Pancreatic fat content is associated with β-cell function and insulin resistance in Chinese type 2 diabetes subjects

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Abstract. The pathogenesis of type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and β-cell dysfunction. Earlier studies reported that increased levels of pancreatic fat may lead to the development of β-cell dysfunction and insulin resistance. The present study aimed to demonstrate the relationship between pancreatic fat content (PFC) and insulin secretion and insulin resistance in Chinese subjects with T2DM. Seventy-eight T2DM subjects and 35 non-diabetic volunteers were recruited in this study. All subjects were subjected to an oral glucose tolerance test (OGTT). We also measured PFC and liver fat content (LFC) by three-point Dixon method (3p-Dixon), and we examined the relations between PFC and OGTT-derived parameters. T2DM subjects had higher PFC than non-diabetic subjects (p < 0.01). PFC was correlated with body mass index (BMI), liver fat content (LFC) and age in two groups, however, it was only positively associated with insulin secretion, insulin resistance, early- and late-phase insulin secretion in male T2DM subjects, but not in non-diabetic and female T2DM subjects. After adjusting for BMI, LFC and age, the association still existed (all p < 0.05). Furthermore, the relationship was more obvious in male T2DM subjects with a shorter course of disease. PFC was associated with β-cell dysfunction and insulin resistance in subjects with T2DM and was more obvious in male T2DM subjects with shorter duration of diabetes. Therefore, PFC might represent a potential risk factor for the development of T2DM.

Key words: Type 2 diabetes mellitus, Pancreatic fat, Three-point Dixon, Pancreatic β-cell function

THE PREVALENCE of diabetes has increased significantly in recent decades and the type 2 diabetes mellitus (T2DM) has increasing to 11.6% in China [1]. This is related to the increase in obesity, especially central obesity, in China [2]. The increased evidence demonstrated that the exaggerated accumulation of fat in non-adipose tissues, such as skeletal muscle and liver is related to the occurrence of T2DM [3-5]. However, the specific role of the different fat depots in the development of diabetes is still not fully understood. As an important organ of metabolism, pancreas has recently gained much attention [6-8]. Some studies showed that PFC correlated with insulin resistance and insulin sensitivity, which may lead to the occurrence of T2DM [9-11], yet they were focused on the metabolically healthy obese (MHO) individuals, prediabetes, and newly diagnosed diabetes. Information regarding the pathological relevance of PFC in Chinese subjects with T2DM is limited, who always show an early phase secretion defect [12] and are found to have a higher content of visceral fat than the European or American populations even with the same BMI [13]. Pathological biopsy is the gold standard for pancreatic fat quantification [14], however, it is invasive and could not be widely used in clinics. Most of the previous studies used ultrasound, computed tomography (CT) and hydrogen proton magnetic resonance spectroscopy (1H-MRS) to quantify the fat content in the pancreas [10, 11, 15], while the accuracy of ultrasound and CT is poor and 1H-MRS is more accurate to determine the content of liver fat rather than pancreatic fat [16]. With the development of imaging technology, Dixon-based magnetic resonance imaging (MRI) method is able to provide a good quantitative surrogate of fat accumulation in pancreas, and can provide error-free decomposition of water and fat proton images even in the presence of off-resonance conditions resulted from susceptibility differences, demagnetization, or shim errors [17]. Furthermore, three-point Dixon (3p-Dixon) method allows the correction for T2* decay [13, 14] and is therefore more accurate than two-point Dixon [18, 19]. Given these, the aims of this study were to assess the
association of PFC that was quantified by 3p-Dixon technology in Chinese T2DM subjects with the parameters related to insulin secretion and insulin resistance as well as to sort out the potential factors that may influence their associations.

**Subjects**

Seventy-eight Chinese subjects with T2DM, aged 30–70 years, who were diagnosed based on the Guidelines for the prevention and treatment of type 2 diabetes mellitus in China (2010 Edition), were recruited from the Endocrinology Department of Zhongda Hospital from 2015 to 2017. Subjects with the following conditions or diseases were excluded: 1) having been treated with insulin secretagogues or insulin in the past week (considering the half-life of the drug); 2) any acute complications, such as ketoacidosis, hyperosmotic coma; 3) a history of pancreatic cancer or pancreatitis; 4) contraindications to MRI. Thirty-five volunteers who had not been diagnosed with diabetes and prediabetes were considered as healthy controls. All these participants provided written informed consent to participation in the study. The local ethics committee approved the study (2016ZDSYLL061-P01).

**Material and Methods**

**Anthropometric measurements**

Waist circumference (WC) was measured and body mass index (BMI, in kg/m²) was calculated. Systolic and diastolic blood pressures (SBP and DBP, respectively) were recorded in the supine position. After an overnight 12-h fasting, all subjects were taken routine biochemistry, including total cholesterol, triglyceride, high-density lipoprotein, and low-density lipoprotein.

**Quantification of pancreatic fat**

Pancreatic fat was quantified using 3-point Dixon method where three gradient-echo scans are acquired with adjacent out-of-phase and in-phase echoes [20] (repetition time = 9.25 ms; echo times = 2.45, 3.67, 7.35 ms; flip angle = 9°; partition thickness = 5 mms; number of excitations = 1; field of vision = 400*400 mm; bandwidth = 270 Hz/pixel). The subjects were examined in the supine position and required 17 s and 21 s of breath holding. The regions of interest (ROIs) focused on were the caput, corpus, and cauda of the pancreas. A representative MRI highlighting the pancreatic fat area measured is presented in Fig. 1 (The white circle in picture A represents the caput, and in picture B represent the corpus and cauda, respectively). The mean fat fraction of the pancreas was calculated as $I_{\text{fat}} / (I_{\text{fat}} + I_{\text{water}}) \times 100\%$. ($I_{\text{fat}}$ represents the signal value of the image fat; $I_{\text{water}}$ represents the signal value of image water).

**OGTT**

After the MRI measurements, 75-g oral glucose tolerance test (OGTT) were performed, with blood samples drawn at 0, 30, 60, 120, and 180 min to measure serum C-peptide level at every time-point. Blood samples at time-points of 0- and 120-min were used to measure blood glucose levels.

**β-Cell function parameters**

Because of the good correlation between the HOMA formula and the glucose clamp test, it has been widely applied in clinical and scientific research. However, the validity of insulin-based HOMA formula may be limited in some patients, particularly those with decreased β-cell function, high fasting glucose levels, and long-term use of insulin which are quite common in Chinese T2DM patients [21]. Insulin and C-peptide are co-secreted from the pancreas in an equimolar ratio. Unlike insulin, C-peptide is not significantly cleared by the liver and it has more steady clearance, what’s more, it has lower within-subject and between-subject variation than insulin [22]. So some researchers thought C-peptide-based index were better indices than insulin based indices for the evalua-
tion of pathophysiology of diabetes [23, 24]. Therefore, our study used C-peptide-based index to reflect β-cell function and insulin resistance. The insulin secretion index (HOMA-β) was calculated as: 20 × Fasting C-peptide (C₀ min)/(fasting blood glucose (FBG)-3.5). The early- and late-phase insulin secretion indices were calculated as the C-peptide integrals from 0 to 30 min (C-peptide AUC 0–30) and 30 to 120 min (C-peptide AUC30–120), respectively. The insulin resistance index (HOMA-IR) was calculated as: FBG × C₀ min/22.5 [22]. The indices of pancreatic β-cell function and insulin resistance above were converted into a normal distribution using logarithmic transformation.

Statistical analysis

Results are presented as means ± SE. Differences between groups were calculated using Student’s t test for unpaired comparisons. Non-normally distributed data were log-transformed. Multiple linear regression analysis was used to analyze the factors of pancreatic fat. The relations between pancreatic fat content and β-cell function or insulin resistance were assessed by Pearson and Partial correlation analysis. P < 0.05 was considered statistically significant.

Results

The baseline characteristics of the study population are presented in Table 1. The T2DM and non-diabetic groups did not differ significantly with respect to age, sex, WC, blood pressure, and total cholesterol (TC) and triglyceride (TG) concentrations (p > 0.05). However, T2DM subjects had higher BMI, FBG, fasting serum C-peptide, liver fat content (LFC) and lower high-density lipoprotein (HDL)-cholesterol (p < 0.05). The median PFC was significantly higher in patients with T2DM compared with the non-diabetic subjects: 7.06% vs. 5.36% (p < 0.01). Importantly, PFC was correlated with BMI, LFC and age by multiple linear regression analysis. Some of the patients with T2DM used lipid-lowering (23/78; 29.5%) and/or metformin medication (34/78; 43.6%), yet PFC in treatment group was still higher than that of non-diabetic group (7.10% vs. 5.37%, p < 0.05) (data not shown).

Unadjusted correlations analysis are presented in Table 2. The results showed that PFC was significantly and positively associated with insulin resistance, early- and late-phase insulin secretion in T2DM patients (all p < 0.05). In contrast, it was absent in non-diabetic subjects. Furthermore, the relations were greater in male

<table>
<thead>
<tr>
<th>Table 1 General characteristics of study subjects</th>
<th>Non-diabetic</th>
<th>Type 2 diabetic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>35</td>
<td>78</td>
<td>—</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>17/18</td>
<td>47/31</td>
<td>0.32</td>
</tr>
<tr>
<td>Duration (yr)</td>
<td>0</td>
<td>8.77 ± 6.84</td>
<td>—</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>58.33 ± 7.09</td>
<td>58.19 ± 9.62</td>
<td>0.95</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.00 ± 2.60</td>
<td>25.66 ± 4.17</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>90.77 ± 11.14</td>
<td>94.14 ± 11.09</td>
<td>0.15</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>128.62 ± 16.17</td>
<td>135.15 ± 16.49</td>
<td>0.15</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.25 ± 5.18</td>
<td>81.49 ± 9.52</td>
<td>0.07</td>
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<tr>
<td>FBG (mmol/L)</td>
<td>5.23 ± 0.63</td>
<td>8.15 ± 2.43</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Fasting C-peptide (nmol/L)</td>
<td>0.66 ± 0.23</td>
<td>1.19 ± 1.37</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.96 ± 0.96</td>
<td>4.63 ± 0.98</td>
<td>0.13</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.90 ± 1.59</td>
<td>2.03 ± 1.59</td>
<td>0.06</td>
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<tr>
<td>HDL (mmol/L)</td>
<td>1.44 ± 0.31</td>
<td>1.18 ± 0.25</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.88 ± 0.66</td>
<td>2.79 ± 0.73</td>
<td>0.58</td>
</tr>
<tr>
<td>PFC (%)</td>
<td>5.36 ± 1.20</td>
<td>7.06 ± 1.70</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>LFC (%)</td>
<td>3.05 ± 1.62</td>
<td>5.81 ± 4.62</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

Data are means ± SD. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressures; DBP, diastolic blood pressures; FPG, fasting plasma glucose concentration; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PFC, pancreatic fat content; LFC, liver fat content.

*: p < 0.05  **: p < 0.01
T2DM subjects when compared to female ones.

Table 3 shows the adjusted correlations analysis between PFC and OGTT-based parameters. After adjusting for BMI (Model 1), BMI and age (Model 2), BMI, age and LFC (Model 3), which related to PFC by multiple linear regression analysis, PFC remained positively correlated with insulin resistance, early- and late-phase insulin secretion in male T2DM patients (all $p < 0.05$).

We further analyzed these relations under different duration in male T2DM subjects found that PFC was significantly and obviously correlated with β-cell dysfunction and insulin resistance in male T2DM subjects with a course of disease less than 4 years, importantly, the relations were stronger than before (Table 4).

### Discussion

Our study aims to evaluate PFC by 3p-Dixon technology in Chinese T2DM patients, and to demonstrate the relationships between PFC and insulin secretion and insulin resistance. We found T2DM patients had higher PFC, LFC and BMI than non-diabetic subjects, importantly, we found PFC was positively associated with BMI, age and LFC by multiple linear regression analysis. The result suggests that with the occurrence of obesity, pancreas is also prone to fatty infiltration, which was consistent with previous researches [15, 25, 26].
Interestingly, we found that the relations between PFC and insulin sensitivity and insulin resistance are only observed in male T2DM subjects, but not in female T2DM subjects and non-diabetic subjects. Ou H-Y [10] thought that the lack of this correlation in women could be explained by the gender difference in ectopic fat deposition in pancreas, of particular note is the fact that, there was no significant difference in PFC between different gender in our study (6.83% vs. 7.40%, p = 0.15). Another researcher thought the reason for this discrepancy may come from the different activity of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) protein in the muscles [30]. Yong Wu [31] found PTEN plays a role in the transmission of signals of free fatty acids and induction of endothelial oxidative stress, and Samaan [30] demonstrated that women have lower muscle PTEN protein expression when compared to men. However, whether the PTEN protein is the cause of the differences among Chinese T2DM patients is worth further study. In addition, whether gender-related sex hormone levels cause the difference between men and women is worth further exploring.

As previous studies showed that ectopic lipid deposition in liver is an important risk factor for insulin resistance and β-cell dysfunction [32]. To explore whether PFC is an independently determinant of β-cell dysfunction and insulin resistance, we performed a partial correlation analysis to adjust for BMI, age and LFC found that the relations still exist. Therefore, it is possible to speculate that PFC plays an important role in β-cell dysfunction and insulin resistance independently, and may represents a better predictor of diabetes. However, a cohort study showed that strength of the positive association between PFC and insulin resistance is weakened when making further adjustment for visceral fat [15]. As a result, the independent function of pancreatic fat remains to be further studied.

Soo Lim et al. found that as the duration of T2DM increased, pancreatic volume decreased and PFC increased [33]. Partly in agree with this finding, we found that the relationship between PFC and β-cell function was changed with the duration of diabetes. It was more obvious when the course of disease was less than 4 years. It is assumed that the increase of pancreatic fat content in central obesity men with shorter duration of diabetes is aggravating or directly leading to β-cell dysfunction and insulin resistance.

There are some limitations of this work. Firstly, the sample size was relatively small and might not be representative of Chinese patients with T2DM. Secondly, some of the subjects had been treated with insulin, which might have affected the accuracy of the β-cell function determination [34], although insulin secretagogues and insulin have been discontinued one week before the experiment. Thirdly, in this study, only 0-min and 120-min blood glucose was measured, so the index of DI, an index of insulin secretion adjusted for insulin sensitivity could not be calculated. The use of HOMA-β to respond to insulin secretion may affect the results of the experiment [35].

In conclusion, our study found that the pancreas is also an important organ of visceral fat deposition. Pancreatic fat content measured by 3p-Dixon method is related to β-cell dysfunction and insulin resistance in Chinese T2DM patients, and is more obvious in central obesity male patients with a shorter course of disease. Therefore, PFC might represent a potential pathogenetic factor leading to T2DM. Further studies investigating the impacts of lifestyle interventions on PFC and its association with the onset of T2DM might be required.

Acknowledgments

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Conflict of Interest

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

References