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ORIGINAL ARTICLE

Pancreatic β-cell dysfunction in normoglycemic patients and risk factors

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Abstract

Aims To evaluate pancreatic β-cell function (βf) in patients with normoglycemia (NG) and normal glucose tolerance (NGT) and related risk factors.

Methods An observational and comparative study in 527 patients with NG and NGT that were divided by quartiles of βf according to the disposition index derived from OGTT. Anthropometrical, clinical, nutritional, and biochemical variables were measured and associated with βf.

Results Quartiles of βf were $Q1 = \text{DI} < 1.93$ $n = 131$, $Q2 = \text{DI}$ 1.93–2.45 $n = 134$, $Q3 = \text{DI}$ 2.46–3.1 $n = 133$, and $Q4 = \text{DI} > 3.1$ $n = 129$. There was a progressive reduction in pancreatic β-cell function and it is negatively correlated with age, weight, BMI, total body fat and visceral fat, waist circumference, total cholesterol, LDL, and triglycerides ($p < 0.01$). Glucose levels during OGTT had a negative correlation with βf; the product of fasting glucose by 1-h glucose had the best correlation with βf ($r = 0.611$, $p < 0.001$) and was the best predictor of βdf (AUC 0.816, CI 95% 0.774–0.857), even better than 1-h glucose ($r = 0.581$, $p < 0.001$). Energy, fat, and carbohydrate intake were negatively correlated with βf ($p < 0.05$). Glucose levels at 1-h OGTT > 110 mg/dl were positively associated with pancreatic βdf (OR 6.85, CI 95% 3.86–12.4). In the multivariate analysis, glucose levels during OGTT, fasting insulin, and BMI were the main factors associated with βf.

Conclusions A subgroup of patients with NG and NGT may have a loss of 40% of their βf. Factors related to this βdf were age, adiposity, glucose during OGTT, and the product of fasting and 1-h glucose, as well as food intake.

Keywords β-Cell function · β-Cell dysfunction · Normoglycemic · Normotolerant · Diabetes risk factors

Introduction

Type 2 diabetes (T2D) is a worldwide health problem affecting up to 18% in Mexico, and at least 50% of them are undiagnosed [1–4]. T2D is directly related to obesity, physical inactivity, and the coexistence of multiple risk factors [5–7]. Several pathophysiological abnormalities are involved in the development of hyperglycemia and T2D, including insulin resistance, impaired insulin secretion, and pancreatic β-cell dysfunction. At early stages, if the insulin resistance persists, pancreatic β-cells increase insulin secretion as an adaptive response to maintain normal glucose levels [7–12].

Progression from the NGT to T2D is related to several factors, but pancreatic β-cell function is one of the main factors involved in the development of prediabetes and T2D progression [13, 14]. Different factors have been involved in the development of pancreatic β-cell dysfunction: glycotoxicity, lipotoxicity, insulin resistance, systemic inflammation, incretin defect, amyloid deposits, and age, among others [7, 9, 12–17].

Previous studies have reported that pancreatic β-cell dysfunction appears at very early stages in the natural history of T2D [18, 19]. Normotolerant patients with obesity who were

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in the upper tertile group of the 2-h OGTT glucose levels (120–139 mg/dl) have already lost two-thirds of their pancreatic β-cell function. Those patients, who were diagnosed with impaired glucose tolerance and are within the upper tertile group (180–199 mg/dl), have lost 80 to 85% of their pancreatic β-cell function. Thus, patients with T2D at the time of diagnosis have lost more than 80% of the pancreatic β-cell function [19].

There are different methods to evaluate pancreatic β-cell function. OGTT has been a reliable tool for the diagnosis of glucose abnormalities, and the shape of glucose levels during the OGTT has been considered as an indicator of risk for the development of hyperglycemia [20]. β-Cells respond to an increase in glucose (ΔG) with an increase in insulin (ΔI); therefore, a better measure of the function of β-cells is the index $\Delta I/\Delta G$. However, β-cells are affected by the body's sensitivity to insulin and adjusted its insulin secretion to maintain normoglycemia; thus, to take into consideration whole-body insulin sensitivity, an improved method to measure β-cell function is insulin secretion/insulin resistance (I/G/IR), called the disposition index [21].

The goal of this work was to characterize the pancreatic β-cell function in normoglycemic and normotolerant subjects and to evaluate the association/correlation of clinical, biochemical, and anthropometrical variables with β-cell dysfunction in these patients.

Materials and methods

Study design and participants

This was an observational, prospective, and comparative study performed in patients that were selected from a metabolic cohort study performed at the Metabolic Research Laboratory in the University of Guanajuato, México. For this particular study, 930 patients were screened with anthropometrical, nutritional, biochemical, and metabolic evaluation, including oral glucose tolerance test. Patients were eligible for enrollment in the study according to the following criteria: (1) normoglycemia and normal glucose tolerance [FG 70 < 100 mg/dl, 2-h glucose < 140 mg/dl (< 7.8 mmol per liter)] during a single oral glucose tolerance test and (2) age between 18 and 65 years; exclusion criteria were (1) treatment with drugs affecting glucose levels during the previous 3 months, (2) previous pathological conditions affecting glucose metabolism or body weight (thyroid disease, Cushing's syndrome, Acromegaly), and (3) pregnancy.

Anthropometrical and body composition measurements Weight was measured while participants were barefoot and wearing minimal clothing. Height was obtained while the participants were standing barefoot with their shoulders in a normal position. BMI (kg/m^2) was obtained from standardized measurements of weight and height and was computed as a ratio of weight (kg) to height squared (m^2). Waist circumference was measured at the high point of the iliac crest at the end of normal expiration to the nearest 0.1 cm. Body composition was assessed with electrical bioimpedance through a Tanita Scale SC-240.

Nutritional evaluation A 24-h recall food consumption was applied to evaluate dietary intake in a subgroup of patients ($n = 160$), and it was analyzed with Nutrikcal® software version 4.0.

Oral glucose tolerance test (OGTT) All subjects were admitted to the Metabolic Research Laboratory at the University of Guanajuato the day of the study between 7 and 8 a.m. After the intravenous catheter was placed and the first blood sample was drawn, the patients ingested 75 g of glucose. Serum samples for glucose and insulin measurement were drawn at − 15 and 0 min and every 30 min thereafter for 2 h.

Measurements Glucose was measured by colorimetric glucose oxidase (Vitros 5600; Ortho Clinical Diagnostics). Lipid levels were measured by dry chemistry with colorimetric method (Vitros 5600; Ortho Clinical Diagnostics). Insulin was measured at 0, 30, 60, 90, and 120 min during OGTT by chemiluminescent immunometric assay (IMMULITE 2000 Immunoassay system, Siemens).

Calculations The incremental AUC for glucose and insulin during the OGTT were calculated according to the trapezoidal rule. Insulin secretion was calculated dividing the $AUCinsulin_{0\_120}$ by the $AUCglucose_{0\_120}$ and dividing the change in insulin from 0 to 30 min by the change in glucose from 0 to 30 min (AIR acute insulin response = Δins0_30/Δgluc0_30) during the OGTT. The insulin secretion/insulin resistance (IS/IR) index (disposition index = DI) during OGTT was calculated as ($AUCinsulin_{0\_120}/AUCglucose_{0\_120}$)*Matsuda index and by AIR * Matsuda index; oral disposition index was calculated dividing the AIR by 1/fasting insulin. Insulin sensitivity during OGTT was calculated from the Matsuda index and at fasting with the homeostasis model assessment (HOMA-IR). According to the pancreatic β-cell function (DI), patients were divided in quartiles in order to have a similar sample size per group ($Q1$ DI: < 1.93 $n = 131$, $Q2$ DI: 1.93–2.45 $n = 134$, $Q3$ DI: 2.46–3.1 $n = 133$, and $Q4$ DI: > 3.1 $n = 129$), and those who fall on the first quartile were considered to have pancreatic β-cell dysfunction.

Statistical analysis

Considering that we wanted to find a minimal correlation coefficient of 0.15 between pancreatic β-cell function with the rest of the variables, we needed at least 378 patients, with an alpha error of 0.05 and a β value of 0.10 (power

90%). Numerical variables were compared with analysis of variance (ANOVA) with a Tukey's post hoc test between the groups divided by quartiles of pancreatic β-cell function. Non-numerical variables were compared between groups with the Chi-squared test. Pearson and Spearman correlation coefficients were determined to evaluate the relation between variables, and multiple regression analysis was used to identify the main variables related to βf. Odds ratio (OR) was calculated to evaluate the association between different categorical variables with the pancreatic β-cell dysfunction (*Q*1). ROC analysis with sensitivity, specificity, and predictive values was performed to evaluate the usefulness of different metabolic variables to identify pancreatic β-cell dysfunction. A paired analysis by insulin sensitivity (Matsuda index) was used to verify the differences between the study groups independent of the degree of insulin sensitivity.

Statistical analyses and graphics were performed using SPSS version 21.0 (SPSS Inc) and GraphPad Prism 5.0. Statistical significance was considered when p value was less than 0.05.

Results

The study was performed between September 4, 2014, and August 30, 2018. A total of 930 patients were evaluated, but only 527 met the established inclusion criteria: 375 (71.1%) women and 152 (28.9%) men.

Pancreatic β-cell function was measured with the insulin disposition index, and four groups of patients were defined based on quartiles, going from the worst (*Q*1) to the best pancreatic β-cell function (*Q*4): *Q*1 DI < 1.93 ($n = 131$), *Q*2 DI 1.93–2.45 ($n = 134$), *Q*3 DI 2.46–3.1 ($n = 133$), and *Q*4 DI > 3.1 ($n = 129$). In Table 1, the characteristics and the comparisons between the study groups are shown.

As expected, there was a progressive reduction in pancreatic β-cell function as age, weight, BMI, total body fat, and visceral fat were increased ($p < 0.001$). Interestingly,

Table 1 Characteristics between the study groups according to the quartiles of pancreatic β-cell function (DI)

Variable	Total ($n = 527$)	*Q*1 ($n = 131$)	*Q*2 ($n = 134$)	*Q*3 ($n = 133$)	*Q*4 ($n = 129$)	*P*
Sex (M/F)	152/375	48/83	36/98	43/90	25/104	0.014
Age (years)	36 ± 11	40 ± 11	36 ± 11[a]	34 ± 12[a]	34 ± 11[a]	< 0.001
Weight (kg)	63 ± 13	76 ± 16	73 ± 15	70 ± 15[a]	63 ± 13[abc]	< 0.001
BMI (kg/m^2)	26.4 ± 5.07	28.2 ± 4.8	27.2 ± 5.1	26.2 ± 4.7[a]	24.04 ± 4.5[abc]	< 0.001
WC (cm)	85.4 ± 13.5	90.7 ± 12.2	87.2 ± 13.7[a]	84.3 ± 13.7[a]	79.2 ± 11.6[abc]	< 0.001
Body fat (%)	32.07 ± 8.8	34.3 ± 8.8	33.3 ± 8.7	31.7 ± 8.3[a]	28.7 ± 8.5[abc]	< 0.001
Visceral fat (au)	6.9 ± 7.1	9.8 ± 12.1	7.05 ± 3.9[a]	6.4 ± 6.5[a]	4.7 ± 3.3[ab]	< 0.001
SBP (mmHg)	112 ± 13	116 ± 14	112 ± 11[a]	112 ± 13[a]	107 ± 10[abc]	< 0.001
DBP (mmHg)	74 ± 10	77 ± 10	74 ± 10[a]	75 ± 10[a]	71 ± 7[abc]	< 0.001
FG (mg/dl)	88 ± 6	92 ± 5	91 ± 5	87 ± 6[ab]	83 ± 6[abc]	< 0.001
Glucose 30 min (mg/dl)	130 ± 24	146 ± 23	135 ± 21[a]	125 ± 20[ab]	115 ± 21[abc]	< 0.001
Glucose 60 min (mg/dl)	123 ± 31	148 ± 31	128 ± 24[a]	113 ± 24[ab]	102 ± 25[abc]	< 0.001
Glucose 90 min (mg/dl)	107 ± 25	126 ± 25	114 ± 19[a]	99 ± 19[ab]	88 ± 18[abc]	< 0.001
Glucose 120 min (mg/dl)	100 ± 19	113 ± 15	108 ± 17[a]	93 ± 16[ab]	83 ± 14[abc]	< 0.001
AUCglucose OGTT (mg/dl/120 min)	13,677 ± 2359	15,759 ± 2129	14,337 ± 1712[a]	12,878 ± 1623[ab]	11,699 ± 1733[abc]	< 0.001
Pancreatic β-cell function (*Matsuda*(AUCins*$_{0\text{-}120}$*/AUCgluc*$_{0\text{-}120}$*)*	2.6 ± 0.9	1.57 ± 0.2	2.1 ± 0.1[a]	2.7 ± 0.1[ab]	3.9 ± 0.7[abc]	< 0.001
HOMA-β	143 ± 116	199 ± 172	134 ± 82[a]	134 ± 94[a]	106 ± 68[a]	< 0.001
Matsuda index	6.2 ± 3.8	4.5 ± 2.9	5.2 ± 2.9	6.6 ± 3.7[ab]	8.7 ± 4.1[abc]	< 0.001
HOMA-IR	2.1 ± 1.8	3.5 ± 2.7	2.2 ± 1.1[a]	1.8 ± 1.03[ab]	1.1 ± 0.6[abc]	< 0.001
Cholesterol (mg/dl)	180 ± 38	193 ± 37	181 ± 37[a]	176 ± 42[a]	170 ± 30[ab]	< 0.001
HDL (mg/dl)	45 ± 13	43 ± 16	44 ± 13	45 ± 12	47 ± 11[a]	< 0.001
LDL (mg/dl)	108 ± 35	118 ± 35	108 ± 36[a]	108 ± 37[a]	100 ± 30[a]	< 0.001
Triglycerides (mg/dl)	131 ± 65	154 ± 75	140 ± 66	118 ± 56[ab]	112 ± 50[ab]	< 0.001

WC waist circumference, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *OGTT* oral glucose tolerance test, *AUC* area under the curve, *HOMA* homeostatic model assessment for insulin resistance, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol

$a = p < 0.05$ versus *Q*1, $b = p < 0.05$ versus *Q*2, $c = p < 0.05$ versus *Q*3

blood pressure was also inversely related to the pancreatic β-cell function ($p < 0.001$). Regarding glucose levels during OGTT, it is important to note that only patients from $Q4$ had a 2-h glucose value similar to the fasting glucose value, and in patients from $Q3$ to $Q1$, all glucose levels during the OGTT as well as the AUCglucose0_120 min were progressively increasing ($p < 0.001$), with the worst OGTT profile in $Q1$ group. HOMA-β was inversely correlated with βf by the disposition index, showing the lowest level in $Q4$ and the highest in $Q1$ of βf. In addition, total cholesterol, LDL, and triglycerides were significantly higher in the lowest quartiles ($p < 0.001$), while HDL levels were lower in these quartiles ($p < 0.001$). In most of the variables, the post hoc analysis showed a difference between $Q1$ and $Q3$–4. Insulin levels were higher in $Q1$ and $Q2$ (Fig. 1a, $p < 0.05$); incremental and AUC for insulin during OGTT tended to be also higher in $Q1$ and $Q2$ (Fig. 1b, c, p NS); however, when insulin levels were adjusted by glucose levels during OGTT (Fig. 1d, e), it was lower in $Q1$ and $Q2$, as well as the first phase insulin secretion when measured by AIR, oral disposition index, and AIR * Matsuda index (Fig. 1f–h, $p < 0.05$).

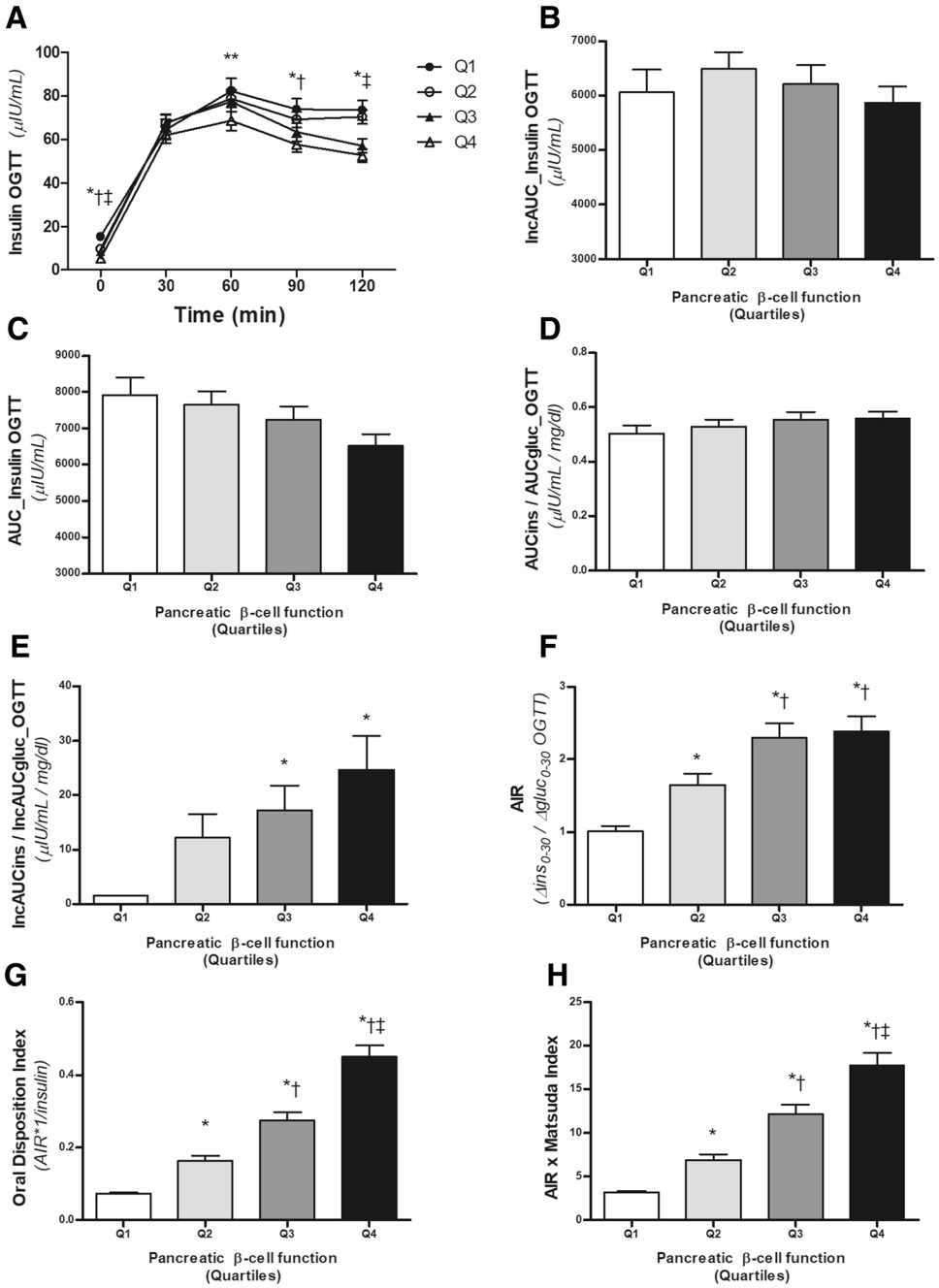

Fig. 1 Insulin levels (**a**), incremental AUC (**b**), AUC for insulin during OGTT (**c**), insulin secretion (**d**, **e**), acute insulin response (**f**), oral disposition index (**g**), and first phase disposition index (**h**) between the study groups. *$p < 0.05$ versus $Q1$, † $p < 0.05$ versus $Q2$, ‡ $p < 0.05$ versus $Q3$

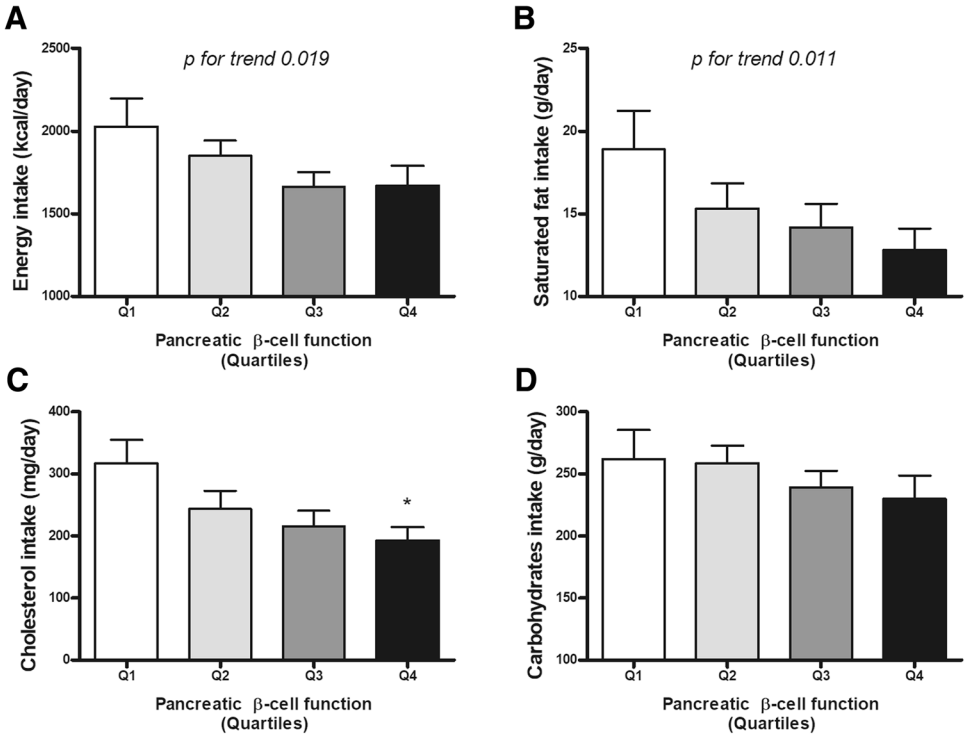

Fig. 2 Total energy (**a**), saturated fat (**b**), cholesterol (**c**), and carbohydrate (**d**) intake in a subgroup of patients according to quartiles of pancreatic β-cell function ($Q1$ $n=34$, $Q2$ $n=45$, $Q3$ $n=42$, $Q4$ $n=39$)

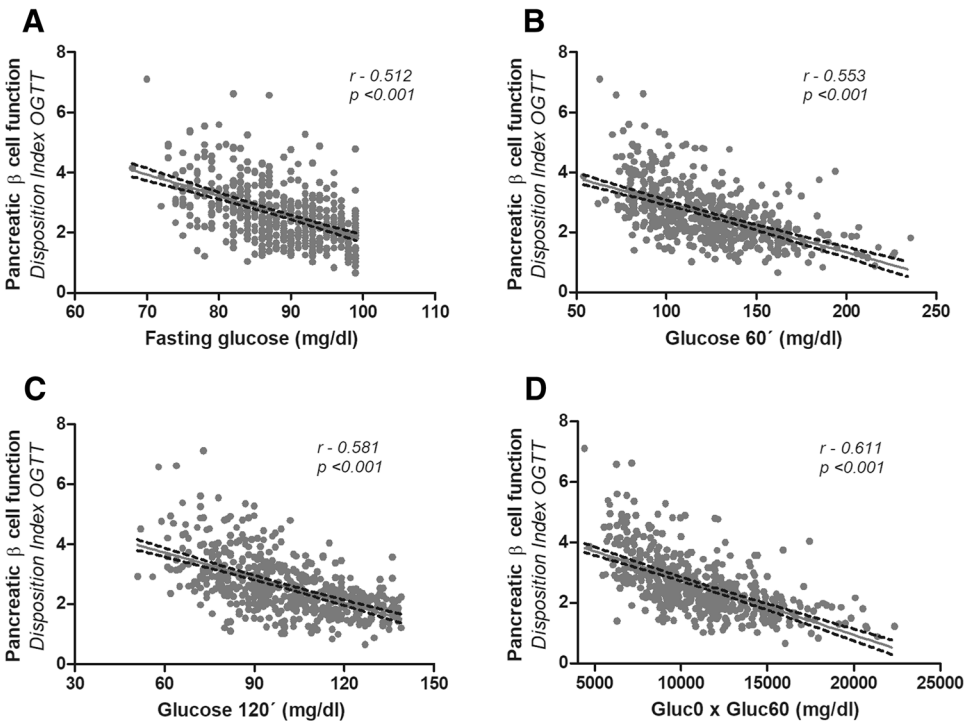

Fig. 3 Correlation coefficient between fasting glucose (**a**), 60 min (**b**), and 120 min (**c**) with the pancreatic β-cell function, as well as the correlation between the product of fasting glucose by 60-min glucose with the pancreatic β-cell function (**d**). *OGTT: oral glucose tolerance test*

On the other hand, the 24-h dietary recall showed that patients in $Q1$ had the highest consumption of energy (Fig. 2a, p for trend < 0.05), saturated fat (Fig. 2b, p for trend < 0.05), total cholesterol (Fig. 2c, $p < 0.05$ $Q1$ vs. $Q4$), and carbohydrates (Fig. 2d, p NS).

Correlation coefficient analysis showed a negative correlation between pancreatic β-cell function and age, body weight, BMI, waist circumference, body fat, visceral fat, blood pressure, total cholesterol, LDL and triglycerides (Suppl. Table 2, $p < 0.01$), and a positive correlation with

Table 2 Variables associated with pancreatic β-cell dysfunction

Variable	Odds ratio (OR)	CI 95%
Male	1.61	1.06–2.46
Age > 45 years	1.63	1.06–2.51
Hypertension	2.36	1.14–4.85
BMI > 27 kg/m^2	2.07	1.39–3.10
Hypertension MS	2.07	1.39–3.10
Waist circumference	2.56	1.66–3.96
Fasting glucose > 90 mg/dl	3.53	2.28–5.46
Fasting glucose > 95 mg/dl	2.96	1.92–4.59
Glucose 60-min OGTT > 110 mg/dl	6.85	3.86–12.40
Glucose 60-min OGTT > 120 mg/dl	7.25	4.36–12.05
Glucose 60-min OGTT > 125 mg/dl	6.81	4.25–10.90
Glucose 60-min OGTT > 130 mg/dl	5.90	3.82–9.12
Glucose 60-min OGTT > 135 mg/dl	6.44	4.18–9.90
Glucose 60-min OGTT > 140 mg/dl	6.11	3.97–9.39
Glucose 60-min OGTT > 145 mg/dl	6.22	3.99–9.69
Glucose 60-min OGTT > 150 mg/dl	7.15	4.48–11.41

BMI body mass index, *MS* metabolic syndrome criteria, *OGTT* oral glucose tolerance test

Matsuda index and HDL (Suppl. Table 1, $p < 0.01$). As expected, glucose levels during OGTT had a negative correlation with pancreatic β-cell function; the best correlation was with the 1-h glucose ($r = 0.581$, $p < 0.001$, Fig. 3); however, there was an even stronger negative correlation between the product of fasting glucose and 1-h glucose and β-cell function ($r = -0.611$ $p < 0.001$, Fig. 3D). Energy and fat intake were negatively correlated with pancreatic β-cell function (Suppl. Table 1, $p < 0.05$), and there was also a negative correlation between quartiles of pancreatic β-cell function and carbohydrate intake (Spearman rho −0.156, p 0.049).

Male sex, age over 45 years, high blood pressure, BMI > 27 kg/m^2, and high waist circumference were positively associated with pancreatic β-cell dysfunction (Table 2). Fasting glucose ˃ 90 and ˃ 95 mg/dl were also associated with a higher risk of pancreatic β-cell dysfunction (OR 3.53 IC 95% 2.28–5.46), and values above 90 mg/dl had a sensitivity and specificity of 65 and 61%, respectively, to predict pancreatic β-cell dysfunction (Fig. 4b). Glucose levels at 1-h OGTT above 110 mg/dl were positively associated with an increased risk of > 6 times of pancreatic β-cell dysfunction (OR 6.85, CI 95% 3.86–12.4). Again, the product of fasting glucose and 1-h glucose (Gluc0 × Gluc60) had the best AUC to predict pancreatic β-cell dysfunction (AUC 0.816, CI 95% 0.774–0.857), in comparison with the other glucose values during the OGTT, even better than glucose at 1 h (AUC 0.791, CI 95%, 0.747–0.835, Fig. 4). Of note, glucose levels at 1-h OGTT > 155 mg/dl had a sensitivity and specificity of 42 and 92%, respectively, while a 1-h glucose value > 125 mg/dl had a sensitivity and specificity of 78 and 65%, respectively, and a better negative predictive value (Fig. 4e, f). A Gluc0 × Gluc60 value > 11,000 mg/dl had a sensitivity and specificity of 82 and 65% and an acceptable negative predictive value (Fig. 4k, l).

Of note, insulin sensitivity, measured by the Matsuda index, was directly related to the pancreatic β-cell function ($p < 0.001$), which could partially explain the differences in pancreatic βf; to control the effect of insulin sensitivity between the study groups, we performed a paired analysis by insulin sensitivity, and we found that most of the differences persisted after controlling insulin sensitivity (Suppl. Table 2); the only differences that disappear after the insulin sensitivity paired analysis were in sex, blood pressure, HOMA-β, and cholesterol levels. In the multiple regression analysis, only glucose and insulin levels during OGTT and BMI were associated with pancreatic βf (R^2 0.795, $p < 0.001$, Suppl. Table 3); after removing glucose and insulin at 30, 60, 90, and 120 min from the analysis, fasting glucose, BMI, and triglyceride levels were the main factors associated with pancreatic βf (R^2 0.414, $p < 0.001$, Suppl. Table 3).

Discussion

In this study, we have characterized the pancreatic β-cell function in Hispanic patients with normoglycemia and normal glucose tolerance by dividing the groups in quartiles of pancreatic β-cell function and showing that there is a subgroup of patients ($Q1$) who have lost around 40% of their pancreatic β-cell function and the first phase insulin secretion besides having normal glucose levels. Furthermore, different clinical, nutritional, and biochemical variables were associated with this pancreatic β-cell dysfunction, including glucose at 1 h and the product of fasting glucose and 1-h glucose, regardless of the degree of insulin sensitivity.

It has been shown that age is related to pancreatic β-cell function and that this function decreases with time [18], which is consistent with what was found in our study, since the group with the lowest pancreatic β-cell function was older than the other groups and there was a negative correlation between age and pancreatic β-cell function. However, patients in $Q1$ were around 40 years of age, which is in agreement with the early starting of the physiopathology of hyperglycemia, although age was not related to pancreatic βf in the multivariate analysis in our population.

Body weight and BMI inversely correlated with the pancreatic β-cell function, as it has been previously reported in normoglycemic subjects with obesity, who have only 50% of their pancreatic β-cell function compared to lean patients; in our study, besides that patients do not reach the obesity range by the BMI, there was a clear reduction in pancreatic

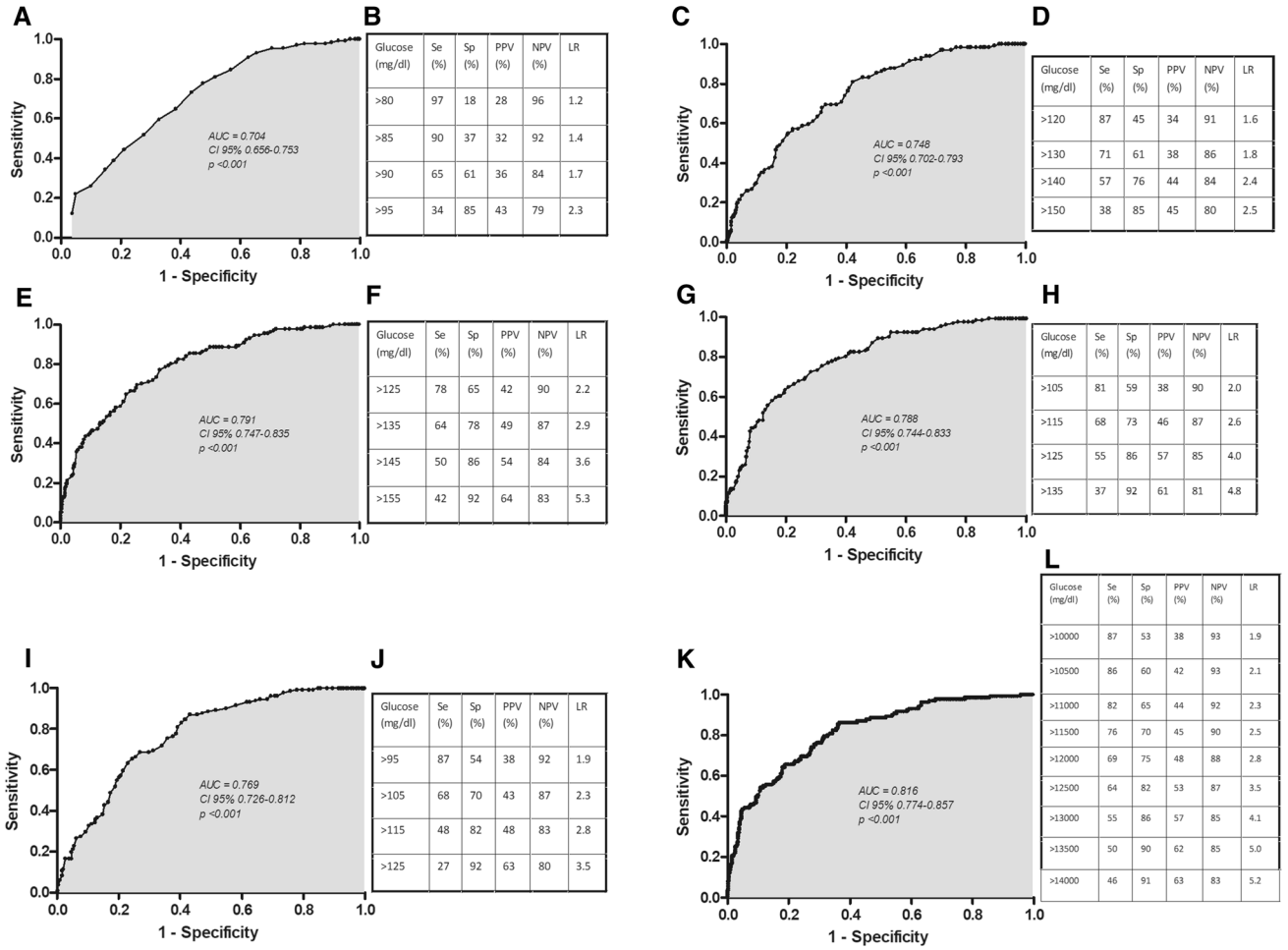

Fig. 4 Area under the curve (AUC), sensitivity, specificity, and predictive values for glucose at fasting (**a**, **b**), 30 min (**c**, **d**), 60 min (**e**, **f**), 90 min (**g**, **h**), 120 min (**i**, **j**), and the product of fasting glucose by glucose at 60 min (**k**, **l**) to diagnose and predict pancreatic β-cell dysfunction in normoglycemic and normotolerant patients

β-cell function [19, 22–25]. However, more than body weight, body fat and visceral fat are variables directly related to greater insulin resistance, lipotoxicity, and greater pancreatic β-cell dysfunction, as well as to an increase in waist circumference that has been associated with an increased risk of developing T2DM [26, 27]. Here, we found that normoglycemic patients with pancreatic β-cell dysfunction had a greater waist circumference, body fat, and visceral fat, in comparison with the patients with a better pancreatic β-cell function (*Q*2–*Q*4), and BMI was one of the significant variables associated with βf in the multivariate analysis [6, 16, 23, 24, 26].

A significant difference in the systolic and diastolic blood pressure was found between *Q*1 and *Q*4, and a slight negative correlation was observed between them with pancreatic β-cell function. Several studies have studied and integrated the relationship between blood pressure and insulin resistance [28, 29], and in our study, patients in *Q*1 were less insulin sensitive.

Lipotoxicity plays an important role in the pathophysiology of β-cell dysfunction [16, 18, 26]. Here, we found higher levels of total cholesterol, LDL, and triglycerides, while HDL was lower in *Q*1, and all of them were correlated with pancreatic β-cell function. Interestingly, after removing non-fasting glucose and insulin levels from the multivariate analysis, triglyceride levels were significantly associated with βf.

Interestingly, HOMA-β was negatively correlated with βf, demonstrating that HOMA-β is not that useful to measure pancreatic β-cell function in patients with normoglycemia and normal glucose tolerance, since it is based only on fasting measurements; in this way, patients with pancreatic β-cell function (*Q*1) showed the highest HOMA-β level, which could be indicating a stressed β-cell instead of a better β-cell function.

Of note, most of the differences between the study groups persisted after controlling insulin sensitivity pairing the groups by the Matsuda index, and one of the main

alteration was observed in parameters related to the first phase insulin secretion, as it has been reported to be one of the first physiopathological abnormalities observed in early stages of hyperglycemia [8, 13, 14, 18, 19].

As expected, glucose concentrations during OGTT and area under the glucose curve were the main variables that presented a significant difference between the studied groups, with a high correlation with pancreatic β-cell function. From the different times at the OGTT, glucose at 1 h showed the highest difference and the strongest correlation coefficient as compared with the other glucose measurements. Moreover, glucose at 1 h during OGTT showed a moderate usefulness to predict pancreatic β-cell dysfunction, since glucose at 1 h > 55 mg/dl had a sensitivity and specificity of 42 and 92%, respectively, which would bring to the conclusion that more than 50% of the patients as "normal" pancreatic β-cell function; glucose at 1 h > 125 mg/dl had a better sensitivity and specificity (78 and 65%) with an accepted negative predictive value; and even 1-h glucose values above 110 already increase the probability of pancreatic β-cell dysfunction for more than 6 times. This highlights the definition of a cutoff level in 1-h glucose to identify subjects with high risk of T2D in patients with normoglycemic and normal glucose tolerance; perhaps the cutoff level of 1-h glucose > 155 mg/dl that has been previously suggested [30, 31] would be a good option to identify patients with certain degree of hyperglycemia (prediabetes), but in normoglycemic patients, 155 mg/dl at 1 h would be less sensitive to predict pancreatic β-cell dysfunction; furthermore, this cutoff level should be lower, in order to have a better screening capacity. Here, we are reporting also a novel measurement to predict pancreatic β-cell dysfunction, the product of fasting glucose by 1-h glucose (Gluc0 × Gluc60) that showed a better capacity to predict pancreatic β-cell dysfunction, even better than 1-h glucose; a Gluc0 × Gluc60 value ˃ 11,000 mg/dl showed a sensitivity and specificity of 82 and 65% and a good negative predictive value. The best use of this variable could be because it integrates the fasting state and the dynamic response to a glucose challenge. This value could be also useful as a screening test to define in which patients we should focus for an early T2D prevention; it could be calculated from the 1-h OGTT.

To our knowledge, no previous studies have reported data about food consumption in patients with normogycemia and pancreatic β-cell dysfunction; interestingly, we found a negative correlation between energy, saturated fat, and cholesterol intake with pancreatic β-cell function, showing that patients in *Q*1 had the highest energy, fat, cholesterol, and carbohydrate intake per day. We did not find an association between sedentary lifestyle and pancreatic β-cell function (data not shown), which highlights the role of food intake and its connection mainly with lipotoxicity.

In conclusion, our data characterize a subgroup of patients with normoglycemia and normal glucose tolerance, around 40 years of age, who are overweight, that have a relative pancreatic β-cell dysfunction with a loss of approximately 40% of their pancreatic β-cell function and the first phase insulin secretion, and identify different clinical and biochemical variables associated with pancreatic β-cell dysfunction in these patients. The product of fasting and 1-h glucose during OGTT showed the best discriminatory ability to identify pancreatic β-cell dysfunction, showing also an inverse relationship between food intake and pancreatic β-cell function, for the first time. This study highlights also the need of a different cutoff level in 1-h glucose to predict early physiopathological abnormalities in normoglycemic patients and perhaps the validation of different surrogate measurements considering fasting and 1-h glucose.

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Author's contribution RGM and LHVH designed the study; RGM, SSSL, GABM, AKVM, GALP, MEQH, MAGV, RRC, and FAR generated the data; LHVH, RGM, MLRE, AAG, and FF analyzed and interpreted the data. All the authors wrote and reviewed the final manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard statement The protocol was approved by the Institutional Research and Ethical Committee at the University of Guanajuato with the number CIBIUG-P40-2016. All procedures performed were in accordance with the ethical standards of the Institutional Research and Ethical Committee and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Informed consent Informed consent was obtained from all the participants before their inclusion in the present study.

References

1. Alegre-Diaz J, Herrington W, Lopez-Cervantes M et al (2016) Diabetes and cause-specific mortality in Mexico City. N Engl J Med 375(20):1961–1971
2. Rangel-Baltazar E, Cuevas-Nasu L, Shamah-Levy T et al (2019) Association between high waist-to-height ratio and cardiovascular risk among adults sampled by the 2016 half-way national health

and nutrition survey in Mexico (ENSANUT MC 2016). Nutrients 11(6):1402
3. Villalpando S, de la Cruz V, Rojas R et al (2010) Prevalence and distribution of type 2 diabetes mellitus in Mexican adult population: a probabilistic survey. Salud Publica Mex 52(Suppl 1):S19–S26
4. Cho NH, Shaw JE, Karuranga S et al (2018) IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 138:271–281
5. Classification and Diagnosis of Diabetes (2019) Standards of medical care in diabetes. Diabetes Care 42(Suppl 1):S13–S28
6. DeFronzo RA (2009) Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links The Claude Bernard Lecture. Diabetologia 53(7):1270–1287
7. Defronzo RA (2009) Banting Lecture From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 58(4):773–795
8. Ferrannini E, Mari A, Nofrate V, Sosenko JM, Skyler JS; DPT-1 Study Group (2010) Progression to diabetes in relatives of type 1 diabetic patients: mechanisms and mode of onset. Diabetes 59(3):679–685
9. Guardado-Mendoza R, Davalli AM, Chavez AO et al (2009) Pancreatic islet amyloidosis, beta-cell apoptosis, and alpha-cell proliferation are determinants of islet remodeling in type-2 diabetic baboons. Proc Natl Acad Sci USA 106(33):13992–13997
10. Kahn SE, Zraika S, Utzschneider KM, Hull RL (2009) The beta cell lesion in type 2 diabetes: there has to be a primary functional abnormality. Diabetologia 52(6):1003–1012
11. Guardado Mendoza R, Perego C, Finzi G et al (2015) Delta cell death in the islet of Langerhans and the progression from normal glucose tolerance to type 2 diabetes in non-human primates (baboon, Papio hamadryas). Diabetologia 58(8):1814–1826
12. Folli F, La Rosa S, Finzi G et al (2018) Pancreatic islet of Langerhans' cytoarchitecture and ultrastructure in normal glucose tolerance and in type 2 diabetes mellitus. Diabetes Obes Metab 20(Suppl 2):137–144
13. Faerch K, Vaag A, Holst JJ, Hansen T, Jorgensen T, Borch-Johnsen K (2009) Natural history of insulin sensitivity and insulin secretion in the progression from normal glucose tolerance to impaired fasting glycemia and impaired glucose tolerance: the Inter99 study. Diabetes Care 32(3):439–444
14. Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA (2005) beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. J Clin Endocrinol Metab 90(1):493–500
15. Ahren B (2009) Beta- and alpha-cell dysfunction in subjects developing impaired glucose tolerance: outcome of a 12-year prospective study in postmenopausal Caucasian women. Diabetes 58(3):726–731
16. Daniele G, Guardado Mendoza R, Winnier D et al (2014) The inflammatory status score including IL-6, TNF-alpha, osteopontin, fractalkine, MCP-1 and adiponectin underlies whole-body insulin resistance and hyperglycemia in type 2 diabetes mellitus. Acta Diabetol 51(1):123–131
17. Guardado-Mendoza R, Chavez AO, Jimenez-Ceja LM et al (2017) Islet amyloid polypeptide response to maximal hyperglycemia and arginine is altered in impaired glucose tolerance and type 2 diabetes mellitus. Acta Diabetol 54(1):53–61
18. DeFronzo RA (2004) Pathogenesis of type 2 diabetes mellitus. Med Clin North Am 88(4):787–835
19. Gastaldelli A, Ferrannini E, Miyazaki Y, Matsuda M, DeFronzo RA (2004) Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study. Diabetologia 47(1):31–39
20. Hannon TS, Kahn SE, Utzschneider KM et al (2018) Review of methods for measuring beta-cell function: design considerations from the Restoring Insulin Secretion (RISE) Consortium. Diabetes Obes Metab 20(1):14–24
21. Salunkhe VA, Veluthakal R, Kahn SE, Thurmond DC (2018) Novel approaches to restore beta cell function in prediabetes and type 2 diabetes. Diabetologia 61(9):1895–1901
22. Matveyenko AV, Butler PC (2006) Beta-cell deficit due to increased apoptosis in the human islet amyloid polypeptide transgenic (HIP) rat recapitulates the metabolic defects present in type 2 diabetes. Diabetes 55(7):2106–2114
23. Camastra S, Manco M, Mari A et al (2005) Beta-cell function in morbidly obese subjects during free living: long-term effects of weight loss. Diabetes 54(8):2382–2389
24. Camastra S, Manco M, Mari A et al (2007) Beta-cell function in severely obese type 2 diabetic patients: long-term effects of bariatric surgery. Diabetes Care 30(4):1002–1004
25. Furukawa H, Carroll RJ, Swift HH, Steiner DF (1999) Long-term elevation of free fatty acids leads to delayed processing of proinsulin and prohormone convertases 2 and 3 in the pancreatic beta-cell line MIN6. Diabetes 48(7):1395–1401
26. Biggs ML, Mukamal KJ, Luchsinger JA et al (2010) Association between adiposity in midlife and older age and risk of diabetes in older adults. JAMA 303(24):2504–2512
27. Niswender KD, Magnuson MA (2007) Obesity and the beta cell: lessons from leptin. J Clin Invest 117(10):2753–2756
28. Abdul-Ghani MA, Jayyousi A, DeFronzo RA, Asaad N, Al-Suwaidi J (2019) Insulin resistance the link between T2DM and CVD: basic mechanisms and clinical implications. Curr Vasc Pharmacol 17(2):153–163
29. Di Pino A, DeFronzo RA (2019) Insulin resistance and atherosclerosis: implications for insulin sensitizing agents. Endocr Rev. https://doi.org/10.1210/er.2018-00141
30. Jagannathan R, Buysschaert M, Medina JL et al (2018) The 1-h post-load plasma glucose as a novel biomarker for diagnosing dysglycemia. Acta Diabetol 55(6):519–529
31. Abdul-Ghani MA, Lyssenko V, Tuomi T, Defronzo RA (2010) Groop L The shape of plasma glucose concentration curve during OGTT predicts future risk of type 2 diabetes. Diabetes Metab Res Rev 26(4):280–286

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