Oral fat tolerance testing identifies abnormal pancreatic β-cell function and insulin resistance in individuals with normal glucose tolerance

Lifang Liu1,2,3†, Xiaoyu Hou1,2†, An Song4, Yunpeng Guan1,2, Peipei Tian5, Chao Wang6, Luping Ren2, Yong Tang2, Ling Gao7, Xiaoping Xing4, Guangyao Song1,2,*

1Department of Internal Medicine, Hebei Medical University, Shijiazhuang, Hebei, China, 2Department of Endocrinology, Hebei General Hospital, Shijiazhuang, Hebei, China, 3Department of Endocrinology, Baoding First Central Hospital, Baoding, Hebei, China, 4Key Laboratory of Endocrinology, Ministry of Health, Department of Endocrinology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China, 5Department of Endocrinology, Cangzhou Central Hospital, Cangzhou, China, 6Hebei Key Laboratory of Metabolic Diseases, Hebei General Hospital, Shijiazhuang, Hebei, China, and 7Department of Endocrinology and Metabolism, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, China

Keywords
Insulin resistance, Insulin secretion, Triglyceridemia

*Correspondence
Guangyao Song
Tel: +86-0311-8598-8556
E-mail address: sguangyao2@163.com

J Diabetes Investig 2022; 13: 1805–1813
doi: 10.1111/jdi.13867

Clinical Trial Registry
China Clinical Trials Registry
ChiCTR1800019514

ABSTRACT
Aims/Introduction: Insulin sensitivity and β-cell function are affected by lipid metabolism disorders, even before the onset of type 2 diabetes. People are in the postprandial state most of the time. Therefore, identifying postprandial hyperlipemias is important. This study aimed to assess patients with abnormalities in lipid metabolism, but with normal glucose tolerance, using oral fat tolerance testing (OFTT) to identify defects in insulin sensitivity and β-cell function.

Materials and Methods: We included 248 volunteers with normal glucose tolerance who underwent OFTT. They were divided into three groups in accordance with their fasting and 4-h postprandial triglyceride (TG) concentrations. Their lipid concentrations during OFTT were compared. The disposition index (DI) was applied to estimate β-cell function, and the Matsuda insulin sensitivity index (ISIM) was used to assess insulin sensitivity. We used multiple linear regression analysis to estimate the relationships of fasting and postprandial TG concentrations with β-cell function and insulin sensitivity.

Results: The changes in TG concentrations during OFTT were more marked than those in low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol or total cholesterol concentrations. As lipid metabolism deteriorated, the ISIM and the DI gradually decreased. Multiple linear regression analysis showed that fasting and 4-h postprandial TG concentrations affected LnISIM and LnDI.

Conclusions: In individuals with normal glucose tolerance, β-cell function and insulin sensitivity gradually decrease with a deterioration in the lipid profile. Not only fasting TG, but also postprandial TG concentrations are independent risk factors for impaired β-cell function and insulin resistance.

INTRODUCTION
Decreased insulin sensitivity and impaired β-cell function are the two leading aspects that promote the development of type 2 diabetes mellitus. The United Kingdom Prospective Diabetes Study showed that β-cell function begins to decline 10–12 years before the onset of type 2 diabetes mellitus, with an approximate 50% decline in β-cell function at the time of diagnosis1. There are many factors that cause β-cell dysfunction, including glucotoxicity, lipotoxicity, insulin resistance, age and

†These authors contributed equally to this manuscript.
Received 22 December 2021; revised 5 May 2022; accepted 3 June 2022
environmental factors. Therefore, the early detection and prevention of these risk factors are important for the prevention of type 2 diabetes mellitus.

Lipid metabolism disorders are considered to be independent risk factors for type 2 diabetes mellitus. The concept of lipotoxicity was first proposed by Roger Unger in 1994, when his team found that a high plasma non-esterified fatty acid concentration and pancreatic triglyceride (TG) accumulation in Zucker diabetic fatty rats occurred before a deterioration in glucose-stimulated insulin secretion and systemic hyperglycemia. This finding led to the recognition that pancreatic steatosis could lead to β-cell dysfunction. Since then, many studies have confirmed that decreased β-cell function caused by hyperlipidemia predates the development of type 2 diabetes mellitus. Therefore, delaying the development and progression of type 2 diabetes mellitus by the early treatment of lipid metabolism disorders should be possible.

In most clinical studies, fasting lipid concentrations have been measured. However, plasma lipids, especially TG, are affected by food consumption. Therefore, assessing postprandial lipemia is important. Several studies carried out in recent years have shown that non-fasting lipid concentrations are strongly associated with cardiovascular diseases, diabetes mellitus, and other metabolic disorder diseases. Postprandial lipid concentrations are superior to fasting lipids for the prediction of cardiovascular diseases. Furthermore, high postprandial lipid concentrations, especially postprandial TG (PTG) concentrations, might induce n-cell dysfunction in individuals with cardiovascular disease, leading to a rapid decrease in insulin sensitivity, and thus the development of type 2 diabetes mellitus. Nevertheless, the methods and criteria used to assess postprandial lipids have not been standardized. In 2010, Kolovou et al. made several recommendations regarding postprandial hypertriglyceridemia (PHTG), including the use of standardized oral fat tolerance testing (OFTT) for the measurement of postprandial lipemia. Regardless of the time after a high-fat meal, TG concentrations >2.5 mmol/L were identified as PHTG. They also suggested that the measurement of TG concentrations at 4 h of OFTT is a suitable means of assessing PTG.

In the present study, OFTT was carried out in Chinese individuals with normal glucose tolerance (NGT) to evaluate the relationships of changes in TG concentrations after a high-fat meal with β-cell function and insulin sensitivity. We aimed to identify patients with postprandial hyperlipidemia using OFTT, and thereby permit the early treatment of insulin resistance and β-cell dysfunction.

**MATERIALS AND METHODS**

**Study sample**

Volunteers were enrolled from Hebei General Hospital from May 2018 to December 2019, as previously reported. The ethics committee of Hebei General Hospital approved the research protocol (2018 NO.2), which conformed to the provision of the Declaration of Helsinki. This study was also registered with the China Clinical Trial Registry. All of the volunteers gave written informed consent and completed questionnaires as required. In addition, a physical examination, 3-h oral glucose tolerance testing (OGTT) and the measurement of insulin concentrations during OGTT were carried out for all of the volunteers. On the basis of the exclusion criteria that have been reported previously, volunteers with fasting TG concentrations >5.0 mmol/L and prediabetes were also excluded from the present study. Therefore, only volunteers with NGT were included. In accordance with the diagnostic criteria published in 1999 by the World Health Organization, those with fasting plasma glucose (FPG) concentrations <6.1 mmol/L and 2-h plasma glucose concentrations <7.8 mmol/L were categorized as having NGT.

**Oral fat tolerance testing**

The protocol for OFTT has been described recently. All of the volunteers were required to retain their ordinary diet for at least 1 week before OFTT. The test meal used in this research was designed according to the Chinese Food Guide Pagoda. The total energy content of the high-fat meal was prepared based on the OFTT, which was designed to last 10 h. To provide sufficient energy for all of the participants who could not have lunch, and taking the advice of professional nutritionists into consideration, the total energy content of the test meal was set at 1,500 kcal. In this meal, 60% was provided by fat (900 kcal), 20% by carbohydrate (300 kcal) and 20% by protein (300 kcal). Venous blood samples were collected in the morning (at 0 h) after a 10-h overnight fast, and then the participants ingested the high-fat meal in 10 min. At 2, 4, 6, 8 and 10 h after the high-fat meal, venous plasma was collected, centrifuged and then stored at −80°C (Haier MDR-382E; Haier, Qingdao, China).

**Anthropometric and biochemical measurements**

The volunteers’ demographic data were collected by trained professionals. These data included age, sex, height, waist circumference (WC), body mass, diastolic blood pressure and systolic blood pressure. Plasma glucose concentrations during OGTT and TG, total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C) concentrations in the fasting state and at 2, 4, 6, 8 and 10 h after the meal were measured using a biochemical analyzer (Hitachi 7600; Hitachi, Tokyo, Japan). A chemiluminescence method was used to measure the plasma insulin concentrations (Cosbas E601; Roche, Basel, Switzerland).

Body mass index (BMI) was calculated as the body mass divided by height squared (kg/m²). The areas under the curves for glucose (AUC<sub>glu</sub>), insulin (AUC<sub>ins</sub>) and TG (AUC<sub>TC</sub>), and the incremental areas under the curves for glucose (iAUC<sub>glu</sub>), insulin (iAUC<sub>ins</sub>) and TG (iAUC<sub>TC</sub>) were calculated by the trapezoidal method.
Indices of insulin resistance/sensitivity and β-cell function

1. Homeostasis model assessment of insulin resistance (HOMA-IR): HOMA-IR = FPG (mmol/L) × fasting insulin (FINS, μIU/mL) / 22.513, which mainly reflects hepatic insulin sensitivity.

2. Matsuda insulin sensitivity index (ISIM): ISIM = 104 / [FPG (mg/dL) × FINS (μIU/mL) × mean blood glucose (mg/dL) × mean insulin (μIU/mL)]0.514, which reflects systemic insulin sensitivity.

3. Homeostasis model assessment for β-cell function index (HOMA-β): HOMA-β = 20 × FINS (μIU/mL)/[FPG (mmol/L) - 3.5]13, which only reflects β-cell function in the fasting state.

4. Insulinogenic index at 30 min (IGI30): IGI30 = ΔINS0-30 / ΔGlucose0-30, which is used to evaluate early-phase insulin secretion function.

5. Disposition index (DI): DI = IGI30 / HOMA-IR17, which is used for the evaluation of β-cell function relative to insulin resistance.

Definition of hypertriglyceridemia

Fasting TG concentrations ≥1.7 mmol/L were identified as hypertriglyceridemia (HTG) based on the 2016 Chinese Guidelines for the Prevention and Treatment of Adult Dyslipidemia18. According to the expert panel statement of 201111, PTG concentrations ≥2.5 mmol/L at 4 h were identified as PHTG.

Statistical analysis

We used SPSS 26.0 (IBM Corp., Armonk, NY, USA) for statistical analysis. Data with a normal distribution for continuous variables are expressed as mean ± standard deviation (SD), and data with a non-normal distribution are represented by the median (P25, P75). One-way analysis of variance and Bonferroni post-hoc analysis were carried out for comparisons of multiple groups. Data with a non-normal distribution were compared among the groups using the Kruskal–Wallis H test. Repeated measures analysis of variance was used to compare glucose, insulin and lipid concentrations at the various time points in OGTT or OFITT among the groups. Categorical datasets were compared by the χ2-test. The variables ISIM and DI, which are the non-normal distribution, were converted into LnISIM and LnDI. Using LnISIM and LnDI as dependent variables, univariate linear regression analysis was carried out to determine the variables affecting LnISIM and LnDI. Because the ISIM and DI were calculated from insulin and glucose concentrations, we did not consider these parameters in the regression analysis. On the basis of adjustment for potential confounding factors, fasting and 4 h TG concentrations were included in multiple linear regression analysis, respectively, to evaluate the relationships of fasting and postprandial TG concentrations with insulin sensitivity (ISIM) and β-cell function (DI). P < 0.05 (two-tailed) was considered to represent statistical significance.

RESULTS

The volunteers were divided into three groups in accordance with their fasting TG and 4-h PTG concentrations: (i) normal fat tolerance (NFT) group (fasting TG concentrations <1.7 mmol/L and 4-h PTG concentrations ≤2.5 mmol/L); (ii) impaired fat tolerance (IFT) group (fasting TG concentrations <1.7 mmol/L and 4-h PTG concentrations >2.5 mmol/L); and (iii) HTG group (fasting TG concentrations ≥1.7 mmol/L and <5.0 mmol/L).

Comparisons of the basic characteristics of the groups

We enrolled 248 participants of whom 98 were in the NFT group, 78 were in the IFT group and 72 were in the HTG group. We did not observe any significant differences in age or sex among the three groups (Table 1). With a deterioration in the lipid profile, WC, BMI, systolic blood pressure, diastolic blood pressure, and FPG, FINS, TG, TC and LDL-C concentrations gradually increased, whereas HDL-C concentrations decreased (all P < 0.05) in the three groups. WC, BMI, and TG, TC, LDL-C, FPG and FINS concentrations were significantly higher (all P < 0.05), whereas HDL-C concentrations were lower (P < 0.05) in the HTG group than in the NFT group. BMI, WC, and FPG, FINS, TG, TC and LDL-C concentrations were significantly higher in the HTG group than in the IFT group (all P < 0.05). There were significant differences in BMI, and TG, HDL-C and FINS concentrations between the IFT and NFT groups (all P < 0.05). These data showed that adiposity was greater in participants in the HTG group than in those in the NFT and IFT groups. Additionally, participants in the HTG group had high FPG concentrations and abnormal lipid metabolism.

Comparison of the lipid profile of each group during OFTT

In the NFT group, TG concentrations peaked 2 h after the meal (P < 0.001 vs 0 h), and then gradually decreased and returned to the baseline value at 10 h. In the IFT group, TG concentrations were increased at 2 h after the lipid load (P < 0.001 vs 0 h), peaked at 4 h (P < 0.001 vs 0 h), and then gradually decreased and returned to the baseline value at 10 h. In the HTG group, the mean TG concentration at 2 h was significantly higher than that at baseline (P < 0.001) and peaked at 6 h (P < 0.001 vs 0 h), but had not returned to baseline by 10 h. The differences in TG concentrations among the three groups at any time point were significant (all P < 0.001; Table 2). Furthermore, the AUCTG significantly differed among the groups (P < 0.001). Nevertheless, no significant difference was observed in the iAUCTG between the IFT and HTG groups (P = 0.148; Figure S1a,b).

At each time point, TC and LDL-C concentrations gradually increased with a decrease in lipid tolerance, whereas HDL-C concentrations decreased (Table 2). In the NFT group, TC concentrations were significantly higher than those at baseline only at 8 h (P < 0.001). However, in the IFT and HTG groups, TC concentrations were high at 6 h and peaked at 8 h (both
Comparison of basic characteristics of the three study groups

| Group       | NFT (n = 98) | IFT (n = 78) | HTG (n = 72) | P      |
|-------------|--------------|--------------|--------------|--------|
| Age (years) | 42.11 ± 12.0 | 43.78 ± 12.51| 46.03 ± 10.75| 0.108  |
| Male, n (%) | 42 (42.86)   | 39 (50.00)   | 40 (55.56)   | 0.253  |
| BMI (kg/m²)| 24.17 ± 3.18 | 25.55 ± 3.18*| 27.65 ± 4.15***| <0.001 |
| WC (cm)     | 82.96 ± 10.96| 86.30 ± 9.10 | 93.25 ± 9.76***| <0.001 |
| SBP (mmHg)  | 122.65 ± 13.19| 125.90 ± 14.74| 129.11 ± 13.99*| 0.012  |
| DBP (mmHg)  | 76.81 ± 8.88 | 77.01 ± 9.49 | 80.56 ± 8.72*| 0.016  |
| Hypertension, n (%) | 13 (13.3) | 17 (21.8) | 22 (30.6)*| 0.023  |
| TG (mmol/L) | 0.91 ± 0.27  | 1.26 ± 0.24***| 2.61 ± 0.80***†| <0.001 |
| TC (mmol/L) | 4.49 ± 0.92  | 4.69 ± 0.84  | 5.08 ± 0.97***†| <0.001 |
| LDL-C (mmol/L) | 2.77 ± 0.67 | 2.99 ± 0.61  | 3.30 ± 0.66***†| <0.001 |
| HDL-C (mmol/L) | 1.36 ± 0.29 | 1.23 ± 0.23* | 1.14 ± 0.24***| <0.001 |
| FPG (mmol/L) | 5.00 ± 0.42  | 5.02 ± 0.36  | 5.17 ± 0.41†*| 0.012  |
| FINS (µU/mL)| 7.77 (5.69, 10.46) | 9.17 (6.72, 13.00)*| 11.50 (8.71, 17.05)**| <0.001 |

*P < 0.05 versus the normal fat tolerance (NFT) group; **P < 0.001 versus the NFT group; †P < 0.05 versus the impaired fat tolerance (IFT) group; ‡P < 0.001 versus the IFT group.

Comparisons of the glucose and insulin concentrations during OGTT

With the deterioration in lipid metabolism, plasma glucose concentrations gradually increased at each time point, but the difference in plasma glucose concentrations was only significant at 1 h (P < 0.001; Table 3). Furthermore, the AUC$_{\text{glu}}$ significantly differed among the three groups (P < 0.001), but no significant difference was observed in the iAUC$_{\text{glu}}$ between the IFT and HTG groups (P = 0.127; Figure S2a, b).

Serum insulin concentrations were also measured at various time points after the oral administration of 75 g of glucose (Table 3). Insulin concentrations in the NFT group peaked 0.5 h after the glucose load, with a 9.79-fold increase. Insulin concentrations in the IFT and HTG groups peaked at 1 h, with a 9.63-fold and 9.72-fold increase, respectively. Significant differences were observed in insulin concentrations only at 0 h and 1 h after the glucose load among the three groups (both P < 0.001). In addition, the AUC$_{\text{ins}}$ significantly differed among the three groups (P < 0.001), whereas no significant difference was observed in the iAUC$_{\text{ins}}$ between the NFT and IFT groups (P = 0.063; Figure S2c, d).

Comparison of insulin sensitivity and β-cell function among the groups

With a decrease in lipid tolerance, HOMA-IR gradually increased and ISI$_{M}$ gradually decreased (P < 0.05, Table 4).

However, no significant difference in IG$_{H30}$ was found among the three groups (P = 0.933). However the DI, which reflects β-cell function after adjustment for insulin resistance, gradually decreased with a decrease in lipid tolerance (P < 0.05).

Relationships of clinical parameters and the lipid profile with insulin sensitivity and β-cell function

According to the expert panel statement of 2011, PTG concentrations >2.5 mmol/L at 4 h were identified as PHTG, so we chose 4 h TG concentrations as the representative of PTG in this research.

Univariate linear regression analysis showed that age, BMI, and 0-h HDL-C, 0-h LDL-C, 0-h TG and 4-h TG concentrations were independent risk factors for LnISI$_{M}$ (Table S1). Male sex, age, BMI, hypertension, and 0-h HDL-C, 0-h TG and 4-h TG concentrations were independent risk factors for LnDI (Table S2).

In multiple linear regression analysis, after adjustment for age, BMI, 0-h HDL-C and 0-h LDL-C concentrations, fasting and 4 h TG concentrations were independent risk factors for LnDI, respectively (all P < 0.05; Table 5). After adjustment for age, sex, hypertension, BMI and 0-h HDL-C concentrations, fasting and 4 h TG concentrations were independent risk factors for LnISI$_{M}$, respectively (all P < 0.05).

**DISCUSSION**

In the present study, 248 volunteers with NGT underwent OFTT. We found that β-cell function and insulin sensitivity gradually decreased along with a deterioration in lipid metabolism in participants with NGT. Additionally, fasting and 4-h postprandial TG concentrations were independent risk factors for β-cell function and insulin sensitivity. These results suggest...
| Table 2 | Comparison of postprandial lipid concentrations during the oral fat tolerance testing in the three study groups |

|        | 0 h      | 2 h      | 4 h      | 6 h      | 8 h      | 10 h     |
|--------|----------|----------|----------|----------|----------|----------|
| TC (mmol/L) |          |          |          |          |          |          |
| NFT    | 0.91±0.27| 1.52±0.52| 1.63±0.70| 1.53±0.72| 1.46±0.66| 1.38±0.46|
| IFT    | 1.26±0.24| 2.53±0.72| 3.46±1.03| 3.00±1.15| 2.20±1.32| 1.38±0.66|
| HTG    | 2.61±0.80| 3.74±1.16| 4.93±1.64| 5.14±2.05| 4.72±1.90| 3.13±1.41|
| HDL-C (mmol/L) |        |          |          |          |          |          |
| NFT    | 4.49±0.92| 4.43±0.89| 4.47±0.90| 4.63±0.94| 4.60±0.90|          |
| IFT    | 4.69±0.84| 4.86±0.81| 4.70±0.82| 4.83±0.90| 4.84±0.92|          |
| HTG    | 3.08±0.92| 5.03±0.91| 5.12±0.96| 5.26±0.91| 5.35±0.95|          |
| LDL-C (mmol/L) |        |          |          |          |          |          |
| NFT    | 2.77±0.67| 2.70±0.66| 2.66±0.64| 2.72±0.64| 2.79±0.69| 2.80±0.67|
| IFT    | 2.99±0.61| 3.20±0.61| 3.24±0.59| 3.28±0.61| 3.29±0.63| 3.35±0.62|

*P < 0.05 versus the normal fat tolerance (NFT) group; **P < 0.01 versus the NFT group; ***P < 0.001 versus the NFT group; †P < 0.05 versus 0 h in the same group; †††P < 0.001 versus 0 h in the same group. HDL-C: high-density lipoprotein-cholesterol; HTG: hypertriglyceridemia; LDL-C: low-density lipoprotein-cholesterol; TC: total cholesterol; TG: triglyceride.

Postprandial hyperlipidemia is characterized as the accumulation of TG-rich lipoproteins in plasma after a meal. Its severity is related to the magnitude and duration of the postprandial increase in TC and TG concentrations. As TC concentrations do not significantly change in the present results, we found that after adjusting for potential confounding variables, not only the increase in TG concentrations was an important factor affecting glucose tolerance. At present, obtaining fasting blood samples to evaluate the increase in TG concentrations after a meal is a simple and effective method for detecting PHTG.

Lipid metabolism abnormalities in β-cell function and insulin resistance are independent risk factors for type 2 diabetes mellitus. PHTG is observed not only in patients with type 2 diabetes mellitus but also in individuals with different types of dyslipidemia. For example, a higher risk of developing type 2 diabetes mellitus is observed in patients with increased cholesterol and triglyceride levels. Therefore, people with normal fasting TG concentrations but PHTG might benefit from more vigorous insulin sensitizing and β-cell function therapies. Increasing attention to postprandial lipids, a type 2 diabetes mellitus risk factor, might prevent or slow the progression of type 2 diabetes mellitus.
Therefore, individuals with NGT were selected in this study, and they were divided into three groups according to the OFT. With a decrease in lipid tolerance, the ISM (reflecting insulin sensitivity) and the DI (reflecting β-cell function) gradually decreased. The damage to insulin sensitivity and β-cell function was already present in the participants with PHTG alone.

Persistent postprandial hyperlipidemia, especially PHTG, can lead to an increase in free fatty acid concentrations in the circulation, which exceed the oxidation ability of tissue and the storage ability of adipose tissue. Free fatty acid transfer to non-adipose tissue, which is called ectopic fat deposition, can lead to chronic damage and dysfunction of target tissues and cells. In insulin target organs, such as the liver, and skeletal muscle, excessive fat deposition leads to insulin receptor substrate and effector protein binding disorders, blockage of insulin signaling pathways, and reduced the biological effects of insulin, resulting in a decline in insulin sensitivity. Lipotoxicity not only aggravates insulin resistance, but also aggravates β-cell dysfunction. When β-cells are continuously exposed to high free fatty acid concentrations, the number and function of β-cells can be affected by various molecular mechanisms, such as endoplasmic reticulum stress, oxidative stress, inflammation, autophagy and dedifferentiation, resulting in insulin secretion disorder. Additionally, during TG metabolism, its products (e.g., ceramide and diacylglycerol) accumulate, affecting insulin sensitivity and β-cell function.

Therefore, insulin resistance can be caused or aggravated by postprandial hyperlipidemia alone.

To maintain blood glucose concentrations in the normal range, β-cells secrete excessive insulin, and hyperinsulinemia occurs to compensate for the decline in insulin biological effects in peripheral tissues, which can prevent hyperglycemia. However, β-cells are continuously in a state of high-load secretion, together with the direct effects of lipotoxicity. Over time, β-cell dysfunction will occur and then develop into type 2 diabetes mellitus.

Several guidelines and recommendations consider the postprandial lipids as a useful subject to evaluate cardiovascular diseases risks and so on. In the present study, PTG was found to be associated with β-cell function and insulin resistance.

In summary, to the best of our knowledge, this is the first large-sample study carried out in the Chinese population with NGT to assess postprandial blood lipids based on OFT. The present study aimed to clarify the associations between PTG and β-cell function, as well as the insulin sensitivity. PHTG might occur in individuals with fasting TG in the normal range. Thus, by PTG detection, early stages of β-cell dysfunction and insulin resistance can be identified, which might be beneficial to type 2 diabetes mellitus prevention and intervention.

The present study had several limitations. First, even though the hyperinsulinemic-euglycemic clamp and the hyperglycemic

### Table 3 | Comparison of glucose and insulin concentrations during oral glucose tolerance testing in the three study groups

|          | 0 h      | 0.5 h    | 1 h      | 2 h      | 3 h      |
|----------|----------|----------|----------|----------|----------|
| Glu (mmol/L) |          |          |          |          |          |
| NFT      | 5.00 ± 0.42 | 8.28 ± 1.19 | 7.25 ± 1.61 | 5.77 ± 0.97 | 4.12 ± 0.93 |
| IFT      | 5.02 ± 0.36 | 8.69 ± 1.33 | 7.88 ± 1.60 | 5.93 ± 0.78 | 4.43 ± 0.52 |
| HTG      | 5.17 ± 0.41*| 9.11 ± 1.31***| 8.53 ± 1.60***| 6.32 ± 0.86***| 4.53 ± 0.77***|
| INS (IU/mL) |          |          |          |          |          |
| NFT      | 7.77 (5.69, 10.46) | 76.04 (51.81, 100.83) | 59.78 (40.04, 101.03) | 41.27 (26.54, 62.17) | 8.67 (5.02, 14.12) |
| IFT      | 9.17 (6.72, 13.00)* | 83.78 (60.54, 105.77) | 88.28 (54.43, 126.85)* | 46.75 (25.35, 77.47) | 11.88 (7.02, 16.33)* |
| HTG      | 11.50 (8.71, 17.05)***| 89.20 (66.92, 135.75) | 111.75 (72.50, 153.85)***| 60.17 (39.46, 91.95)***| 12.71 (8.87, 20.27)***|

*P < 0.05 compared with the normal fat tolerance (NFT) group; ***P < 0.001 compared with the NFT group; †P < 0.05 compared with the impaired fat tolerance (IFT) group. Glu, glucose; HTG, hypertriglyceridemia; Ins, insulin.

### Table 4 | Comparison of insulin sensitivity and β-cell function in the three study groups

|          | NFT      | IFT      | HTG      | P       |
|----------|----------|----------|----------|---------|
| HOMA-IR  | 1.76 (1.25, 2.40) | 2.29 (1.70, 3.62)* | 3.17 (2.09, 4.51)***| <0.001 |
| ISM      | 5.46 (4.14, 7.49) | 4.42 (3.42, 6.48)* | 3.48 (2.34, 5.28)***| <0.001 |
| HOMA-β   | 111.20 (83.00, 142.80) | 118.27 (92.32, 201.11) | 142.13 (105.31, 189.96)* | <0.003 |
| DI       | 12.76 (7.83, 19.48) | 8.43 (5.32, 13.56)* | 6.31 (4.63, 11.28)*** | <0.001 |
| IG30     | 22.85 (14.07, 33.72) | 20.46 (12.30, 32.48) | 20.25 (15.08, 29.28) | 0.933 |

*P < 0.05 versus the normal fat tolerance (NFT) group; ***P < 0.001 versus the NFT group; †P < 0.05 versus the impaired fat tolerance (IFT) group. DI, disposition index; HTG, hypertriglyceridemia; IG30, insulinogenic index at 30 min; ISM, Matsuda insulin sensitivity index.
clamp are considered as the gold standards for measuring insulin sensitivity and β-cell function, respectively, they are complex to carry out and difficult to be widely popularized in clinic. Therefore, the commonly used HOMA-IR and HOMA-β, as well as the ISIM and DI based on OGTT, were chosen in the present study. However, the results from these methods are likely to be less accurate than those with the clamp test. Second, the energy of the test meal in this study was high, and the range of the test time was relatively fixed, which could have affected the results to some extent. In the future, our group will carry out further clinical trials to investigate suitable diagnostic criteria for PHTG in the Chinese population. We have gained approval for this future study from the National Natural Science Foundation of China, and it is registered in the China Clinical Trials Registry (registration number: ChiCTR2100048497).

ACKNOWLEDGMENTS
All authors thank the staff of the Research Institution of Endocrine and Metabolic Diseases in Hebei of China for their assistance with this research. We thank Ellen Knapp, PhD, from Liwen Bianji (Edanz; www.liwenbianji.cn/), for editing the English text of a draft of this manuscript.

The Research Fund from Hebei Provincial Government of China provided funding for this research.

DISCLOSURE
The authors declare no conflict of interest.

Approval of the research protocol: The ethics committee of Hebei General Hospital approved the research protocol (approval number: 2018 NO.2, approval date: 26 February 2018)

Informed consent: All volunteers gave informed consent.

Registry and the registration no. of the study/trial: The research proposal was registered with the China Clinical Trial Registry. (registration date: 15 November 2018, registration number: ChiCTR1800019514).

Animal studies: N/A.

REFERENCES
1. U.K. prospective diabetes study 16. Overview of 6 years’ therapy of type II diabetes: a progressive disease. U.K. prospective diabetes study group. Diabetes 1995; 44: 1249–1258.

2. Cernea S, Dobreanu M. Diabetes and beta cell function: from mechanisms to evaluation and clinical implications. Biochem Med (Zagreb) 2013; 23: 266–280.
3. Lee Y, Hirose H, Ohneda M, et al. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. Proc Natl Acad Sci USA 1994; 91: 10878–10882.

4. Zheng S, Xu H, Zhou H, et al. Associations of lipid profiles with insulin resistance and β cell function in adults with normal glucose tolerance and different categories of impaired glucose regulation. PLoS One 2017; 12: e0172221.

5. Ma M, Liu H, Yu J, et al. Triglyceride is independently correlated with insulin resistance and islet beta cell function: a study in population with different glucose and lipid metabolism states. Lipids Health Dis 2020; 19: 121.

6. Sasson S. Nutrient overload, lipid peroxidation and pancreatic beta cell function. Free Radic Biol Med 2017; 111: 102–109.

7. Langsted A, Nordestgaard BG. Nonfasting versus fasting lipid profile for cardiovascular risk prediction. Pathology 2019; 51: 131–141.

8. Pozuelo-Sanchez I, Villasanta-Gonzalez A, Alcala-Diaz JF, et al. Postprandial lipemia modulates pancreatic alpha-cell function in the prediction of type 2 diabetes development: the CORDIOPREV study. J Agirc Food Chem 2020; 68: 1266–1275.

9. Pedrini MT, Niederwanger A, Kranebitter M, et al. Postprandial lipaemia induces an acute decrease of insulin sensitivity in healthy men independently of plasma NEFA levels. Diabetologia 2006; 49: 1612–1618.

10. Aslam M, Aggarwal S, Sharma KK, et al. Postprandial hypertriglyceridemia predicts development of insulin resistance glucose intolerance and type 2 diabetes. PLoS One 2016; 11: e0145730.

11. Kolovou GD, Mikhailidis DP, Kovar J, et al. Assessment and clinical relevance of non-fasting and postprandial triglycerides: an expert panel statement. Curr Vasc Pharmacol 2011; 9: 258–270.

12. Hou X, Guan Y, Tang Y, et al. A correlation study of the relationships between nonalcoholic fatty liver disease and serum triglyceride concentration after an oral fat tolerance test. Lipids Health Dis 2021; 20: 54.

13. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412–419.

14. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: Comparison with the euglycemic insulin clamp. Diabetes Care 1999; 22: 1462–1470.

15. Haffner SM, Miettinen H, Gaskill SP, et al. Decreased insulin secretion and increased insulin resistance are independently related to the 7-year risk of NIDDM in Mexican-Americans. Diabetes 1995; 44: 1386–1391.

16. Oka R, Yagi K, Sakurai M, et al. Insulin secretion and insulin sensitivity on the oral glucose tolerance test (OGTT) in middle-aged Japanese. Endocr J 2012; 59: 55–64.

17. Jensen CC, Cnop M, Hull RL, et al. Beta-cell function is a major contributor to oral glucose tolerance in high-risk relatives of four ethnic groups in the U.S. Diabetes 2002; 51: 2170–2178.

18. Hu DY. New guidelines and evidence for the prevention and treatment of dyslipidemia and atherosclerotic cardiovascular disease in China. Zhonghua Xin Xue Guan Bing Za Zhi 2016; 44: 826–827. (Chinese)

19. Peng J, Zhao F, Yang X, et al. Association between dyslipidemia and risk of type 2 diabetes mellitus in middle-aged and older Chinese adults: a secondary analysis of a nationwide cohort. BMJ Open 2021; 11: e042821.

20. Kane JP, Pullinger CR, Goldfine ID, et al. Dyslipidemia and diabetes mellitus: role of lipoprotein species and interrelated pathways of lipid metabolism in diabetes mellitus. Curr Opin Pharmacol 2021; 61: 21–27.

21. Guo H, Ma C, Wu X, et al. Functional status of pancreatic β and a cells in type 2 diabetes mellitus patients with different plasma triglyceride levels: a retrospective analysis. Int J Endocrinol 2021; 2021: 9976067.

22. Fiorentino TV, Succurro E, Marini MA, et al. HDL cholesterol is an independent predictor of β-cell function decline and incident type 2 diabetes: a longitudinal study. Diabetes Metab Res Rev 2020; 36: e3289.

23. Seghieri M, Tric D, Natali A. The impact of triglycerides on glucose tolerance: lipotoxicity revisited. Diabet Metab 2017; 43: 314–322.

24. Zhao Y, Liu L, Yang S, et al. Mechanisms of atherosclerosis induced by postprandial lipemia. Front Cardiovasc Med 2021; 8: 636947.

25. Nakamura K, Miyoshi T, Yunoki K, et al. Postprandial hyperlipidemia as a potential residual risk factor. J Cardiol 2016; 67: 335–339.

26. Tomlinson B, Chan P, Lam CKW. Postprandial hyperlipidemia as a risk factor in patients with type 2 diabetes. Expert Rev Endocrinol Metab 2020; 15: 147–157.

27. Hallcs CJ, Van Wijk JP, Ribalta J, et al. Diurnal triglyceridaemia and insulin resistance in mildly obese subjects with normal fasting plasma lipids. J Intern Med 2004; 255: 74–81.

28. Moro E, Gallina P, Pais M, et al. Hypertriglyceridaemia is associated with increased insulin resistance in subjects with normal glucose tolerance: evaluation in a large cohort of subjects assessed with the 1999 World Health Organization criteria for the classification of diabetes. Metabolism 2003; 52: 616–619.

29. Zheng S, Zhou H, Han T, et al. Clinical characteristics and beta cell function in Chinese patients with newly diagnosed type 2 diabetes mellitus with different levels of serum triglyceride. BMC Endocr Disord 2015; 15: 21.

30. Natali A, Baldi S, Bonnet F, et al. Plasma HDL-cholesterol and triglycerides, but not LDL-cholesterol, are associated with insulin secretion in non-diabetic subjects. Metabolism 2017; 69: 33–42.
31. Madhu S, Sinha B, Aslam M, et al. Postprandial triglyceride responses and endothelial function in prediabetic first-degree relatives of patients with diabetes. J Clin Lipidol 2017; 11: 1415–1420.
32. Yazc D, Sezer H. Insulin resistance, obesity and lipotoxicity. Adv Exp Med Biol 2017; 960: 277–304.
33. Prentki M, Peyot ML, Masiero P, et al. Nutrient-induced metabolic stress, adaptation, detoxification, and toxicity in the pancreatic β-cell. Diabetes 2020; 69: 279–290.
34. Benito-Vicente A, Jebari-Benslaiman S, Galicia-Garcia U, et al. Molecular mechanisms of lipotoxicity-induced pancreatic β-cell dysfunction. Int Rev Cell Mol Biol 2021; 359: 357–402.
35. Chaurasia B, Summers SA. Ceramides - lipotoxic inducers of metabolic disorders. Trends Endocrinol Metab 2015; 26: 538–550.
36. Kaneko YK, Ishikawa T. Diacylglycerol signaling pathway in pancreatic β-cells: an essential role of diacylglycerol kinase in the regulation of insulin secretion. Biol Pharm Bull 2015; 38: 669–673.
37. Mandal N, Grambergs R, Mondal K, et al. Role of ceramides in the pathogenesis of diabetes mellitus and its complications. J Diabetes Complications 2021; 35: 107734.
38. Berglund L, Brunzell JD, Goldberg AC, et al. Evaluation and treatment of hypertriglyceridemia: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2012; 97: 2969–2989.
39. Lipid Modification: Cardiovascular risk assessment and the modification of blood lipids for the primary and secondary prevention of cardiovascular disease. 2014.
40. Catapano AL, Graham I, De Backer G, et al. 2016 ESC/EAS guidelines for the Management of Dyslipidaemias. Eur Heart J 2016; 37: 2999–3058.
41. Kolovou GD, Watts GF, Mikhailidis DP, et al. Postprandial Hypertriglyceridaemia revisited in the era of non-fasting lipid profile testing: a 2019 expert panel statement. Curr Vasc Pharmacol 2019; 17: 498–514.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Changes in postprandial triglycerides concentrations during oral fat tolerance testing in the three study groups.

Figure S2 | Changes in glucose and insulin concentrations during oral glucose tolerance testing in the three study groups.

Table S1 | Univariate linear regression analysis of variables that were independently related to Log,Matsuda insulin sensitivity index

Table S2 | Univariate linear regression analysis of variables that were independently related to Log,Disposition index.