Association Between Serum Adipsin Levels and Insulin Resistance in Subjects With Various Degrees of Glucose Intolerance

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Context: The association between adipsin and glucose metabolism in human subjects remains unclear.

Objective: We investigated the associations between adipsin and insulin resistance/β-cell function in subjects with various degrees of glucose intolerance.

Design: Fasting blood samples were collected for measurements of fasting plasma glucose (FPG), insulin, and adipsin. An oral glucose tolerance test was conducted in subjects with no history of diabetes.

Setting: This study was conducted at a medical center.

Patients: We enrolled 240 subjects with no history of diabetes and 80 patients with known type 2 diabetes (T2D) on diet control or metformin monotherapy.

Main Outcome Measure: β-cell function and insulin resistance were assessed using the homeostasis model assessment (HOMA-β and HOMA-IR, respectively).

Results: Levels of serum adipsin were higher in subjects with normal glucose tolerance (4.0 ± 1.1 µg/mL) or prediabetes (4.0 ± 1.5 µg/mL) compared with subjects with newly diagnosed diabetes (3.8 ± 1.1 µg/mL) or with known T2D on diet control (3.4 ± 1.0 µg/mL) or metformin monotherapy (3.0 ± 1.0 µg/mL, $P < 0.001$). There was no significant association between adipsin and HOMA-β. In contrast, there was an independent negative association between adipsin and HOMA-IR (β coefficient −0.414, 95% CI −0.720 to −0.109, $P = 0.008$). The association was more prominent in subjects with a body mass index (BMI) ≥25 kg/m^2 or an FPG ≥100 mg/dL (P interaction < 0.001 and 0.014, respectively).

Conclusions: Serum adipsin levels were negatively associated with insulin resistance, especially in subjects with a BMI ≥25 kg/m^2 or an FPG ≥100 mg/dL.

Adipsin is an adipokine that was first described in 1987 [1]. Despite being one of the major proteins secreted by adipocytes [2], a paradoxical decline in adipsin has been noted in animal models of obesity and diabetes [3]. The main function of adipsin is to catalyze the breakdown of complement factor C3 into C3a [4] and activate the complement alternative pathway [5]. Adipsin was later identified as complement factor D [6], which plays an important role in the immune system [7]. Obesity and type 2 diabetes (T2D) are associated with chronic inflammation [8]. Despite an increasing awareness of the interplay between immune system and adipose cell biology [9], the role of adipsin in glucose homeostasis remains unclear.

Activation of the complement pathway has been consistently observed in *ob/ob* mice and high-fat diet-induced obese mice [10]. In contrast, protection against obesity, reduction in adipose inflammation, and improvement in insulin sensitivity were observed in studies using mice deficient in C3aR1 or treated with an antagonist of the receptor [11, 12]. Lo *et al.* [13] recently reported that ablation of adipsin in diabetic mice led to insulinopenia and exacerbation of diabetes, whereas restoration of adipsin augmented insulin secretion and improved glucose homeostasis. The authors observed that patients with T2D and β-cell function failure (on insulin therapy) had a lower circulating adipsin level than patients with T2D on metformin therapy, and concluded that adipsin improved β-cell function in diabetes.

Given the aforementioned inconsistent findings, and the limited data in human subjects [14–16] regarding the effects of adipsin on glucose metabolism, more studies are needed to elucidate the mechanism by which adipsin may affect glucose homeostasis. In this study, we aimed to investigate the association between adipsin and insulin resistance/β-cell function in patients with no history of diabetes who underwent an oral glucose tolerance test (OGTT).

1. Subjects and Methods

We enrolled subjects with no history of diabetes who underwent an OGTT to screen for abnormal glucose regulation at our outpatient clinic. Outpatients with known T2D on diet control or metformin monotherapy were enrolled to compare serum adipsin level between patients with known and newly diagnosed abnormal glucose regulation. A fasting blood sample was collected from all study subjects for the measurement of serum adipsin. Written informed consent was provided by all study subjects. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital, Taichung, Taiwan, and was conducted in accordance with the Declaration of Helsinki.

A standard 75-g OGTT [17] was conducted in subjects with no history of diabetes after an overnight fast. A fasting blood sample was collected for the measurement of glycated hemoglobin (HbA1c), insulin, and adipsin. For outpatients with known T2D, levels of serum adipsin were determined using fasting blood samples collected for measurements of fasting plasma glucose (FPG) and HbA1c at their outpatient clinic visit. Relevant clinical parameters, such as body mass index (BMI), systolic and diastolic blood pressure, duration of diabetes, and lipids profiles, were collected from hospital records.

Serum adipsin was determined using an ELISA kit (Quantikine® Human Complement Factor D Immunoassay, Catalog Number DFD00; R&D Systems, Minneapolis, MN) [18] following the manufacturer's instructions. The mean minimal detectable concentration of adipsin was 0.013 ng/mL, and the intra- and interassay coefficients of variation were <6.4% and <9.0%, respectively. Plasma glucose was measured using the glucose oxidase-peroxidase method (Wako Diagnostics, Tokyo, Japan). The intra- and interassay coefficients of variation for glucose (range, 0 to 800 mg/dL) were both <1.5%. Plasma insulin was determined using an

electrochemiluminescence immunoassay (Elecsys 2010; Roche Diagnostics, Indianapolis, IN). The intra- and interassay coefficients of variation for insulin were 1.8% and 2.5%, respectively. HbA1c was measured by boronate-affinity high-performance liquid chromatography (CLC385TM, Primus Corporation, Kansas City, MO). The intra- and interassay coefficients of variation for HbA1c (range, 4.2% to 19.6%) were <0.9% and <2.9%, respectively.

For subjects with no history of diabetes, their glucose regulation status was determined according to the results of OGTT and HbA1c, as recommended by the American Diabetes Association [19]. We assessed insulin resistance and β-cell function using the homeostasis model assessment [20] of insulin resistance and β-cell function (HOMA-IR and HOMA-β, respectively). HOMA-IR = fasting insulin [μU/L] * fasting glucose [mmol/L] / 22.5. HOMA-β = $20 \times$ fasting insulin [μU/L] / (fasting glucose [mmol/L] − 3.5).

The Statistical Package for the Social Sciences (IBM SPSS version 22.0; International Business Machines Corp, NY) was used for all of the statistical analyses. Continuous variables are reported as mean ± SD and categorical data are given as number (percentage). The Student t test or one-way ANOVA (posthoc analysis using the Bonferroni test) was used to test statistically significant differences in continuous variables between groups, whereas the χ^2 test was used for categorical variables. A generalized linear model with fixed effect was used to examine the association of adipsin with insulin resistance (HOMA-IR) and β-cell function (HOMA-β). Interactions of clinical parameters on the association between adipsin and HOMA-IR/HOMA-β were also examined using a generalized linear model. In all of the statistical analyses, a two-sided P value of less than 0.05 was considered statistically significant.

2. Results

From July 2013 to January 2015, a total of 320 subjects were enrolled in this study. Table 1 shows the distribution of the study population. Among the 320 subjects included in this study, 240 subjects with no history of diabetes underwent an OGTT and HbA1c test to determine their glucose regulation status (group 1~group 3), whereas 80 subjects with known T2D were on diet control or metformin monotherapy (group 4 and group 5, respectively). Table 2 shows the characteristics of the study subjects according to their glucose regulation status. Subjects with normal glucose tolerance (group 1) had a lower BMI, a lower systolic and diastolic blood pressure, lower levels of triglycerides, FPG, and HbA1c, as well as a higher level of serum adipsin, compared with subjects with prediabetes (group 2), newly diagnosed diabetes (group 3), or known T2D (group 4 and group 5). Among subjects who had undergone an OGTT (group 1~3), 2-hour plasma glucose was lower, whereas HOMA-β was higher in subjects in group 1, compared with subjects in group 2 and group 3. There was no significant between-group difference in HOMA-IR.

Figure 1 shows levels of serum adipsin in subgroups of the study population by gender, BMI, FPG, 2-hour plasma glucose, and HbA1c. Overall, the mean adipsin level was 3.7 μg/mL. There was no significant between-group difference in serum adipsin levels when study subjects were divided into two groups by gender (male vs female) or BMI (<25 vs ≥25 kg/m2).

Table 1. Distribution of the Study Population

All Participants	N = 320
Participants with no history of diabetes	n = 240
Normal glucose tolerance by OGTT and HbA1c (group 1, n = 58)	
Prediabetes by OGTT and HbA1c (group 2, n = 140)	
Newly diagnosed diabetes by OGTT and HbA1c (group 3, n = 42)	
Participants with known T2D	n = 80
On diet control (group 4, n = 40)	
On metformin monotherapy (group 5, n = 40)	

Table 2. Characteristics of the Study Population According to Their Glucose Regulation Status

| Variable | Subjects With No History of Diabetes | | | Subjects With Known T2D | | P |
	Group 1 (NGT)	Group 2 (Prediabetes)	Group 3 (New Diabetes)	Group 4 (on Diet Control)	Group 5 (on Metformin)	
N	58	140	42	40	40	
Age, y	58.6 ± 12.1	61.3 ± 11.5	63.0 ± 12.1	62.3 ± 10.5	63.7 ± 10.1	0.180
Male, n (%)	43 (74.1)	117 (83.6)	36 (85.7)	34 (85.0)	34 (85.0)	0.468
BMI, kg/m^2	24.9 ± 3.1	26.1 ± 3.7	27.1 ± 3.7	26.9 ± 3.5	26.6 ± 3.4	0.017
Systolic BP, mm Hg	122 ± 19	128 ± 18	129 ± 19	131 ± 19	133 ± 11	0.047
Diastolic BP, mm Hg	73 ± 10	75 ± 11	74 ± 10	80 ± 14	79 ± 9	0.007
Smoking, n (%)	7 (12.1)	12 (8.6)	6 (14.3)	5 (12.5)	7 (17.5)	0.565
Diabetes duration, y	N/A	N/A	N/A	4.4 ± 5.3	4.8 ± 6.8	<0.001
Total cholesterol, mg/dL	177 ± 50	168 ± 33	165 ± 40	184 ± 42	175 ± 36	0.154
LDL cholesterol, mg/dL	115 ± 45	106 ± 30	100 ± 28	114 ± 38	110 ± 34	0.187
HDL cholesterol, mg/dL	48 ± 14	47 ± 14	43 ± 11	47 ± 12	46 ± 10	0.289
Triglycerides, mg/dL	128 ± 72	132 ± 74	171 ± 156	203 ± 176	147 ± 63	0.002
Fasting PG, mg/dL	88 ± 6	94 ± 7	108 ± 19	132 ± 46	139 ± 33	<0.001
OGTT 2-h PG, mg/dL	112 ± 21	144 ± 31	229 ± 45	N/A	N/A	<0.001
HbA1c, %	5.3 ± 0.2	5.7 ± 0.4	6.4 ± 0.7	7.0 ± 1.4	7.3 ± 1.4	<0.001
HOMA-IR[a]	2.3 ± 1.2	2.6 ± 2.0	3.0 ± 1.9	N/A	N/A	0.129
HOMA-β[a]	152 ± 82	128 ± 93	121 ± 110	N/A	N/A	0.009
Adipsin, µg/mL[a]	4.0 ± 1.1	4.0 ± 1.5	3.8 ± 1.1	3.4 ± 1.0	3.0 ± 1.0	<0.001

Values are mean ± SD or n (%).
Abbreviations: BP, blood pressure; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; IR, insulin resistance; LDL, low-density lipoprotein; N/A, not applicable; NGT, normal glucose tolerance; PG, plasma glucose.
[a]Log transformed (base 10) before analysis.

When we grouped study subjects according to their FPG, 2-hour plasma glucose, and HbA1c levels, we observed that mean serum adipsin level was higher in subjects with a FPG <100 mg/dL or an HbA1c <5.7%, compared with those who had a FPG ≥100 m/dL or an HbA1c ≥5.7%. However, there was no significant between-group difference in serum adipsin level when study subjects were grouped according to their 2-hour plasma glucose (<140 vs ≥140 mg/dL).

We examined the associations of serum adipsin level with HOMA-β and HOMA-IR. There was no significant association between adipsin and HOMA-β (β coefficient, −0.320; 95% CI, −0.645 to 0.006; $P = 0.054$). In contrast, there was a significant negative association between adipsin and HOMA-IR (β coefficient, −0.449; 95% CI, −0.762 to −0.135; $P = 0.005$, Table 3). The negative association between adipsin and HOMA-IR remained significant after adjustment for confounders (β coefficient, −0.414; 95% CI, −0.720 to −0.109; $P = 0.008$; Table 3), such as age, gender, and BMI.

We examined the effects of gender, BMI, FPG, 2-hour plasma glucose, and HbA1c on the negative association between adipsin and HOMA-IR. As shown in Table 4, we found that gender, 2-hour plasma glucose, and HbA1c had no significant effect on the negative association between adipsin and HOMA-IR (P interaction > 0.05). In contrast, both BMI and FPG had a significant effect (P interaction < 0.001 and 0.014, respectively). A negative association between adipsin and HOMA-IR was observed in subjects with a BMI ≥25 kg/m^2 or a FPG ≥100 mg/dL.

3. Discussion

In this study, we demonstrated that serum adipsin was negatively associated with insulin resistance (HOMA-IR), especially in subjects with a BMI ≥25 kg/m2 or a FPG ≥100 mg/dL.
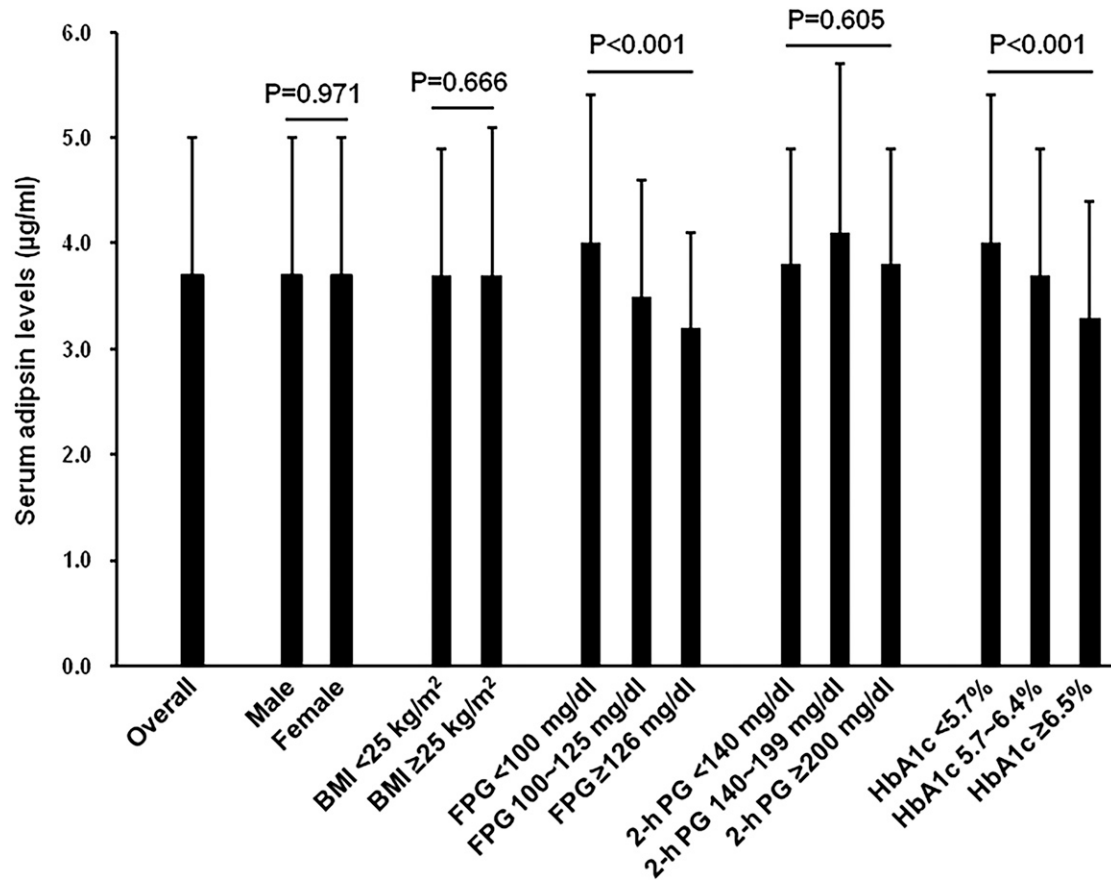
Figure 1. Levels of serum adipsin[a] in subgroups of the study population. Error bars represent one SD. 2-h PG, OGTT 2-h plasma glucose. [a]Log transformed (base 10) before analysis.

Lo *et al.* [13] recently reported that adipsin improved β-cell function in diabetic mice, and a lower circulating adipsin level was observed in patients with T2D and β-cell function failure (on insulin therapy), compared with those with T2D on metformin therapy. However, they did not report an association between circulating adipsin and β-cell function/insulin resistance in human subjects. In this study, we observed that serum adipsin level was highest in subjects with normal glucose tolerance, and was lower in subjects with newly diagnosed diabetes, with known T2D patients having the lowest level (Table 2). Nevertheless, we found that adipsin

Table 3. Generalized Linear Model With HOMA-IR[a] as the Dependent Variable

Independent Variable	β Coefficient	95% CI	*P*
Adipsin (µg/mL)[a]			
Model 1	−0.449	−0.762, −0.135	0.005
Model 2	−0.477	−0.773, −0.180	0.002
Model 3	−0.471	−0.777, −0.165	0.003
Model 4	−0.414	−0.720, −0.109	0.008

Model 1, unadjusted; Model 2, adjusted for age, gender, and BMI; Model 3, adjusted for variables in Model 2 plus systolic blood pressure, smoking, total cholesterol, and triglycerides; Model 4, adjusted for variables in Model 3 plus FPG and HbA1c.
Abbreviations: HOMA, homeostasis model assessment. IR, insulin resistance.
[a]Log transformed (base 10).

Table 4. Association[a] Between Adipsin and HOMA-IR[b] in Subgroups of the Study Population

Independent Variable	β Coefficient	95% CI	P Interaction
Adipsin (μg/mL)[b]	−0.414	−0.720, −0.109	
Subgroups			
Gender			0.307
Male	−0.563	−0.901, −0.225	
Female	0.134	−0.667, 0.936	
BMI			<0.001
<25.0 kg/m^2	0.318	−0.143, 0.778	
≥25.0 kg/m^2	−0.840	−1.273, −0.406	
FPG			0.014
<100 mg/dL	−0.244	−0.599, 0.112	
≥100 mg/dL	−0.704	−1.332, −0.076	
2-h plasma glucose			0.122
<140 mg/dL	−0.427	−0.839, −0.014	
≥140 mg/dL	−0.441	−0.908, 0.027	
HbA1c			0.338
<5.7%	−0.144	−0.566, 0.278	
≥5.7%	−0.789	−1.249, −0.329	

Abbreviations: HOMA, homeostasis model assessment; IR, insulin resistance.
[a]Adjusted for age, gender, BMI, systolic blood pressure, smoking, total cholesterol, triglycerides, FPG, and HbA1c.
[b]Log transformed (base 10).

was not associated with β-cell function (HOMA-β). In contrast, adipsin was negatively associated with insulin resistance (HOMA-IR), independent of several confounders (Table 3).

Consistent with our results, Zhou *et al.* [16] recently reported that levels of serum adipsin were lower in Chinese subjects with newly diagnosed T2D and impaired glucose tolerance, compared with those with normal glucose tolerance. Although the total number of patients in their study was relatively small (n = 137), the authors observed a positive correlation between adipsin and HOMA-β, whereas a negative correlation between adipsin and HOMA-IR. Their finding of the association between circulating adipsin levels and insulin resistance was not consistent with previous reports. In a study conducted in 74 pregnant women [14], the authors reported a positive correlation between fetal adipsin concentrations and fetal/maternal HOMA-IR. In contrast, another study conducted in 379 adults [15] reported no significant association between circulating adipsin and HOMA-IR. The inconsistent findings might be explained in part by the different study populations. Our study has several strengths. First, we recruited a relatively large number of study subjects. Second, we enrolled subjects with no history of diabetes and subjects with known T2D for comparison. Third, we conducted an OGTT in subjects with no history of diabetes to confirm their glucose regulation status. The negative association between adipsin and HOMA-IR in subjects with no history of diabetes suggests that adipsin may be involved in the pathogenesis of abnormal glucose metabolism.

The mechanism by which adipsin was negatively associated with HOMA-IR might be related to inflammation. T2D is a chronic inflammatory disease [21, 22], whereas insulin resistance is a key factor that leads to deterioration of glucose homeostasis in the early course of the disease [23, 24]. It has been reported that inflammatory cytokines are elevated in patients with T2D. For example, IL-17, which was reported to suppress differentiation of preadipocytes and impair adipsin expression in adipocytes [25], was elevated in patients with T2D [26, 27]. Furthermore, IL-17 has been associated with insulin resistance in animal models [28]. The aforementioned results in previous studies may help to explain our findings that showed decreased adipsin in subjects with newly diagnosed diabetes and known T2D (Table 2), and there was an independent negative association between adipsin and HOMA-IR (Table 3). Interestingly, the negative association between adipsin and HOMA-IR was observed in subjects with a BMI ≥25 kg/m2 or a FPG ≥100 mg/dL (Table 4). This finding may not be surprising, as both obesity and FPG are known to be associated with insulin resistance [29, 30] in human subjects.

There are several limitations in this study. First, the cross-sectional study design did not allow us to draw conclusions about adipsin in the pathogenesis of T2D. This issue needs to be addressed in prospective studies. Second, we did not measure inflammatory markers or cytokines. Thus, the association between inflammation, adipsin, and insulin resistance could not be investigated in this study. Finally, we enrolled subjects with no history of diabetes who underwent an OGTT to screen for abnormal glucose regulation. Our results may not be generalized to other populations, such as patients with long-standing diabetes or chronic diabetes complications, and therefore further studies are needed.

In summary, we demonstrated that serum adipsin was negatively associated with insulin resistance (HOMA-IR), especially in subjects with a BMI $\geq$25 kg/m2 or a FPG $\geq$100 mg/dL. Our findings suggest that adipsin may be involved in the pathogenesis of abnormal glucose metabolism, and therefore further investigation is warranted.

Acknowledgments

References and Notes

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