Relationship between serum secreted frizzled-related protein 4 levels and the first-phase of glucose-stimulated insulin secretion in individuals with different glucose tolerance

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Abstract. Recent evidence suggests that serum secreted frizzled-related protein (SFRP) 4 may affect β-cell function. In a cross-sectional clinical study, 56 subjects with type 2 diabetes mellitus (T2DM), 52 subjects with impaired glucose tolerance (IGT) and 42 normal glucose tolerance (NGT) subjects were enrolled to investigate the relationship between SFRP4 levels and the first-phase of glucose-stimulated insulin secretion, glucose metabolism and inflammation. Intravenous glucose tolerance tests were conducted, and acute insulin response (AIR), the area under the curve of the first-phase (0-10 min) insulin secretion (AUC), and the glucose disposition index (GDI) were calculated. The serum levels of SFRP4, IL-1β, plasma glucose, serum lipid, and glycated hemoglobin (HbA1c) were measured. Levels of serum SFRP4 and IL-1β in the T2DM group and IGT group were significantly higher than those in the NGT group ($P < 0.01$). The AIR, AUC and GDI between the three groups showed a progressive decrease from the NGT to IGT groups with the lowest value in the T2DM groups ($P < 0.01$). The serum SFRP4 levels were negatively correlated with AIR, AUC, GDI and HOMA-β ($P < 0.01$) and were positively correlated with fasting plasma glucose, HbA1c, hs-CRP, and IL-1β ($P < 0.01$). Our study provides evidence that the concentrations of serum SFRP4 in T2DM and IGT subjects were increased and were correlated closely with glycose metabolic disorder, the first-phase of glucose-stimulated insulin secretion and chronic low-grade inflammation. SFRP4 may participate in the development of type 2 diabetes mellitus.

Key words: Secreted frizzled-related protein 4, First-phase of insulin secretion, Intravenous glucose tolerance test, IL-1β, Diabetes

TYPE 2 DIABETES MELLITUS (T2DM) is a heterogeneous disorder characterized by β-cell dysfunction and insulin resistance. A defect in β-cell function can be found in patients with impaired glucose tolerance (IGT) and with newly diagnosed T2DM [1]. Progressive β-cell dysfunction occurs in the natural course of T2DM, but the detailed molecular mechanisms involved are not entirely understood.

Increasing evidence suggests that chronic low-grade inflammation, which is activated by metabolic stress, plays a crucial role in the development of T2DM and

is closely linked with glucotoxicity, lipotoxicity, oxidative stress, and endoplasmic reticulum (ER) stress in the pathogenesis of type 2 diabetes [2-5]. Several studies have found abnormally high circulating levels of proinflammatory cytokines, such as IL-1β, IL-6, and CRP, which may induce the activation of the innate immune system in type 2 diabetic patients and potentiate the inflammatory mechanisms in pancreatic islets as well as in peripheral tissues [6]. On the one hand, hyperglycemia and/or elevated free fatty acid levels induce the production and secretion of various proinflammatory mediators, such as IL-1β, TNF-a, IL-6, and IL-1 dependent cytokines in pancreatic islets [4, 7]. IL-1β is a pivotal cytokine that stimulates β-cells to produce and secrete more IL-1β and increases nitric oxide production, leading to β-cell dysfunction and reduced insulin secretion [8]. On the other hand, dysfunctional adipocytes in obesity also contribute to elevated circulating inflammatory cytokines, potentially affecting β-cell

function [9]. Adipocytokines are uniquely produced by adipocytes, such as leptin, adiponectin, omentin, and visfatin, and may contribute to β-cell dysfunction during insulin resistance and connect adipose tissue inflammation to β-cell failure [10, 11].

T2DM may be referred to as a chronic auto-inflammatory disease, and inflammatory cytokines play an important role in the process of β-cell dysfunction [12]. Therefore, anti-inflammatory drugs may offer attractive advantages to preserving β-cell mass and function rather than just palliating the hyperglycemia. Consequently, there is a need for a better understanding of mechanisms that may help identify new therapeutic targets for the treatment of β-cell dysfunction.

A new inflammation mediator, called secreted frizzled-related protein (SFRP) 4, was recently found, which affects insulin secretion in human and mouse β-cells as an extracellular regulator of the Wingless (Wnt) pathway. SFRP4 expression and release from islets is stimulated by IL-1β [13] and is connected to chronic low-grade inflammation and β-cell dysfunction. Adipose tissue contributes by elevating circulating SFRP4 in obesity [14]. SFRP4 may be a new adipocytokine, which also relates adipose tissue inflammation to β-cell dysfunction. Thereby, SFRP4 may be a biomarker of β-cell dysfunction and a potential therapeutic target for preserving β-cell function. However, until now, available data on SFRP4 have been limited, especially the relationship between serum SFRP4 and β-cell function in human subjects. One of the most reliable methods of measuring β-cell function is the intravenous glucose tolerance test (IVGTT). The first-phase of insulin secretion (0-10 min) is particularly important in terms of β-cell function, and the acute insulin response may be a better indicator of early β-cell dysfunction than HOMA-β [1, 15]. Therefore, we initiated a study to investigate the relationships of serum SFRP4 levels with inflammation and the first-phase of glucose-stimulated insulin secretion from pancreatic β-cells in individuals with different glucose tolerance.

Materials and Methods

Subjects

A total of 150 Chinese volunteers were enrolled in the study and were divided into three groups according to their glucose tolerance status: normal glucose tolerance (NGT) (n =42), impaired glucose tolerance (IGT) (n =52), or Type 2 diabetes mellitus (T2DM) (n =56).

All subjects also underwent intravenous glucose tolerance testing (IVGTT) to assess the first-phase of glucose-stimulated insulin secretion. The IGT and T2DM patients were newly diagnosed and did not receive any treatment, including diet, exercise and medication. The diagnoses of T2DM and IGT were based on the diagnostic criteria of the World Health Organization (WHO) in 1999 [16].

Exclusion criteria

(1) Acute and chronic complications of diabetes.
(2) Hepatic or renal disease, sustained hypertension, or coronary heart disease.
(3) History of tumor diseases.
(4) Systemic corticosteroid treatment.
(5) Women who were currently pregnant, breastfeeding or taking contraceptive pills.

The study was approved by the Ethical Committee of Chongqing Medical University. Signed informed consent was obtained from all participants in this study.

Clinical and biochemical evaluations

Body weight, height, waist and hip circumferences, and blood pressure (BP) were measured in all subjects with 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 subjects seated. The body mass index (BMI) and the waist to hip ratio (WHR) were also calculated.

Blood samples were collected after overnight fasting. Serum samples were obtained by centrifugation at 4°C and were stored at -80°C. Plasma glucose was determined by the glucose oxidase method. Fasting insulin (FINS) was measured by the chemiluminescence method. HbA1c was measured by isoelectric focusing. The triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and high-sensitivity C-reaction protein (hs-CRP) concentrations were assayed by enzymatic methods.

Assessment of serum SFRP4 and IL-1β concentrations

Serum SFRP4 and IL-1β levels were determined by enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions (Human ELISA kit, Wuhan USCN Science Co., Ltd., China). The intra-assay coefficient of variation was 10%, and the inter-assay coefficient of variation was 12%. No sig-

nificant cross-reactivity or interference was observed.

Intravenous glucose tolerance test (IVGTT)

IVGTT was performed to estimate the first-phase of insulin secretion after an overnight fast. Subjects had a diet containing at least 150 g of carbohydrate/day for the 3 days before the test. A 50% glucose solution was infused as a square wave bolus over 3 min (min) ±15 s with a dose of 300 mg/kg body weight glucose (maximum dose 35 g). Repeat blood samples for the determination of the glucose and insulin levels were drawn before (0 min) and at 3, 5, 8, and 10 min after glucose infusion [15, 17].

Related calculation formulas

BMI was expressed as the weight per height squared (kg/m^2); WHR was calculated as the ratio of the waist and hip circumferences.

The homeostasis model assessment of IR (HOMA-IR) and β-cell function (HOMA-β) were calculated from the fasting insulin and glucose levels using the following equation: HOMA-IR =Fasting insulin (μU/mL) × Fasting plasma glucose (mmol/L)/22.5, HOMA-β =20×Fasting insulin (μU/mL)/[Fasting plasma glucose (mmol/L) − 3.5].

The insulin secretory capacity was evaluated by the acute insulin response (AIR), the area under the curves (AUC) of the plasma insulin concentration during IVGTT and the glucose disposal index (GDI), which was used to adjust the insulin secretion for the degree of insulin sensitivity. AIR was calculated as the mean plasma insulin concentration at 3 and 5 min after the administration of glucose, and this value correlates with the MINMOD-derived AIR [18]. The AUC for the plasma insulin concentrations was calculated by the linear trapezoid method. GDI was calculated as $\log_{10}$[AIR×Fasting plasma glucose (mmol/L)/ Fasting insulin (μU/mL)], which is similar to the correction of AIR using the insulin sensitivity index that is used in the minimal model intravenous glucose tolerance test for glucose utilization [19, 20].

Statistical analysis

The results were presented as the mean and the standard deviation (SD) or as the median (interquartile range) when there was a skewed distribution. To improve the discrimination and calibration of the models and to minimize the influence, continuous variables with skewed distribution were log-transformed for statistical analysis. One-way analysis of variance (ANOVA) was used for group comparisons. Interrelationships between variables were analyzed by Pearson correlation analysis and partial correlation analysis. All calculations were performed by SPSS 19.0 for Windows. *P* values <0.05 (two tailed) were reported as statistically significant.

Results

The clinical characteristics

The anthropometric and the biochemical characteristics of 150 subjects, according to glucose tolerance status, are shown in Table 1. There were no differences regardless of age and gender between all groups (*P*>0.05). However, the T2DM groups showed a higher value of BMI than the NGT group (*P*<0.05), and WHR showed a significant difference between the NGT and T2DM groups, the IGT and T2DM groups, and the NGT and IGT groups with the highest value of WHR in the T2DM groups (*P*<0.05 or *P*<0.01). There was a statistically significant difference in the diastolic blood pressure and systolic blood pressure between the NGT and T2DM groups (*P*<0.01), and the systolic blood pressure showed a significant difference between the NGT and IGT groups (*P*<0.01). Diastolic blood pressure showed a significant difference between the IGT and T2DM groups (*P*<0.05). There was a statistically significant difference of FPG, 2hPG, HbA1c, HOMA-IR, hs-CRP, and IL-1β between the three groups, with a progressive increase from the NGT to IGT groups with the highest value in the T2DM groups (*P*<0.01). Compared to the NGT groups, the IGT groups had lower HOMA-β levels with the lowest levels in the T2DM groups (*P*<0.01). The TC and LDL-C concentrations showed no significant difference between all groups (*P*>0.05). However, the TG levels were higher in the T2DM groups and IGT groups than in NGT groups (all *P*<0.01). HDL-C concentrations were significantly lower in the T2DM groups than in the NGT and IGT groups (all *P*<0.01).

Serum SFRP4 levels in different glucose tolerance status

Fig. 1 shows the serum levels of SFRP4 according to glucose tolerance status. The serum SFRP4 concentrations showed a significant difference between the NGT and T2DM groups, the NGT and IGT groups, and the IGT and T2DM groups (all *P*<0.01). There was a progressive increase from the NGT to IGT groups with

Table 1 The clinical characteristics and the biochemical indicators of the study subjects

Factor	NGT	IGT	T2DM
n (M/F)	42 (20/22)	52 (27/25)	56 (29/27)
Age (year)	51.33 ± 11.54	53.21 ± 11.08	50. 59 ± 10.98
BMI (Kg/m^2)	22.91 ± 2.56	23.10 ± 3.20	24.20 ± 3.53^a
WHR	0.87 ± 0.05	0.90 ± 0.06^a	0.93 ± 0.07^{bd}
SBP (mm Hg)	120.26 ± 11.24	129.37 ± 15.59^b	131.05 ± 15.04^b
DBP (mm Hg)	79.95 ± 7.80	81.35 ± 8.00	84.93 ± 8.92^{bc}
FPG (mmol/L)*	5.17 (4.85-5.29)	6.29 (5.79-6.50)^b	8.40 (7.59-9.86)^{bd}
2hPG (mmol/L)*	4.61 (4.24-5.06)	8.40 (8.15-8.97)^b	10.02 (8.54-11.31)^{bd}
FINS (uU/mL)*	4.99 ± 1.27	5.60 ± 1.36	6.58 ± 3.06^{bc}
HbA$_1$c (%)*	5.70 (5.60-5.80)	6.10 (5.90-6.48)^b	9.20 (7.48-10.60)^{bd}
HOMA-IR*	1.14 ± 0.31	1.53 ± 0.43^b	2.57 ± 1.15^{bd}
HOMA-β*	61.19 (50.39-76.73)	41.01 (36.77-53.32)^b	24.96(17.23-33.65)^{bd}
TG (mmol/L)*	1.15 (1.00-1.48)	1.56 (1.11-1.82)^b	1.78 (1.08-2.41)^b
TC (mmol/L)	4.84 ± 0.81	4.92 ± 0.92	4.72 ± 1.00
HDL-C(mmol/L)*	1.47 (1.32-1.49)	1.33 (1.25-1.56)	1.12 (0.97-1.32)^{bd}
LDL-C (mmol/L)	2.69 ± 0.89	2.90 ± 0.63	2.72 ± 0.89
hs-CRP (mg/L)	0.94 ± 0.49	1.64 ± 0.81^b	2.15 ± 1.05^{bd}
IL-1β (pg/mL)	4.32 ± 1.71	6.65 ± 2.42^b	7.81 ± 1.56^{bd}

Data are presented as the means±SD and [$M(P_{25}, P_{75})$] when there was a skewed distribution. NGT, Normal glucose tolerance group; IGT, Impaired glucose tolerance; T2DM, Type 2 diabetes mellitus group; M, Male; F, Female; BMI, Body mass index; WHR, Waist-hip ratio; SBP, Systolic blood pressure, 1 mmHg = 0.133 kPa; DBP, Diastolic blood pressure; FPG, Fasting plasma glucose; *, The values had an abnormal distribution and were statistically analyzed after logarithmic transformation; 2hPG, 2 hour plasma glucose; FINS, Fasting insulin; HOMA-IR, Homeostasis model assessment for insulin resistance; HOMA-β, Homeostasis model assessment β-cell function; TC, Total cholesterol; TG, Triglyeerides; HDL-C, High density lipoprotein-cholesterol; LDL-C, Low density lipoprotien-cholesterol; hs-CRP, High-sensitivity C-reaction protein; ^aP <0.05, ^bP<0.01 compared with NGT. ^cP<0.05, ^dP<0.01 compared with IGT. One-way analysis of variance (ANOVA) was used for group comparison.
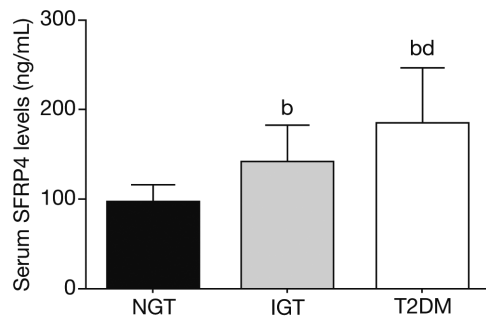

Fig. 1 Bars showing serum levels of SFRP4 according to glucose tolerance status. ^aP<0.05, ^bP<0.01 compared with NGT. ^cP<0.05, ^dP<0.01 compared with IGT. Data are presented as the means ± SD. One-way analysis of variance (ANOVA) was used for group comparison. SFRP4, Secreted frizzled-related protein 4. Groups are NGT, IGT and T2DM.

the highest value in the T2DM group for serum SFRP4 levels. The serum SFRP4 concentrations in the three groups were the following: NGT group 95.46 ± 20.13 ng/mL, IGT group 141.64 ± 40.46 ng/mL, and T2DM group 184.38 ± 61.34 ng/mL. However, there were no significant differences in the serum SFRP4 concen-

trations between men and women (146.25 ±55.16 *vs* 143.04 ± 60.70 ng/mL, *P*=0.735).

The first-phase insulin secretion in different glucose tolerance status groups

Fig. 2 shows the AIR, AUC, and GDI according to glucose tolerance status. AIR, AUC, and GDI showed significant differences between the NGT and T2DM, the NGT and IGT, and the IGT and T2DM groups (all *P*<0.001). The AUC in the three groups were the following: NGT group [408.06 (218.87, 570.55) μU/mL], IGT group [180.41 (131.99, 238.93) μU/mL], and T2DM group [78.19 (43.55, 107.47) μU/mL].

Relationships between the serum SFRP4 levels and the first-phase insulin secretion, metabolic and inflammatory parameters

Bivariate correlation analysis revealed that the serum SFRP4 levels were positively correlated with the levels of WHR, FPG, 2hPG, HbA1c, hs-CRP, and IL-1β (*r*=0.241, 0.587, 0.608, 0.538, 0.424, 0.552, respectively, *P*<0.01) and were correlated negatively significantly with HDL-C, HOMA-β, AIR, AUC, and
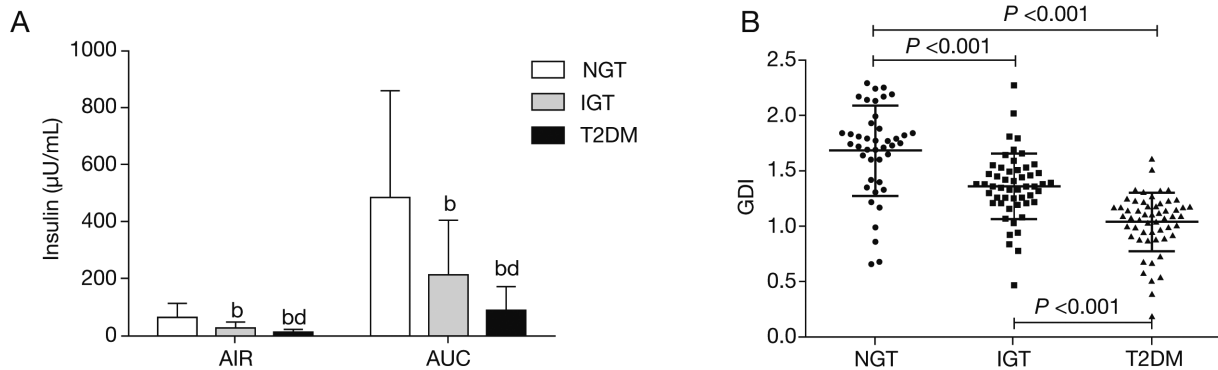
Fig. 2 The data show the first-phase insulin secretion with different glucose tolerance status. ANOVA analysis of the AIR and AUC levels (A) and GDI levels (means ± SD) (B) according to glucose tolerance status. The AIR and AUC had abnormal distributions and were statistically analyzed after logarithmic transformation. [a]$P<0.05$, [b]$P<0.01$ compared with NGT. [c]$P<0.05$, [d]$P<0.01$ compared with IGT. AIR, Acute insulin response; AUC, The area under the curve of the first phase insulin secretion; GDI, Glucose disposal index. Groups are NGT, IGT and T2DM.

GDI (r=-0.215, -0.475, -0.559, -0.595, -0.511, respectively, all $P<0.01$). Serum SFRP4 was also negatively correlated with AUC in the three subgroups (r=-0.346, P=0.02 in the T2DM group; r=-0.442, P=0.001 in the IGT group; r=-0.335, P=0.03 in the NGT group, respectively). The serum SFRP4 levels were also positively correlated with HOMA-IR (r=0.359, $P<0.01$); however, there was no correlation of serum SFRP4 with HOMA-IR in the three subgroups (r=0.250, P=0.856 in the T2DM group; r=0.184, P=0.191 in the IGT group; and r=-0.079, P=0.617 in the NGT group). The positive correlation may be not reliable in all subjects. After adjustment for BMI, WHR, and HOMA-IR, there was a negative correlation of SFRP4 with HOMA-β, AIR, AUC, and GDI [r=-0.445, -0.487, -0.539 (Fig. 3A), -0.399 (Fig. 3B), respectively, all $P<0.01$], and there was a positive correlation of SFRP4 with FPG, 2hPG, IL-1β, hs-CRP, and HbA1c [r=0.482, 0.516, 0.508 (Fig. 3C), 0.322, 0.433 (Fig. 3D), respectively, all $P<0.01$]. AIR, AUC, and HOMA-IR were transformed by a base-10 logarithm.

Discussion

The secreted frizzled-related proteins (SFRPs), a family of soluble proteins, are approximately 30 kDa in size, and each contains a putative signal sequence followed by a frizzled-like cysteine-rich domain (CRD) [21]. There are five known members of the SFRPs family – SFRP1 to SFRP5. SFRPs are characterized as antagonists that bind to Wnt proteins to prevent signal activation through its sequence similarity with the Fz receptors [22]. Recently, Taneera et al. found

that SFRP4 contains a CRD homologous to the putative Wnt–binding site of frizzled protein – G protein-coupled receptor proteins related to the Wnt signaling pathway [23]. Several studies demonstrated that SFRP4 plays an important role in both physiological and pathological states, for example, in the homeostasis of phosphorus and inorganic phosphate, the proliferation and apoptosis of tumor cells and the promotion of epidermal differentiation [24-27]. Furthermore, Mahdi and colleagues identified SFRP4 as an early mediator of pancreatic β-cell dysfunction in T2DM through coupling of the global evaluation of gene expression with the co-expression network analysis of human islets from T2DM patients [13]. However, data on the relationship between the changes of circulating SFRP4 and the first-phase insulin secretion of glucose stimulation are still not distinct.

In our study, we found that subjects with T2DM and IGT showed statistically significant higher levels of serum SFRP4 than those of apparently healthy individuals with NGT (all $P<0.01$). We also observed that the serum SFRP4 concentrations were positively correlated to FPG, 2hPG, and HbA1c. Similarly, gene expression analysis in previous studies revealed that the expression of *SFRP4* in the islets of the donors was higher in hyperglycemia versus normoglycemia and was positively correlated with higher HbA1c levels [13, 23]. Furthermore, Maddi et al. found that T2DM subjects have increased levels of SFRP4 in the blood, even before the development of overt hyperglycemia [13]. These results indicate that the circulating SFRP4 concentrations are correlated with deteriorating glucose metabolism.
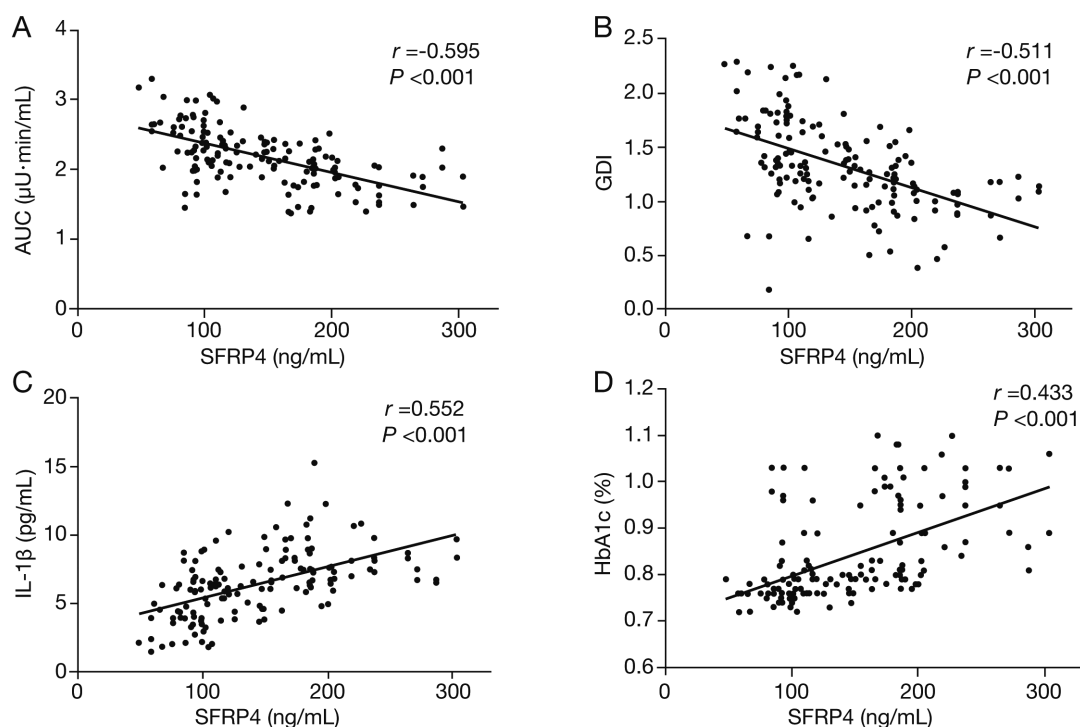
Fig. 3 Correlation between SFRP4 and the measured parameters. Interrelationships between variables were analyzed by Pearson correlation analysis. The variables presented are: AUC (A), GDI (B), IL-1β (C) and HbA1c (D), and the AUC were transformed by base-10 logarithm.

The deficit of first-phase (acute) insulin secretion is the main characteristic in the early stage of T2DM. A previous study indicated that the first-phase insulin secretion is a better indicator of early β-cell dysfunction than HOMA-β, and based on the first-phase insulin secretion results from the Insulin Resistance Atherosclerosis Study (IRAS), a decline in β-cell function may start even earlier (>10 years before diagnosis), and/or the decline in β-cell function over time may be much steeper than suggested from the UK Prospective Diabetes Study (UKPDS) [1]. In our study, we observed that serum SFRP4 levels were negatively and significantly correlated with AIR, AUC, and GDI, and the correlations were also found in the three subgroups. After adjustment for body fat parameters and insulin sensitivity, correlations were still found ($P<0.001$). There are minimal data on the relationship between SFRP4 and the first-phase (acute) insulin secretion in human subjects. However, Taneera *et al.* found that high expression of *SFRP4* in islets was associated with impaired insulin secretion, and recombinant SFRP4 inhibited *in vitro* insulin secretion by 30% and β-cell exocytosis by 50% in human islets [23]. Moreover, silencing of *SFRP4* in the clonal β-cell line

INS832/13 (76% ± 6% mRNA knockdown) enhanced glucose-stimulated insulin release by 25% ($P = 0.029$) [13]. SFRP4 led to an increase of the unphosphorylated β-catenin and TCF/LEF activation, which caused decreased Ca^{2+} channels expression. Moreover, further study showed that SFRP4 decreased expression of Ca^{2+} channels in pancreatic β-cells *via* Wnt signaling to reduce insulin secretion [13, 28]. Our findings confirmed that SFRP4 is associated with first-phase insulin secretion and β-cell dysfunction. The role of SFRP4 as a drug-target to improve first-phase insulin secretion function requires further evaluation. Furthermore, we found that higher serum SFRP4 levels showed the tendency of defective acute insulin secretion in normal glucose tolerance subjects; therefore, SFRP4 may be a simple means to assess acute insulin secretion failure. However, this conclusion requires more experiments in a larger sample size.

There are several morphological and therapeutic intervention studies that indicate a chronic, low-grade inflammatory state in islets of patients with type 2 diabetes characterized by the presence of cytokines, amyloid deposits, fibrosis, immune cells and β-cell apoptosis [12, 29-31]. IL-1β, a prototype proinflam-

matory cytokine, is a pivotal cytokine that regulates β-cell function and triggers and amplifies inflammatory responses [8]. Expression of *SFRP4* is associated with increased expression of inflammatory markers in pancreatic islets of donors. Mahai *et al*. identified SFRP4 was one of the group of T2DM-related genes enriched for IL-1-related genes, and human islets incubated with IL-1β showed a 1.8-fold elevation of SFRP4 mRNA and a 3-fold higher secretion of protein into the medium [13]. Another study found that overexpression of *SFRP4* was associated with increased expression of inflammatory markers in the pancreatic islets of donors [23]. These results revealed that SFRP4 might be an inflammatory mediator and was induced by IL-1β. The present study showed that serum IL-1β levels were higher in T2DM and IGT subjects than in those with normal glucose tolerance. Furthermore, we observed that SFRP4 was positively correlated with IL-1β ($P < 0.001$), which suggests that SFRP4 may be involved in the inhibition of insulin release by IL-1β. It may be linked to the chronic, low-grade inflammation in islets and defective insulin secretion.

In conclusion, our study confirmed that SFRP4 as a cytokine and adipokine was closely associated with the first-phase insulin secretion of pancreatic β-cells, glucose metabolism and inflammation. The levels of serum SFRP4 were increased in patients with T2DM and IGT. Therefore, it may be an important mechanism of β-cell dysfunction. SFRP4 may become a drug-target to improve the insulin secretion function in patients with T2DM and may be a bio-marker of β-cell dysfunction according to the previous proposal [32]. However, this is a cross-sectional clinical study with data presented as comparisons and correlations; the determination of the molecular mechanisms requires more basic experiments. We had only a small cohort of Chinese subjects, so the serum SFRP4 concentrations in a larger sample of multi-ethnic subjects should be measured.

Acknowledgment

Disclosure

No potential conflicts of interest relevant to this article were reported.

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