blood samples were collected either after an overnight fast or 2 h after administration of 75 g OGTT. Plasma samples were collected by centrifugation at 4 °C and kept at -80 °C for further use.

Plasma vaspin levels were measured with a commercial ELISA kit (Adipobiotech, Inc., Beijing, China). The linear range of the assay was 0.2–5.0 μ g/l, and the standard range was 0.13–20 μ g/l. The inter- and intra-assay coefficients of variation were 5 and 10% respectively. Insulin was measured in deproteinized serum by RIA using human insulin as standards (Linco, St. Charles, MO, USA). Free fatty acids (FFA) were measured with a commercial assay kit (Randox Laboratories Ltd, Antrim, UK). Plasma glucose was assayed using the glucose oxidase method. HbA1c was measured by isoelectric focusing. Triglyceride (TG), cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) concentrations were determined enzymatically. Percent body fat was determined by bioelectrical impedance analysis (Tanita, Inc., Tokyo, Japan). The homeostasis model assessment of IR (HOMA-IR) and the HOMA of β -cell insulin secretion (HOMA-IS) were calculated from fasting insulin (FINS) and FBG levels using the following equations (17):

$$\text{HOMA-IR} = \frac{\text{FINS (mU/l)} \times \text{FBG (mmol/l)}}{22.5}$$

$$\text{HOMA-IS} = \frac{(20 \times \text{FINS (mU/l)})}{(\text{FBG (mmol/l)} - 3.5)}$$

Statistical analysis

Statistical analyses were performed using the SPSS 15.0 Software (SPSS, Chicago, IL, USA). Since the distributions of plasma insulin, FFA, HOMA-IR and HOMR-IS

Table 1 Clinical characteristics and vaspin levels of study subjects. Data are means $\pm$ s.d. or medians (interquartile ranges).

Group	NGT	IGT	T2DM
Gender (M:F)	17:21	16:21	14:16
Age (years)	51.5 $\pm$ 9.69	52.8 $\pm$ 8.3	48.9 $\pm$ 1.01
BMI (kg/m ²)	23.99 $\pm$ 4.03	24.29 $\pm$ 2.6	24.02 $\pm$ 2.28
WHR	0.86 $\pm$ 0.08	0.89 $\pm$ 0.07	0.89 $\pm$ 0.04
Body fat (%)	29.12 $\pm$ 8.00	29.95 $\pm$ 5.81	27.72 $\pm$ 2.62
SBP (mmHg)	125.63 $\pm$ 16.88	126.92 $\pm$ 16.64	130.77 $\pm$ 11.39
DBP (mmHg)	80.82 $\pm$ 9.01	77.18 $\pm$ 9.06	80.7 $\pm$ 8.33
TG (mmol/l)	1.66 $\pm$ 1.15	2.28 $\pm$ 1.14	3.74 $\pm$ 1.98 ^{*,†}
TC (mmol/l)	5.01 $\pm$ 0.86	5.22 $\pm$ 1.10	4.71 $\pm$ 2.02
HDL (mmol/l)	1.21 $\pm$ 0.37	1.13 $\pm$ 0.24	1.32 $\pm$ 0.93
LDL (mmol/l)	2.71 $\pm$ 0.72	2.89 $\pm$ 0.74	3.39 $\pm$ 1.14 [*]
FFA (μ mol/l)	0.63 (0.16–2.61)	0.57 (0.23–2.94)	0.95 (0.54–2.55) ^{*,†}
HbA1c (%)	5.58 $\pm$ 0.31	5.97 $\pm$ 0.45	10.5 $\pm$ 1.33 ^{*,†}
FBG (mmol/l)	5.51 $\pm$ 0.76	6.02 $\pm$ 0.49	14.49 $\pm$ 1.60 ^{*,†}
2hPBG (mmol/l)	6.07 $\pm$ 0.92	8.60 $\pm$ 1.22 [*]	21.55 $\pm$ 4.59 ^{*,†}
FINS (mU/l)	7.65 (3.40–60.4)	10.4 (3.9–73.55) [*]	6.7 (2.59–10.37) ^{*,†}
PINS (mU/l)	23.1 (7.09–87.4)	52.6 (12.22–170.8) [*]	20.65 (4.1–56.3) ^{*,†}
HOMA-IR	1.86 (0.71–15.41)	2.76 (1.13–19.84)	4.28 (1.7–6.47) [*]
HOMA-IS	86.94 (32.46–539.29)	85.95 (25.7–572.3)	13.94 (4.09–21.8) ^{*,†}
Vaspin (μ g/l)	0.53 $\pm$ 0.24	0.51 $\pm$ 0.21	1.83 $\pm$ 0.55 ^{*,†}

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WHR, waist-to-hip ratio; fat%, visceral fat %; FBG, fasting blood glucose; 2hPBG, 2 h post-glucose load blood glucose; FINS, fasting plasma insulin; PINS, 2 h plasma insulin after glucose overload; HOMA-IR, HOMA-insulin resistance index; FFA, free fatty acids; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin. ^{*} $P < 0.05$ compared with NGT group. [†] $P < 0.05$ compared with IGT group.

values were skewed, logarithmically transformed values were used for statistical analysis. Baseline characteristics of case and control subjects were compared by *t*-test or ANOVA. The paired *t*-test was used to compare differences in biochemical characteristics and vaspin levels between pre- and post-treatment with CSII in T2DM group. One-way ANOVA and Tukey's honestly significant difference *post-hoc* test were performed to test the changes among the groups. Bivariate correlation and multiple regression analyses were used to examine the association between fasting plasma vaspin levels and the values of other biomarkers. There is no existence of the multicollinearity between all the variables included in the multiple regression analyses. All of the statistical analyses were two-sided, and all data are presented as means $\pm$ s.d. or medians (interquartile ranges) with a *P* value < 0.05 considered statistically significant.

Results

The clinical characteristics and fasting plasma vaspin levels

The clinical characteristics of the three groups did not show significant difference in gender distribution, age, BMI, waist-to-hip ratio (WHR), percent body fat, blood pressure, total cholesterol (TC) and HDL (Table 1). The fasting levels of TG, FFA, FBG, 2hPBG, HbA1c and plasma vaspin in T2DM group were higher than the other two groups (all $P < 0.05$). Compared with

the NGT group, T2DM group had higher LDL levels and HOMA-IR (both $P < 0.05$), but lower fasting insulin, postprandial insulin (PINS) and HOMA-IS (all $P < 0.05$). FINS, PINS and 2hPBG levels in IGT group were significantly higher than in NGT group (all $P < 0.05$). There was no significant difference in fasting plasma vaspin levels between the IGT and NGT groups (0.51 ± 0.21 vs 0.53 ± 0.24 μ g/l, $P > 0.05$).

In NGT subjects and IGT subjects, fasting plasma vaspin levels were significantly higher in female compared with male subjects (0.64 ± 0.26 vs 0.40 ± 0.13 μ g/l in NGT group, 0.61 ± 0.20 vs 0.37 ± 0.13 μ g/l in IGT group, $P < 0.05$; Fig. 1). However, no sexual dimorphism became apparent in patients with T2DM (female versus male: 1.95 ± 0.57 vs 1.70 ± 0.51 μ g/l, $P > 0.05$; Fig. 1).

Relationship between fasting plasma vaspin levels and metabolic parameters

In the NGT group, plasma vaspin levels correlated with gender ($r = 0.809$, $P < 0.05$). Moreover, gender, age, BMI, WHR, TG, TC, LDL, HDL cholesterol, FFA, FBG, PBG, HbA1c, FINS and PINS were included in the multiple regressions. The result showed gender was the only independent predictor of fasting plasma vaspin levels ($r^2 = 0.247$, $B = 0.235$, $P = 0.002$). However, correlation between vaspin and gender was not confirmed in patients with T2DM. In T2DM group, fasting plasma vaspin levels correlated positively with FINS, PINS, HOMA-IR and HOMA-IS ($r = 0.886$, $r = 0.703$, $r = 0.919$ and $r = 0.809$, respectively, all

Figure 1 Fasting plasma vaspin concentrations in the subjects. * $P<0.05$ versus male in NGT group; # $P<0.05$ versus male in IGT group.

$P<0.05$), and correlated negatively with M values ($r=-0.57$, $P<0.05$).

To investigate the association of plasma vaspin and IR, we analyzed patients with T2DM using models with plasma vaspin levels as a dependent variable, and gender, age, BMI, WHR, HbA1c, HOMA-IR and HOMA-IS as independent variables. Result indicates a significant association with HOMA-IR ($r^2=0.845$, $B=0.393$, $P<0.001$). Additionally, multiple regression analyses in the subgroup of patients with EHC revealed a strong association with M values ($r^2=0.565$, $B=-0.266$, $P=0.005$).

The effects of CSII on clinical characteristics and vaspin levels in T2DM patients

All T2DM patients treated with CSII achieved excellent glycemic control within 3–5 days and maintained euglycemia in the following 2 weeks. After 2 weeks of CSII therapy, blood glucose control was remarkably improved. As shown in Table 2, there was a significant decrease in FBG and PBG (both $P<0.05$), and an increase in insulin levels, including FINS and PINS (both $P<0.05$). Treatment with CSII decreased plasma TC and LDL levels (both $P<0.05$). Also, elevated fasting plasma vaspin levels were decreased concomitant with ameliorations in insulin sensitivity and β -cell function, which were shown by a significant decrease in HOMA-IR values and a significant increase in HOMA-IS values (both $P<0.05$).

To directly examine the quantitative effect of CSII treatment on insulin sensitivity, we performed EHC on the same group of patients pre- and post-treatment. CSII treatment resulted in a significant increase in glucose disposal rate shown as M values (5.10 ± 0.51 vs 2.99 ± 0.42 mg/kg per min, $P<0.05$, Table 2) and insulin sensitivity index shown as M/I (0.43 ± 0.09 vs 0.19 ± 0.04 mg/kg per min mU/l, $P<0.05$, Table 2).

We also assessed the associations between the changes of plasma vaspin (Δ vaspin) and several parameters pre- and post- CSII treatment. Interestingly, Δ vaspin was positively associated with Δ FINS ($r=0.576$, $P=0.001$) and Δ HOMA-IR ($r=0.868$, $P<0.001$).

Discussion

Previous studies have indicated the importance of vaspin in IR and obesity in humans. However, the correlation between serum vaspin levels and markers of insulin sensitivity, glucose metabolism, and obesity is still controversial.

In the current study, we found that plasma vaspin levels were elevated in patients with new-onset T2DM compared with the gender-, BMI- and age-matched NGT and IGT subjects. In T2DM patients, elevated vaspin levels are positively associated with metabolic risk factors, including FINS, PINS, HOMA-IR and HOMA-IS, but not in NGT subjects. These findings suggest that vaspin may relate to insulin sensitivity and play a role in a compensatory mechanism associated with severe IR and T2DM. Interestingly, consistent with the results reported by Von Loeffelholz *et al.* (18), there was no significant difference in circulating vaspin concentrations between NGT and IGT subjects, despite

Table 2 Clinical characteristics and vaspin levels pre- and post-treatment with CSII in T2DM group. Data are means $\pm$ s.d. or medians (interquartile ranges).

Group	Pre-treatment	Post-treatment
BMI (kg/m ²)	24.02 $\pm$ 2.28	24.52 $\pm$ 2.26
WHR	0.89 $\pm$ 0.04	0.89 $\pm$ 0.05
Body fat (%)	27.72 $\pm$ 2.62	27.65 $\pm$ 2.57
SBP (mmHg)	130.77 $\pm$ 11.39	123.47 $\pm$ 12.65
DBP (mmHg)	80.7 $\pm$ 8.33	75.37 $\pm$ 5.01
TG (mmol/l)	3.74 $\pm$ 1.98	3.21 $\pm$ 1.51
TC (mmol/l)	4.71 $\pm$ 2.02	3.37 $\pm$ 2.17*
HDL (mmol/l)	1.32 $\pm$ 0.93	1.19 $\pm$ 0.25
LDL (mmol/l)	3.39 $\pm$ 1.14	2.56 $\pm$ 0.71*
FFA (μ mol/l)	0.95 (0.54–2.55)	0.81 (0.12–2.86)
FBG (mmol/l)	14.49 $\pm$ 1.60	6.04 $\pm$ 0.86*
2hPBG (mmol/l)	21.55 $\pm$ 4.59	7.13 $\pm$ 0.69*
FINS (mU/l)	6.7 (2.59–10.37)	8.81 (4.61–26.56)*
PINS (mU/l)	20.65 (4.1–56.26)	45.45 (12.44–92.78)*
HOMA-IR	4.28 (1.7–6.47)	2.3 (1.09–7.2)*
HOMA-IS	13.94 (4.09–21.8)	78.21 (46.75–204.31)*
Vaspin (μ g/l)	1.83 $\pm$ 0.55	1.19 $\pm$ 0.57*
M (mg/kg per min)	2.99 $\pm$ 0.42	5.10 $\pm$ 0.51*
M/I (mg/kg per min (mU/l))	0.19 $\pm$ 0.04	0.43 $\pm$ 0.09*

BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, total triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acids; FBG, fasting blood glucose; 2hPBG, 2 h postprandial blood glucose; FINS, fasting insulin; PINS, 2 h postprandial insulin; HOMA-IR, HOMA-insulin resistance index; HOMA-IS, HOMA- β -cell insulin secretion index; M, whole body glucose uptake rate; M/I, insulin sensitivity index. * $P<0.05$ compared with pre-treatment.

IGT subjects having higher plasma glucose and insulin levels. In contrast, the results of two separate studies in Caucasian and Turkey females, showed no difference in circulating vaspin between NGT and T2DM (8, 9). Multiple linear regression analyses revealed that HOMA-IR was an independent factor associated with plasma vaspin level in T2DM patients. Interestingly, we observed a significant relationship between vaspin and *M* values in T2DM patients with EHC. Taking these findings into consideration, our results support the hypothesis that vaspin may be an insulin-sensitizing adipokine in humans. Therefore, the most likely explanation for these findings is that elevated vaspin levels in T2DM patients might represent a compensatory mechanism in response to decreased insulin sensitivity or impairment of glucose metabolism. These results are in accordance with some (8), but not all previous studies (9). These differences may be due to the different populations studied and the different kits used for the measurement and different protocols and dilutions recommended by the manufacturers. In the current study, we found significantly higher circulating vaspin levels in females versus males in NGT and IGT subjects. Interestingly, these gender differences were abrogated in T2DM patients, suggesting that metabolic alterations in T2DM, including chronic hyperglycemia and decreased insulin sensitivity, modulate serum vaspin concentrations.

In the present study, blood glucose, both postprandial and fasting, were nearly normalized within 7 days and euglycemia was maintained for 2 weeks by CSII therapy in patients with severe new-onset T2DM. Interestingly, along with an improvement in glucose and lipid metabolism, we observed a dramatic decrease in fasting plasma vaspin concentrations after treatment with CSII in these patients. More importantly, the reduction in plasma vaspin levels was found to associate with the amelioration of insulin sensitivity shown by the changes in HOMA-IR. Hence, the regulation of circulating vaspin might have been influenced by metabolic control and IR. Decreased circulating vaspin after CSII treatment could mediate the improvement of IR in the patients with T2DM. These results further confirm that vaspin may play an important role in the development of T2DM and IR and may be a part of the protective mechanisms aimed at reducing IR in humans. Our findings also suggest that vaspin can be used as a novel biomarker for IR syndrome.

In conclusion, our data found that circulating vaspin levels are significantly increased and significantly associated with HOMA-IR and HOMA-IS in new-onset T2DM. More importantly, we present novel data that CSII treatment significantly decreases plasma vaspin levels in T2DM. The reduction in plasma vaspin levels was found to associate with the amelioration of insulin sensitivity shown by the changes in HOMA-IR. These findings suggest that vaspin can be used as a novel biomarker for IR syndrome and T2DM.

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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