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CLINICAL TRIAL

Relationship between serum adipsin and the first phase of glucose-stimulated insulin secretion in individuals with different glucose tolerance

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ABSTRACT

Aims/Introduction: To detect serum adipsin levels in individuals with different glucose tolerance, and investigate the relationship between adipsisn and the first phase of insulin secretion.

Materials and Methods: A total of 56 patients with newly diagnosed type 2 diabetes mellitus, 36 patients with impaired glucose tolerance (IGT) and 45 individuals with normal glucose tolerance were enrolled. Intravenous glucose tolerance tests were carried out to evaluate pancreatic β-cell function. The serum levels of adipsin, interleukin-1β and high-sensitivity C-reactive protein were assayed.

Results: Serum adipsin levels were significantly lower in the type 2 diabetes mellitus and the IGT patients than those in the normal glucose tolerance group ($P < 0.05$). The acute insulin response and area under the curve showed a progressive decrease in the normal glucose tolerance and IGT groups, and decreased to the lowest levels in the type 2 diabetes mellitus group ($P < 0.05$). Adipsin was found to be negatively correlated with waist-to-hip ratio, free fatty acid, fasting plasma glucose, 2-h postprandial plasma glucose, glycated hemoglobin, homeostasis model assessment of insulin resistance, interleukin-1β and high-sensitivity C-reactive protein ($P < 0.05$ or $P < 0.001$), and positively correlated with homeostasis model assessment of β-cell function, high-density lipoprotein cholesterol, the area under the curve of the first phase insulin secretion and acute insulin response ($P < 0.05$ or $P < 0.001$). Stepwise multiple regression analysis showed that homeostasis model assessment for β-cell function and acute insulin response were independently related to adipsin ($P < 0.05$).

Conclusions: Serum adipsin levels were lower in type 2 diabetes mellitus and IGT patients, and correlated with the first phase of insulin secretion. Adipsin might be involved in the pathology of type 2 diabetes mellitus.

INTRODUCTION

The incidence of type 2 diabetes mellitus has risen every year around the world, and has become the third largest non-communicable disease besides cardiovascular diseases and cancers. According to World Health Organization reports, there were 422 million diabetes patients around the world in 2014[1]. Insulin resistance and pancreatic β-cell dysfunction are two major pathological characteristics of type 2 diabetes mellitus. It has been proven that pancreatic β-cell function of newly-diagnosed diabetes patients has fallen 50%[2], and it progressively declines during the pathological process until exhaustion. Insulin resistance is closely related to metabolic diseases, such as

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hypertension and polycystic ovary syndrome, which suggests it is a sufficient condition, but not a necessary condition for the occurrence of diabetes. Hence, preservation, even restoration, of the remaining β-cell function is necessary and essential.

Adipsin, a member of the serine protease family, was the first adipokine found in 3T3 adipocytes[3]. Later studies found adipsin was identified to be complement factor D, which participates in an alternative pathway of the complement system[4]. Recently, adipsin was found to be able to promote insulin secretion and stabilize plasma glucose levels. Furthermore, *db*/*db* mice treated with adipsin showed significant decreases in fasting plasma glucose (FPG) and improvement of glucose clearance, at the same time, fasting and glucose-induced insulin levels were increased[5]. However, the association and possible mechanism between serum adipsin and type 2 diabetes mellitus in humans are still quite unclear.

Assessment of pancreatic β-cell function has always been a topical issue, all the methods we use have limitations. Of note, through carrying out intravenous glucose tolerance tests, early pancreatic β-cell dysfunction can be estimated by the first phase of insulin secretion[6]. Additionally, it has been proven that a deficit of first-phase insulin secretion is a main characteristic[7], but also an independent predictor of type 2 diabetes mellitus[8]. Therefore, we aim to investigate the serum adipsin levels in individuals with different glucose tolerance, and further explore the relationship between adipsin and the first phase of insulin secretion.

METHODS

Participants

A total of 137 participants were recruited and underwent 75-g oral glucose tolerance tests. All individuals were divided into three groups according to the World Health Organization diagnostic criteria of diabetes[9]: normal glucose tolerance (NGT; $n = 45$; aged 52.29 ± 12.16 years; 22 men), impaired glucose tolerance (IGT; $n = 35$; 51.85 ± 8.57 years; 17 men) and type 2 diabetes mellitus ($n = 56$; 50.95 ± 12.48 years; 28 men). All the type 2 diabetes mellitus patients were newly diagnosed without any antidiabetic treatment and other medications that might affect glucolipid metabolism during the past 3 months. Individuals who had type 1 diabetes, gestational diabetes, lactation diabetes, secondary obesity, acute or chronic diabetes complications, acute inflammation, liver or renal disease, cardiovascular disease, corticosteroid treatment, or other known major diseases were excluded. Informed consent was obtained from all the participants. The ethical committee of the First Affiliated Hospital of Chongqing Medical University approved this study. All procedures followed were carried out in accordance with the ethical standards laid down in the 1995 Declaration of Helsinki and its later amendments.

Anthropometric measurements

Height, weight (without shoes and outdoor clothing), waist circumferences, hip circumferences and blood pressure were measured using standard protocols. Height, waist and hip circumferences were measured to a minimum recorded unit of 0.1 cm, and blood pressure was measured twice with a standard mercury manometer with the participants seated. Body mass index (BMI) and waist-to-hip ratio (WHR) were calculated.

Serum biochemistry

All the participants were asked to fast for 12 h before the blood samples were collected. The FPG (glucose oxidase method), fasting insulin (FINS; chemiluminescence method), glycated hemoglobin (HbA1c; isoelectric focusing method), blood lipid (triglyceride [TG], total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, enzymatic method], free fatty acid (FFA; automatic biochemistry analyzer analysis), liver and renal function (automatic biochemistry analyzer analysis) and high-sensitivity C-reaction protein (hs-CRP; enzymatic method) were detected. Serum samples, which were used later to determine the levels of adipsin and interleukin-1β (IL-1β), were obtained by centrifugation at 4°C and stored at −80°C.

Measurement of serum adipsin and IL-1β

Enzyme-linked immunosorbent assay kits were used to detected serum adipsin and IL-1β (Human ELISA kit; USCN, Wuhan, China). The intra- and interassay coefficient of variation was 10 and 12%, respectively. All samples were run in duplicate and repeated if there was a > 15% differences between duplicates.

Intravenous glucose tolerance test

After an overnight fasting, intravenous glucose tolerance test was carried out. All the individuals were required to have a diet containing at least 150 g of carbohydrate/day for 3 days before the test. Glucose solution (50% glucose, 300 mg/kg, maximum dose 35 g) was given intravenously over 3 min ± 15 s. Blood samples were collected at 0, 3, 5, 8 and 10 min after the glucose infusion[10,11]. Glucose and insulin above the time-point were assayed. Homeostasis model assessment of insulin resistance (HOMA-IR) and pancreatic β-cell function (HOMA-β) were calculated[12]. Acute insulin response (AIR) were calculated as the mean plasma insulin concentration at 3 and 5 min after infusion of glucose, and this value correlates with the minimal model-derived AIR[13]. The area under the curve of insulin concentration (AUC) was calculated by linear trapezoid method.

Statistical analysis

SPSS software, version 19.0 (SPSS Inc., Chicago, IL, USA), was used for all statistical analyses. Data are presented as mean ± standard deviation or median (interquartile range 25–75%). Non-normally distributed variables were natural-logarithmically transformed. One-way analysis of variance (ANOVA) was used for groups comparisons. Pearson correlation analysis was used to analyze interrelationships between variables. Stepwise

Table 1 | Comparison of clinical data and biochemical parameters among three groups

Groups	*n* (male/female)	Age (ysears)	BMI (kg/m^2)	WHR	SBP (mmHg)	DBP (mmHg)
NGT	45 (22/23)	52.29 ± 12.16	23.16 ± 1.98	0.89 ± 0.04	121.47 ± 16.40	76.76 ± 9.24
IGT	36 (17/19)	51.85 ± 8.57	23.88 ± 3.20	0.90 ± 0.04	129.69 ± 15.64	84.69 ± 9.02*
Type 2 diabetes mellitus	56 (28/28)	50.95 ± 12.48	25.60 ± 3.69*	0.92 ± 0.06*	132.52 ± 16.59*	86.81 ± 10.32**

	FFA (mmol/L)	FPG (mmol/L)†	2hPG (mmol/L)†	FINS (mU/L)	HbA1c (%)	HOMA-IR†
NGT	0.51 ± 0.12	4.90 (4.80–5.25)	4.47 (4.17–5.16)	5.21 ± 2.32	5.67 ± 0.31	1.12 (0.71–1.51)
IGT	0.69 ± 0.31*	6.42 (6.33–6.66)**	6.76 (6.18–7.20)**	5.20 ± 1.24	6.25 ± 0.56	1.51 (1.20–1.89)*
Type 2 diabetes mellitus	0.88 ± 0.18******	8.88 (7.92–10.23)*******	9.33 (7.674–11.79)*******	7.51 ± 2.32*******	8.39 ± 2.09*******	3.09 (1.97–3.98)*******

	HOMA-β†	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	IL-1β (pg/mL)
NGT	62.96 (43.05–90.31)	1.25 ± 0.51	4.94 ± 0.93	1.42 ± 0.26	2.95 ± 0.84	2.91 ± 0.77
IGT	33.00 (26.86–40.26)**	1.44 ± 0.53	4.61 ± 1.10	1.32 ± 0.17	2.83 ± 0.89	5.04 ± 0.63**
Type 2 diabetes mellitus	27.37 (21.39–35.03)**	1.98 ± 1.24*	4.61 ± 0.84	1.24 ± 0.42	2.79 ± 0.91	6.79 ± 1.19*******

	hs-CRP (mg/L)	Adipsin (ng/mL)†
NGT	0.65 ± 0.43	6,833.04 (5,587.02–10,126.3)
IGT	1.32 ± 0.34**	5,159.40 (2,775.35–6,501.95)*
Type 2 diabetes mellitus	1.82 ± 0.73********	3,200.54 (2,541.94–4,069.56)********

All values are given as mean ± standard deviation or median (interquartile range 25–75th percentile). Compared with normal glucose tolerance (NGT), *$P < 0.05$, **$P < 0.01$; compared with impaired glucose tolerance (IGT), ***$P < 0.05$, ****$P < 0.01$. †Skewed distribution, and are presented as median (interquartile range) and were natural logarithmically transformed for analysis. 2hPG, 2-h postprandial plasma glucose; BMI, body mass index; DBP, diastolic blood pressure; FFA, free fatty acid; FINS, fasting serum insulin; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reaction protein; IL-1β, interleukin-1β; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure (1 mmHg = 0.133 kPa); TC, total cholesterol; TG, triglyceride; WHR, waist-to-hip ratio.

multiple regression analysis was carried out. In all statistical tests, $P < 0.05$ were considered as significant.

RESULTS

Clinical characteristics

The descriptive characteristics of all participants are shown in Table 1.

Serum adipsin levels in different glucose tolerance status

As shown in Table 1, the serum adipsin levels were significantly different between the NGT and IGT groups, the IGT and type 2 diabetes mellitus groups, and the NGT and type 2 diabetes mellitus groups. There is no significant difference in serum adipsin levels between men and women (5,204.35 ng/mL [3,006.04–7,384.63 ng/mL] vs 4,541.89 ng/mL [2,864.30–6,472.90 ng/mL], $P > 0.05$).

First-phase insulin secretion in different glucose tolerance status groups

The AIR and AUC of the three groups are shown in Figure 1. The AIR according to glucose status were: 37.29 (25.14–54.40)

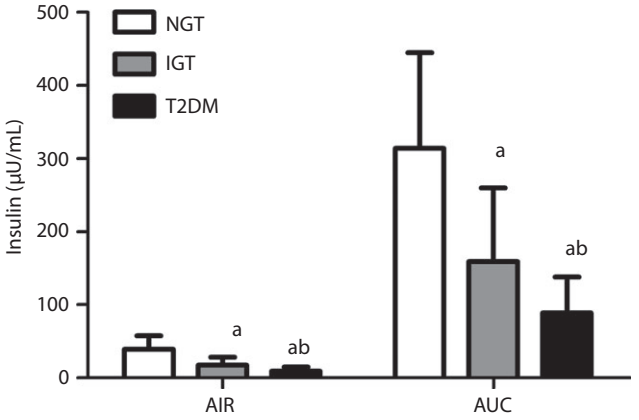

Figure 1 | The data show the first-phase insulin secretion with different glucose tolerance status. The acute insulin response (AIR) and the area under the curve of the first-phase insulin secretion (AUC) had abnormal distributions and were statistically analyzed after logarithmic transformation. [a]$P < 0.05$ compared with normal glucose tolerance (NGT). [b]$P < 0.05$ compared with impaired glucose tolerance (IGT). T2DM, type 2 diabetes mellitus.

vs 17.68 (9.03–19.39) vs 9.73 (5.28–14.09; $P < 0.01$). The AUC were: 285.91 (214.41–405.51) vs 128.64 (84.68–202.76) vs 91.46 (48.47–126.54; $P < 0.01$).

Relationships between the serum adipsin levels and the first phase of insulin secretion and metabolic parameters

Correlations between serum adipsin with glucolipid metabolism, the first phase of insulin secretion and insulin resistance are shown in Table 2. Serum adipsin levels were negatively correlated with WHR, FFA, FPG, 2hPG, HbA1c, HOMA-IR, IL-1β and hs-CRP ($P < 0.05$), whereas serum adipsin levels were positively correlated with HOMA-β, high-density lipoprotein cholesterol, AUC and AIR ($P < 0.05$). However, no significant correlations were found between adipsin levels and age, BMI, SBP, DBP, FINS, TG, total cholesterol and low-density lipoprotein cholesterol ($P > 0.05$).

Stepwise multiple regression analysis was carried out for serum adipsin as a dependent variable, including WHR, FFA, FPG, 2hPG, HbA1c, HOMA-IR, HOMA-β, high-density lipoprotein cholesterol, AUC, AIR, IL-1β and hs-CRP as independent variables. The results showed that HOMA-β and AIR were independently related to serum adipsin levels ($P < 0.05$).

DISCUSSION

Adipsin, a 28-kDa protein found in 3T3 adipocytes[3], was the first adipokine described. In an alternative pathway of complement activation, adipsin is involved in the formation of C3bBb convertase, which cleaves C3 to C3a and C3b, and then, C3a is cleaved by carboxypeptidase to produce C3adeArg, which is also called acylation stimulating protein[14].

Previous studies showed that serum adipsin was significant lower in *db*/*db* and *ob*/*ob* mice, and in hyperglycemic and hyperinsulinemic states induced by continuous infusion of glucose, serum adipsin levels were decreased[15,16]. Lo *et al.*[5] discovered that mice lacking the adipsin gene showed impaired glucose tolerance as a result of inadequate insulin secretion. Diabetic *db*/*db* mice that received adenoviral vectors expressing adipsin showed restoration of glucose tolerance, and fasting glucose levels were significant decreased. To our knowledge, this is the first study to investigate the relationship between serum adipsin levels and the first phase of insulin secretion in humans with different glucose tolerance.

In the present study, serum adipsin was significant lower in IGT and type 2 diabetes mellitus patients compared with NGT individuals, which was similar to the study of Lo *et al.*[5], which found that circulating adipsin was lower in type 2 diabetes mellitus patients with β-cell failure[5]. Nevertheless, they detected circulating adipsin in Caucasian obese type 2 diabetes mellitus men and women (BMI 34.3 ± 4.9 kg/m^2) who underwent open abdominal surgery and had received insulin alone or metformin. Therefore, the discrepancies of the data might be due to the difference of race, degree of obesity, glycemic control, inclusion criteria and the detection methods. Furthermore, consistent with previous studies[17,18], the correlation analysis showed a negative correlation between serum adipsin and FPG and HbA1c. Meanwhile, there was a positive correlation between serum adipsin and HOMA-β, AUC and AIR. Further analysis showed that AIR and HOMA-β were independently associated with serum adipsin levels, which indicated that serum adipsin levels were closely related to first-phase insulin secretion. Lo *et al.*[5] found that adipsin was not produced locally in pancreatic β-cells, but its downstream receptor of C3a, C3aR1, was expressed in β-cells. Further studies showed administration of C3a enhanced insulin secretion by 30–40%, and the underlying mechanisms included increasing [Ca2+]i flux, as well as intracellular adenosine triphosphate levels with adenosine triphosphate-coupled respiration[5], which was in accordance with a previous study that found that C3aR1 could enhance [Ca2+]i

Table 2 | Pearson correlation of variables associated with serum adipsin in all study participants

Variables	BMI	WHR	SBP	DBP	FFA	FPG	2hPG
r	−0.221	−0.285	0.041	−0.219	−0.405	−0.521	−0.532
P	0.119	0.043	0.778	0.122	0.003	<0.001	<0.001

	FINS	HbA1c	HOMA-β	HOMA-IR	TG	TC	HDL-C
r	−0.193	−0.347	0.572	−0.394	−0.142	0.178	0.325
P	0.174	0.012	<0.001	0.004	0.319	0.210	0.020

	LDL-C	AUC	AIR	IL-1β	hs-CRP
r	0.075	0.533	0.577	−0.476	−0.310
P	0.599	<0.001	<0.001	<0.001	0.027

2hPG, 2-h postprandial plasma glucose; AIR, acute insulin response; AUC, the area under the curve of insulin secretion; BMI, body mass index; DBP, diastolic blood pressure; FFA, free fatty acid; FINS, fasting serum insulin; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reaction protein; IL-1β, interleukin-1β; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure (1 mmHg = 0.133 kPa); TC, total cholesterol; TG, triglyceride; WHR, waist-to-hip ratio.

flux. Meanwhile, Song *et al.*[19] found overexpression of adipsin could increase C3a expression and C3aR activation. Collectively, these results suggested that adipsin could improve pancreatic β-cell function through increasing first-phase insulin secretion, and the mechanism at least in part from the interaction of C3a and C3aR1.

It has been clearly shown that type 2 diabetes mellitus is a chronic, low-grade inflammatory disease[20,21]; nuclear factor-κB and other pathways are significantly activated and amplified, leading to rapid progress of type 2 diabetes mellitus. IL-6, IL-1β and transforming growth factor-β are prototype pro-inflammatory cytokines that can induce expression of IL-17[22,23], which promotes inflammation through triggering nuclear factor-κB and other pathways[24]. Zúñiga *et al.*[25] found that IL-17 could impair the expression of adipsin. In airway hyper-responsiveness, Mathews *et al.*[26] found adipsin messenger ribonucleic acid in lung tissue was negatively correlated with IL-17 messenger ribonucleic acid in high-fat diet-fed mice. Furthermore, diabetic *db*/*db* mice that received adenoviral vectors expressing adipsin showed decreased expression of the *Crp* gene[5]. All the aforementioned results suggest that serum adipsin is closely related to inflammation. In the present study, correlation analysis showed serum adipsin was negatively correlated with hs-CRP and IL-1β. However, stepwise multiple regression analysis did not show a relationship between serum adipsin and IL-1β and hs-CRP. As for the cross-sectional and limited sample size, more in-depth research is required to explore the causal relationship and mechanism between serum adipsin and inflammation.

Type 2 diabetes mellitus is often accompanied with obesity and dyslipidemia, which could accelerate the pathological progress and lead to worse outcomes. Previous study showed that adipsin can stimulate glucose transport for triglyceride accumulation in adipose tissue and inhibits lipolysis[27]. It has been well established that excess FFA could aggravate pancreatic β-cell function and insulin resistance. Cianflone *et al.*[28] found adipsin–acylation stimulating protein increased absorption of FFA, thus preventing excess accumulation of FFA in circulation. In the present study, we found serum adipsin levels were negatively correlated with FFA; however, further stepwise multiple regression analysis did not show an independent relationship between serum adipsin levels and FFA. Animal studies showed that serum adipsin levels were lower in genetic and acquired mice[15,28]; however, human studies showed different or even contrary conclusions. Some studies showed that serum adispin levels were higher in obesity[29], and were positively correlated with BMI and TG[30]. Nevertheless, it has been suggested that in mild-to-moderate obese individuals, the circulating adipsin levels are not decreased[31]. However, in the present study, there was no significant relationship between serum adipsin levels and BMI and TG, which was in line with previous studies[32,33]. It has been suggested that in obesity, some transcription factors might regulate adipsin expression[34], and as Lo *et al.*[5] suggested, the high levels of adipsin in obesity might be due to the expansion of fat mass that compensates to keep the serum adipsin levels high. Additionally, the different of race, inclusion criteria and detection methods might, in part, account for the inconsistencies.

Insulin resistance is a main characteristic of type 2 diabetes mellitus, and is closely correlated with obesity. Thyroid hormone receptor associated protein 3 could interact with peroxisome proliferator-activated receptor-γ, thus controlling the diabetic gene programming mediated by peroxisome proliferator-activated receptor-γ. Choi *et al.*[35] found that reduced expression of thyroid hormone receptor associated protein 3 restored the expression of adipsin and adiponectin. In the offspring of obese pregnant women, the circulating adipsin was significantly increased, and was positively correlated with HOMA-IR[36]. The above studies suggested the adipsin might be involved in the physiology of insulin resistance. However, in individuals with non-alcoholic fatty liver disease, a state with remarkable insulin resistance, the serum adipsin levels were not significantly increased[37,38]. In adult Arabic participants, the circulating adipsin did not show a significant correlation with HOMA-IR[39]. In the present study, HOMA-IR was significantly increased from the NGT group to the IGT group, with the highest value in the type 2 diabetes mellitus group. Correlation analysis showed that serum adispin levels were negatively correlated with HOMA-IR; however, further analysis did not show an independent association between serum adipsin and HOMA-IR. The discrepancies of the relationship between adipsin and insulin resistance are not quite clear, and further basic human studies are required to explore the possible mechanism.

In conclusion, the levels of serum adipsin are decreased in patients with IGT and type 2 diabetes mellitus, and are closely associated with the first-phase insulin secretion of pancreatic β-cells and glucose metabolism. Therefore, adipsin might serve as a target to improve pancreatic β-cell dysfunction in patients with type 2 diabetes mellitus. The expression of adipsin in patients with type 2 diabetes mellitus needs to be explored in larger-scale samples, and more in-depth studies are required to probe the detail of the mechanism to make adipsin treatment possible.

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DISCLOSURE

The authors declare no conflict of interest.

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