Effects of a new 75 g glucose- and high fat-containing cookie meal test on postprandial glucose and triglyceride excursions in morbidly obese patients

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Abstract. A new meal tolerance test (MTT) using a 75 g glucose- and high fat-containing meal was applied to classify glucose intolerance in morbidly obese patients. According to the MTT data, the concordance rate of diagnosis was 82.5% compared to the 75 g oral glucose tolerance test (OGTT) in patients with normal glucose tolerance (NGT, $n = 40$). In the NGT patients, the insulinogenic index ($r = 0.833$), Matsuda index ($r = 0.752$), and disposition index ($r = 0.845$) calculated from the MTT data were each significantly ($p < 0.001$) correlated with those derived from the OGTT data. However, in patients with impaired glucose tolerance (IGT, $n = 23$) or diabetes mellitus (DM, $n = 17$), the postprandial glucose levels post-MTT were significantly lower than those post-OGTT, without increases in the postprandial insulin levels post-MTT. Thus, the severity of glucose intolerance measured by the MTT was milder than that indicated by the OGTT. Plasma levels of both glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) were increased at the postprandial state, but only the GIP levels post-MTT were significantly higher than those post-OGTT. The enhancement of glucose disposal rates in patients with NGT or IGT after the MTT was associated with increased GIP levels. The postprandial hypertriglyceridemia induced by the MTT was associated with insulin resistance, but it was not associated with the impaired insulinogenic index or the disposition index. These results indicate that the new MTT is clinically useful to evaluate both abnormal glucose and triglyceride excursions caused by abnormal insulin sensitivity and secretions of insulin and gut hormones in morbidly obese patients.

Key words: Morbidly obese patients, Meal tolerance test, Insulin resistance, β -cell function, Incretin

THE NUMBERS of morbidly obese individuals in Asian countries are rapidly increasing. According to recent Japanese population surveys, the body mass index (BMI) values (kg/m^2) are ≥ 25 in 24% of the population, ≥ 30 in 3%, and ≥ 35 in 0.5%, with the percentage of the population having severe obesity being lower than in the EU or U.S. [1-3]. However, a World Health Organization (WHO) Expert Committee has reported that the incidence of health problems stemming from obesity is higher among Asians [4]. It has been generally accepted that bariatric surgery is more effective for reducing morbid obesity-associated mortality and morbidity [5, 6]

than other intensive medical treatments [7, 8]. It has also been suggested that type 2 diabetes mellitus (T2DM) is frequently resolved following gastric bypass surgery, with 60% of patients achieving remission of the diabetic state [9]. Laparoscopic sleeve gastrectomy (LSG) effectively reduces body weight to the same extent as a Roux-en-Y gastric bypass (RYGB) [10, 11]. The LSG is a restrictive surgery and since April 2014 it has been the only approved procedure covered by Japan's national health insurance system. According to the 2018 surgery record of the Japanese Society for Treatment of Obesity, 98% of the bariatric surgeries in Japan are LSGs.

Using the standard 75 g oral glucose tolerance test (OGTT), we recently demonstrated that the LSG procedure efficiently improves glycemic control in Japanese T2DM patients with morbid obesity [12]. The patients who achieved diabetes remission in our series had certain clinical characteristics, such as lower HbA1c levels,

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higher C-peptide values, shorter diabetes duration, lower insulin requirement, and higher insulin levels in response to the oral glucose loading, suggesting that preserving insulin secretion is a major determinant of diabetes remission [12]. The results of that study clearly demonstrated that an additional protocol beyond the standard OGTT will be needed to further define and measure glucose and lipid metabolism and confirm their complete normalization.

Glucose intolerance in morbidly obese patients with or without T2DM has been evaluated before and after medical or surgical treatments by using the standard 75 g OGTT. This test has shown postprandial hyperinsulinemia-related hypoglycemia after oral glucose-loading in a few patient populations (*e.g.*, patients with dumping syndrome). However, postprandial hyperinsulinemia-related hypoglycemia is not a rare complication among morbidly obese patients who undergo bariatric surgery [13–15].

In the present study, therefore, we attempted to devise a new meal tolerance test (MTT) that differs from the standard OGTT and meets three objectives: achieving a more precise measurement of glucose intolerance than that provided by the 75 g OGTT; preventing postprandial hyperinsulinemia-related hypoglycemia; and identifying postprandial lipid metabolism in morbidly obese individuals. We created the new MTT using a cookie meal containing a high amount of fat (43.6% of total energy), 75 g of glucose-containing carbohydrate (51%), and a low amount of protein (5.4%), and administered it to morbidly obese patients who were scheduled to undergo an LSG. A same cookie meal test was used to assess glucose intolerance, insulin resistance, postprandial hyperinsulinemia, and hypertriglyceridemia in healthy control volunteers [16], and this specific type of MTT contains the same amount of glucose as used in the standard 75 g OGTT. We speculated that this high-fat test meal might modify the *in vivo* glycemic excursion by modulating incretin secretion and gastric emptying in the postprandial state, compared with the same amount of a single 75 g glucose loading test, as suggested in previous studies [17–19].

Before the clinical application of this cookie meal test to morbidly obese patients after their bariatric surgeries, we needed to clarify the differences between the standard OGTT and the new MTT in terms of (1) the concordance rate for the accurate classification of glucose intolerance, (2) the concordance rates for clinical parameters determining glucose intolerance, such as insulin resistance and impaired insulin secretion, and (3) the ability to identify high fat-induced hypertriglyceridemia. We thus conducted the present study to evaluate the clinical usefulness of the new MTT by serially administering both

types of glucose tolerance test in each patient.

Materials and Methods

Study participants

Eighty-five morbidly obese patients (age range: 22–62 years old) on the waiting list for bariatric surgery were recruited from the outpatient obesity clinic at Omi Medical Center for medical and surgical management of morbidly obese patients with or without type 2 diabetes mellitus (T2DM) during the period from Oct 1, 2017–June 30, 2021. Each patient's BMI was treated to bring his or her body weight as close as possible to 35 kg/m² by nutritional and behavior intervention for at least 3 months before enrollment in the present study. Five insulin-treated T2DM patients were excluded from the present study, leaving a final series of 80 patients for the analyses.

All patients were hospitalized for at least 6 days to evaluate body composition, and to perform a physical examination and baseline blood testing, under the conditions of stable nutritional management (the planned dietary energy intake was 1,400 kcal/day) and daily physical activity. Informed written consent for study participation and data publication was obtained from all patients. The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of Kusatsu General Hospital (protocol no. 2017-0317-05; dated March 24, 2017). The baseline clinical and metabolic characteristics of the patients are summarized in Table 1.

75 g oral glucose tolerance test (OGTT)

On the 2nd day after admission, after completing a 12-h overnight fast, the patients underwent a 75 g OGTT. A 20-gauge polyethylene cannula was inserted into an antecubital vein for blood sampling. A baseline blood sample was drawn for measurements of fasting plasma glucose (FPG), immunoreactive insulin (IRI), serum triglyceride (TG), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and estimated glomerular filtration rate (eGFR). Each participant consumed, within 5 min, a 225 mL beverage that contained a partial starch hydrolysate, and then blood samples were drawn at 0, 30, 60, and 120 min before and after the consumption of a beverage containing 75 g glucose for the measurements of plasma glucose, IRI, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), and serum triglyceride (TG) concentrations. Blood samples were centrifuged for 15 min (3,000 g, at 4°C) and frozen at –80°C before analysis. The glucose tolerance, homeo-

Table 1 Anthropometric and metabolic characteristics of morbidly obese patients

	Total	NGT	IGT	DM
No. males/females	80, 19/61	40, 9/31	23, 4/19	17, 6/11
Age, yrs	41.2 ± 9.0	39.5 ± 8.9	42 ± 7.3	43.9 ± 10.4
BMI, kg/m ²	40.3 (37.1–45.2)	40.4 (37.6–44.6)	39.4 (36.0–47.0)	40.8 (37.5–44.1)
% body fat, %	47.4 ± 5.0	48.0 ± 5.3	48.2 ± 3.3	44.8 ± 5.1
Visceral fat amount, g	1,272.8 ± 251.6	1,235.4 ± 225.9	1,227.1 ± 253.9	1,414.3 ± 272.2*
s-BP/d-BP, mmHg	141.9/85.4 ± 17.0/16.5	139.2/82.7 ± 17.6/16.4	142/85.1 ± 15.8/17.3	146.7/92.2* ± 15.8/13.5
FPG, mg/dL	90.2 ± 11.7	84.3 ± 7.9	91.0 ± 8.6**	103.1 ± 12.3***
IRI, μU/mL	10.9 ± 4.6	10.9 ± 5.2	10.5 ± 4.0	11.2 ± 3.8
HbA1c, %	6.3 ± 1.3	5.7 ± 0.3	5.9 ± 0.6	8.2 ± 1.4***
HOMA-IR	2.4 ± 1.2	2.3 ± 1.2	2.4 ± 1.0	2.9 ± 1.2
Triglycerides, mg/dL	157 (105–207)	152 (95–200)	159 (122–226)	163 (119–237)
HDL-cholesterol, mg/dL	52.1 ± 10.2	53.3 ± 10.4	51.0 ± 10.8	50.8 ± 9.7
LDL-cholesterol, mg/dL	106.4 ± 32.3	106.3 ± 92.2	103.6 ± 22.2	110.5 ± 42.0
AST, IU/L	20.0 (17–31)	20 (17–26)	19 (18–27)	40 (30–51)***
ALT, IU/L	28 (20–47)	25 (18–36)	27 (19–39)	64 (37–89)***
Creatinine, mg/dL	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.7 ± 0.3

Data are means ± SD or medians (*Quartile 1–Quartile 3*).

s-BP/d-BP, systolic blood pressure/diastolic blood pressure; FPG, fasting plasma glucose; IRI, immunoreactive insulin

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. NGT

static model assessment of insulin resistance (HOMA-R), insulinogenic index, Matsuda index, and disposition index were calculated.

Meal tolerance test (MTT)

The new test meal consists of 75 g glucose (85% flour starch and 15% maltose), 28.5 g butter fat (saturated fat, 43.3% of total energy), and 8.0 g protein with total energy of 592 kcal, and is commercially available as a 12 pieces of cookie meal (Saraya Co., Osaka, Japan). After the patients underwent a 12 h-overnight fast, they were instructed to consume the 12-pieces of cookie test meal with 200 mL of water within 10–15 min at the 4th day after admission. For measurements of postprandial TG levels after the MTT, blood samples were also drawn for measurements of the same metabolic parameters detected by 75 g OGTT. We then compared the values of each parameter obtained by the MTT with those obtained by the OGTT.

Biochemical and body composition analyses

Body composition was measured by the bioelectrical impedance method using an InBody 770 device (In Body Japan, Tokyo) [20]. Plasma glucose (PG) was determined using the standard enzymatic method and plasma insulin levels were measured using a chemiluminescence

immunoassay (*CLIA*) kit (Abbott, Japan). Plasma GLP-1 levels were quantified by a total GLP-1(9-36/37) Assay enzyme-linked immunosorbent assay (*ELISA*) kit (*Code No. 27788*) (Immuno-Biological Laboratories Co., Fujioka, Japan). The antibody used in this assay is reported to be specific for total GLP-1 (9-36/37) and has no cross-reactivity with GLP-2, GIP, glucagon, or oxyntomodulin. Total GIP concentrations were measured by a sandwich *ELISA* kit (*Code No. 27203*) (Immuno-Biological Laboratories Co.). In the present study, we intended to measure the changes in total GLP-1 and GIP secretory responses to the OGTT and MTT, respectively. Thus, we measured both inactive forms of GLP-1 and total GIP concentrations, since plasma concentrations of inactive GLP-1 forms were nearly equal to the total GLP-1, where active forms were rapidly metabolized into inactive forms at the present blood sampling and the total GIP measurement included both active and inactive forms of GIP. Serum LDL-C, HDL-C, TG, ALT, AST, and creatinine concentrations were detected in the central laboratory by routine methods. In the present study, only data for 57 of the 63 patients with NGT or IGT were available for full set analysis of triglyceride excursion after the MTT; the data for 6 patients were missing due to measurement error or hemolysis of blood samples. The GLP-1 and GIP concentrations were evaluated in

only 37 patients, because these measurements were initiated at a midpoint during the study period.

Mathematical modeling and the calculation of indices determining glucose tolerance curves

We estimated the baseline insulin resistance of morbidly obese patients by homeostasis model assessment of insulin resistance (HOMA-R) [21, 22] using the following equation: $\text{HOMA-R} = \text{FPG (mg/dL)} \times \text{F-IRI } (\mu\text{U/mL})/405$. $\text{HOMA-R} \geq 2.5$ indicates the presence of insulin resistance in the Japanese population. The insulin sensitivity index was evaluated by glucose and insulin excursions after the OGTT and MMT, and we estimated it by the Matsuda index, which was calculated as follows; Matsuda index (ISI-M) = $10,000/(\text{GLU}_0 \times \text{INS}_0 \times \text{GLU}_{\text{mean}} \times \text{INS}_{\text{mean}})^{1/2}$, where GLU_0 and GLU_{mean} are the fasting and mean glucose levels during the OGTT and MTT, and INS_0 and INS_{mean} are the fasting and mean insulin levels during 75 g OGTT and the MTT, respectively. A Matsuda index value <2.5 or <4.3 indicates the presence of insulin resistance, depending on the study population [22–24]. In the present study, we defined insulin resistance as a Matsuda index <2.5 . The insulinogenic index was measured as $[\text{IRI}_{30} - \text{IRI}_0 (\mu\text{U/mL})]/[\text{GLU}_{30} - \text{GLU}_0 (\text{mg/dL})]$, where IRI_0 and IRI_{30} are the IRI levels at fasting and 30 min after the OGTT and MTT, and GLU_0 and GLU_{30} are the plasma glucose levels at fasting and 30 min after the OGTT and MTT, respectively. An insulinogenic index value less than 0.4 was defined as an impairment of acute insulin secretory activity. The disposition index was calculated as the product of insulin sensitivity and the amount of acute insulin secretion in response to increases in blood glucose levels. Thus, the disposition index was expressed as insulin sensitivity index $\times$ acute insulin response, and a value ≤ 1.0 was defined as abnormal [22, 25].

Statistics

Continuous variables are presented as the mean (standard deviation [SD]) and median (interquartile range; Quartile 1 [Q1] and Quartile 3 [Q3]). Normally distributed data were analyzed by parametric tests. Non-normally distributed data were analyzed by the Wilcoxon rank-sum test. The total area under the curves (AUC) for insulin and for glucose were calculated using the trapezoid rule. Differences between the data from the OGTT and MTT were analyzed using a paired Student-test. We also calculated Pearson's correlation to assess the relationships among the insulinogenic index, Matsuda index, and disposition index based on data from the OGTT and MMT. Pearson's correlation was also used to analyze the correlation between the fasting TG levels and 2h-TG levels after the MMT and correlations between the Log_{10}

2h-TG levels and the Matsuda index, insulinogenic index, and disposition index using the MTT data, respectively. p values of <0.05 were considered significant.

Results

Baseline clinical and metabolic characteristics in morbidly obese patients

The baseline clinical and metabolic characteristics of the patients in this study are shown in Table 1. The 80 patients were classified into three groups: NGT ($n = 40$), IGT ($n = 23$), and DM ($n = 17$) based on the OGTT data according to the current Japanese diagnostic criteria for diabetes [26]. Diagnosis of diabetes was made based on either the use of oral glucose-lowering drugs or the patient's OGTT data. In the patients with DM, the oral glucose tolerance curves were thus modified by those glucose-lowering drugs. Glucose intolerance was classified using the 2h-PG values after the OGTT and after the MTT. NGT was diagnosed 2h-PG values <140 mg/dL, IGT was diagnosed based on 2h-PG ≥ 140 mg/dL and <200 mg/dL, and DM was diagnosed based on 2h-PG ≥ 200 mg/dL. The clinical characteristics in patients with NGT, IGT, and DM were also compared in Table 1. As shown in Table 2, the PG levels at 30 min and 60 min after the MTT were both significantly lower than those obtained by the OGTT ($p < 0.01$). However, there was no significant difference in PG values at 120 min between the MTT and the OGTT. As a result, the area under the curve (AUC) of glucose excursion after MTT was 11.9% lower ($p < 0.01$) than that after OGTT. The insulin levels at 60 min and 120 min after the MTT were also lower than those of the OGTT ($p < 0.05$). As a result, the AUC of insulin after the MTT was 17.9% lower ($p < 0.05$) than that of the OGTT. Thus, the insulin sensitivity index (Matsuda index) and the glucose disposition rate (disposition index) were each significantly higher ($p < 0.05$) in the MTT as compared with the OGTT. The insulinogenic index did not differ significantly between the OGTT and MTT (Table 2).

Concordance rate of the glucose tolerance staging between the MTT and the OGTT

As shown in Table 3, according to the MTT data, 82.5% of NGT patients were classified as having NGT and 17.5% of NGT patients were classified as having IGT, which suggested that the NGT patients showed a sufficient coincidence rate with the results from OGTT according to the variability of the test. However, according to their MTT data, 47.8% of IGT patients were classified as having IGT and 52.2% of IGT patients were classified as having NGT. Compared with the results from the OGTT, 29.4% of DM patients were classified as

Table 2 Plasma glucose and insulin excursions during oral challenges and parameters of insulinogenic index, Matsuda index, and disposition index after oral challenge data between the 75 g OGTT and MTT

<i>n</i> = 80	(min)	OGTT	MT
PG, mg/dL	0	90.2 ± 11.7	89.6 ± 12.5
	30	145.5 ± 30.7	128.3 ± 22.6**
	60	172.9 ± 42.6	143.2 ± 33.1**
	120	148.6 ± 48.2	139.1 ± 36.2
PG-AUC, mg/dL × min		17,954 ± 3,889	15,810 ± 3,152**
IRI, μU/mL	0	10.9 ± 4.6	10.4 ± 5.2
	30	61.7 ± 51.1	58.8 ± 36.7
	60	85.6 ± 53.9	66.4 ± 42.9*
	120	83.4 ± 53.4	67.0 ± 38.0*
Insulin-AUC, μU/mL × min		8,475 ± 4,675	6,960 ± 3,927*
Insulinogenic index		1.1 ± 1.3	1.5 ± 1.4
Matsuda index		3.8 ± 2.0	4.6 ± 2.0*
Disposition index		4.1 ± 5.0	6.5 ± 7.5*

Data are means ± SD, ** $p < 0.01$, * $p < 0.05$ compared with the data from OGTT.

PG, plasma glucose; IRI, immunoreactive insulin; AUC, area under the curve.

Table 3 The classification of NGT, IGT, and DM made by the 75 g OGTT and the meal tolerance test (MTT) and parameters of insulinogenic index, Matsuda index, HOMA-R, and disposition index in morbidly obese patients

Glucose tolerance (from OGTT)		NGT	IGT	DM	Total
No.		40	23	17	80
From MTT data	NGT	33 (82.5%)*	12 (52.2%)*	2 (11.8%)*	47
	IGT	7 (17.5%)*	11 (47.8%)*	10 (58.8%)*	28
	DM	0	0	5 (29.4%)*	5
Insulinogenic index					
Abnormal	≤0.4	4 (10%)**	2 (8.6%)**	10 (58.8%)**	16
Normal	>0.4	36	21	7	64
Matsuda index					
Abnormal	≤2.5	10 (26.3%)**	5 (21.7%)**	5 (31.3%)**	20
Normal	>2.5	28	18	11	57
HOMA-R					
Abnormal	≤2.5	27 (67.5%)**	16 (69.6%)**	6 (35.2%)**	49
Normal	>2.5	13	7	11	31
Disposition index					
Abnormal	≤1.0	2 (5.3%)**	1 (4.3%)**	7 (43.8%)**	10
Normal	>1.0	36	22	9	67

The MTT was performed using a meal consisting of 75 g glucose-containing carbohydrate (85% flour starch and 15% maltose), 28.5 g of butter fat (43.3% of total energy), and 8.0 g of protein.

* The percentage indicates the correct diagnosis of glucose tolerance after the MTT compared with that of the 75 g OGTT.

** The percentage of patients with abnormal values

*** Insulinogenic index, Matsuda index, and disposition index were calculated from OGTT data.

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus; OGTT, oral glucose tolerance test; HOMA-R, homeostatic model assessment of insulin resistance

Fig. 1 The postprandial glucose (A–C) and insulin (D–F) excursions were compared between the 75 g OGTT and MTT in patients with NGT, IGT, and DM.

The postprandial glucose excursion after the MTT (red line) was significantly lower than that of the OGTT (blue line) in NGT, IGT, and DM patients, respectively. However, the postprandial insulin excursion after the MTT was not significantly different from that after the OGTT. Data are means $\pm$ SD, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ as compared with each MTT data.

having DM, 58.8% of DM patients were classified as having IGT, and 11.8% of DM patients were classified as having NGT after the MTT. These results suggested that the 2h-PG levels in the MTT were significantly lower than those in the OGTT for both patients with IGT and DM. In addition, the DM patients had a numerically higher frequency of abnormal insulinogenic index and disposition index values, compared to the patients with NGT or IGT. In contrast, 20–30% of morbidly obese patients showed abnormal Matsuda index values and 35–70% of those patients showed abnormal HOMA-R values regardless of the severity of glucose intolerance.

Consistently, as shown in Fig. 1, the postprandial PG levels at 30 and 60 min after the MTT in the NGT, IGT, and DM groups were significantly lower than those obtained from the OGTT. However, the 2h-PG levels in NGT and DM patients were not significantly different between the OGTT and MTT, with the exception that the 2h-PG levels after the MTT were significantly lower compared with those after the OGTT in patients with IGT ($p < 0.001$) (Fig. 1A–C). The postprandial plasma insulin levels in the MTT group were tended to be lower

than that of the OGTT group, regardless of glucose intolerance of the patients (Fig. 1D–F).

Correlation of parameters related to insulin resistance and to insulin secretion between those values calculated from the MTT and OGTT data

As shown in Fig. 2, the correlation between the insulinogenic indices derived from the OGTT and MTT data was significant in both the NGT ($r = 0.883$) and IGT groups ($r = 0.702$) (both $p < 0.001$) (Fig. 2A, 2B), but not in the DM group (Fig. 2C). The correlation between the Matsuda indices derived from the OGTT and MTT data were also significant in the NGT ($r = 0.752$) and IGT groups ($r = 0.648$) (both $p < 0.001$) (Fig. 2D, 2E), but not in the DM group (Fig. 2F). The correlation between the disposition indices ($r = 0.845$) derived from the OGTT and MTT data was significant ($p < 0.001$) in the NGT group (Fig. 2G), but not in either the IGT or DM group (Fig. 2H, 2I).

As shown in Table 4, the insulinogenic index based on the MTT data was significantly higher than that based on the OGTT data in the NGT group ($p < 0.05$), but the dif-

Fig. 2 Correlations of the insulinogenic index (A–C), Matsuda index (D–F), and disposition index (G–I) between the values after the OGTT and the MTT in patients with NGT (A, D, G), IGT (B, E, H), and DM (C, F, I).

ference was not significant in the IGT or DM group. The Matsuda indices derived from the MTT and the OGTT data were not significantly different in any patient groups. Finally, the disposition index derived from the MTT data was significantly higher than that from the OGTT data in both the NGT and IGT groups (both $p < 0.05$), but the difference was not significant between the two tests in the DM group.

Differences in postprandial plasma GLP-1 and GIP concentrations between the MTT and the OGTT

The postprandial plasma GLP-1 levels in the NGT, IGT, and DM groups were significantly increased or tended to be increased after both MTT and OGTT, without significant differences in the values obtained by the two tolerance tests (Fig. 3A–C). However, the postprandial plasma GIP levels in the NGT and IGT groups were significantly higher than those obtained by the OGTT at all time points (Fig. 3D, 3E). The postprandial plasma GIP levels in the DM group were also increased after the

Table 4 Comparison of the insulinogenic index, Matsuda index, and disposition index among the patients according to the glucose tolerance (NGT, IGT, and DM) classified by the OGTT and MTT

Glucose tolerance	Oral loading test	Insulinogenic index	Matsuda index	Disposition index
NGT	OGTT	1.3 ± 1.2	4.3 ± 2.3	5.2 ± 5.2
	MTT	2.0 ± 1.8*	5.1 ± 2.5	9.8 ± 9.4*
IGT	OGTT	1.0 ± 0.8	3.3 ± 1.1	3.0 ± 1.8
	MTT	1.2 ± 0.7	3.9 ± 1.2	4.3 ± 2.1*
DM	OGTT	0.8 ± 1.8	3.4 ± 1.4	3.0 ± 7.2
	MTT	0.6 ± 0.4	4.3 ± 1.6	2.3 ± 1.5

Data are means ± SD. * $p < 0.05$ compared with that of the OGTT

Data were analyzed using a paired t -test

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus

Fig. 3 The plasma GLP-1 and GIP levels during the OGTT and the MTT in patients with NGT, IGT, and DM. The postprandial plasma GLP-1 levels (A–C) tended to increase or significantly increased at some time points after the OGTT (blue line) and after the MTT (red line), without any significant differences between the two tolerance tests. The postprandial plasma GIP levels (D–F) were significantly higher in the MTT than the OGTT. Data are means ± SD. † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ compared with the baseline values. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$ compared with the OGTT data.

two treatments, and the values at 120 min after the MTT were significantly higher than those of the OGTT (Fig. 3F).

The insulin resistance state was associated with postprandial hypertriglyceridemia induced by the MTT

The baseline and postprandial TG levels after the MTT were measured in 57 morbidly obese patients with NGT or IGT. We classified the 57 patients into two

Table 5 Differences in clinical characteristics and HOMA-R, insulinogenic index, Matsuda index, and disposition index calculated from the OGTT and MTT data based on the baseline TG levels in morbidly obese patients

Baseline TG values		Lower TG <150 mg/dL	Higher TG ≥150 mg/dL
No. of patients, female/male		<i>n</i> = 44, 7/37	<i>n</i> = 13, 4/9
BMI (kg/m ²)		40.4 (36.8–47.9)	38.2 (36.8–42.2)
% body fat (%)		49.0 ± 4.5	45.6 ± 4.3
Visceral fat amount (g)		1,225 ± 253	1,240 ± 215
Lean body mass/height, (kg/m ²)		19.9 ± 3.8	20.0 ± 2.9
HbA1c, (%)		5.7 ± 0.4	6.0 ± 0.8
s-BP/d-BP (mmHg)		138.8/82.7 ± 17.0/14.9	143.5/84.3 ± 20.4/24.9
FPG (mg/dL)		85.0 ± 7.2	89.8 ± 8.6*
IRI (μU/mL)		9.2 ± 3.3	13.9 ± 7.5***
TG, (mg/dL) before and after 30–120 min MTT	0 min	104 (77–118)	181 (167–201)****
	30	105 (86–122)	187 (168–208)****
	60	113 (100–131)	200 (182–238)****
	120	134.5 (111–148)	227 (201–242)****
ΔTG (mg/dL)		35 (17–44)	41 (26–69)
HDL-C (mg/dL)		55 ± 11	48 ± 8*
LDL-C (mg/dL)		105 ± 32	106 ± 27
HOMA-R	OGTT	2.1 ± 1.0	3.1 ± 1.3***
	MTT	1.9 ± 0.7	3.2 ± 2.0***
Matsuda index	OGTT	4.1 ± 1.9	3.0 ± 1.2
	MTT	5.1 ± 2.2 [†]	3.5 ± 1.4*
Insulinogenic index	OGTT	1.3 ± 1.2	1.0 ± 0.6
	MTT	1.8 ± 1.7	1.9 ± 1.3 [†]
Disposition index	OGTT	4.9 ± 5.0	2.8 ± 1.6
	MTT	9.0 ± 9.3 [†]	5.6 ± 4.3 [†]
PG-AUC, (mg/dL × min)	OGTT	16,469.0 ± 2,839.1	17,224.6 ± 2,747.8
	MTT	14,545.6 ± 2,271.2 ^{†††}	15,436.2 ± 2,330.7
Insulin-AUC, (μU/mL × min)	OGTT	8,355.2 ± 4,218.6	10,269.9 ± 4,678.8
	MTT	6,552.1 ± 2,698.5 [†]	8,988.8 ± 4,134.2*

ΔTG was serum TG values at 120 min post-MTT above baseline values.

Data are means ± SD and median (Q1–Q3).

Statistical significance of changes in TG levels was analyzed using Wilcoxon rank-sum test.

The other parameters were analyzed by paired *t*-test.

**** *p* < 0.001, *** *p* < 0.01, ** *p* < 0.02, * *p* < 0.05 compared with that of the lower baseline TG group.

^{††††} *p* < 0.001, ^{†††} *p* < 0.01, ^{††} *p* < 0.02, [†] *p* < 0.05 compared with that of OGTT data.

FPG, fasting plasma glucose; IRI, immunoreactive insulin; PG-AUC, plasma glucose–area under the curve; HOMA-R, homeostatic model assessment of insulin resistance; s-BP, systolic blood pressure; d-BP, diastolic blood pressure

groups: a higher baseline TG group (TG ≥150 mg/dL, *n* = 13, 23% of the total patient group) and a lower baseline TG group (TG <150 mg/dL *n* = 44, 77% of the total). As shown in Table 5, there were significantly higher baseline IRI levels and lower HDL-C levels in the higher baseline TG group compared with the lower base-

line TG group. The TG levels at 30–120 min after MTT were significantly higher in the higher baseline TG group compared to the lower baseline TG group (*p* < 0.001). The patients in the higher baseline TG group also showed significantly higher HOMA-R (*p* < 0.01) and significantly lower Matsuda index (*p* < 0.05) compared

to the lower baseline TG group.

The postprandial TG values at 2 h after the MTT were also classified into two groups based on the median values (142 mg/dL) (Table 6). The higher 2h-TG group (TG $\geq$ 142 mg/dL) showed significantly ($p < 0.05$) lower percentages of body fat, higher FPG, higher IRI, and lower HDL-C levels, and significantly ($p < 0.001$) higher

postprandial 2h-TG levels at 30–120 min after the MTT than those of the lower 2h-TG group (TG <142 mg/dL). In addition, the increment of 2h-TG levels above the basal TG values after the MTT was significantly higher in the higher 2h-TG group than in the lower 2h-TG group ($p < 0.001$). The higher 2h-TG group after the MTT also showed significantly ($p < 0.05$) higher

Table 6 Differences in clinical characteristics and HOMA-R, insulinogenic index, Matsuda index, and disposition index calculated from the OGTT and MTT data based on the TG levels at 120 min after MTT in morbidly obese patients with either NGT or IGT

Median postprandial TG levels		Lower TG <142 mg/dL	Higher TG $\geq$ 142 mg/dL
No. of patients, females/males		$n = 28, 3/25$	$n = 29, 8/21$
BMI (kg/m ²)		40.1 (37.4–45.3)	38.4 (36.6–45.0)
% body fat (%)		49.6 $\pm$ 3.5	46.8 $\pm$ 5.7*
Visceral fat amount (g)		1,177.7 $\pm$ 224.6	1,283.0 $\pm$ 255.3
Lean body mass/height (kg/m ²)		19.3 $\pm$ 3.9	20.6 $\pm$ 3.2
HbA1c (%)		5.7 $\pm$ 0.4	5.9 $\pm$ 0.6
s-BP/d-BP (mmHg)		138.5/84.5 $\pm$ 19.3/15.3	141.3/84.5 $\pm$ 16.3/19.5
FPG (mg/dL)		83.8 $\pm$ 6.8	88.4 $\pm$ 8.0*
IRI (μ U/mL)		8.8 $\pm$ 3.3	11.7 $\pm$ 5.7*
TG (mg/dL) before and 30–120 min after MTT	0 min	86.5 (67–103.5)	142 (118–175)****
	30	96 (68–105)	147 (132–181)****
	60	104 (82–113)	163 (133–200)****
	120	120 (98–133)	197 (164–227)****
Δ TG (mg/dL)		27.5 (15–35.3)	48 (31–68.0)****
HDL-C (mg/dL)		56.4 $\pm$ 11.0	50.2 $\pm$ 9.2*
LDL-C (mg/dL)		108.4 $\pm$ 34.7	102.7 $\pm$ 26.5
HOMA-R	OGTT	1.8 $\pm$ 0.7	2.8 $\pm$ 1.2****
	MTT	1.8 $\pm$ 0.69	2.6 $\pm$ 1.5*
Matsuda Index	OGTT	4.3 $\pm$ 1.5	3.3 $\pm$ 2.0*
	MTT	5.4 $\pm$ 2.4 [†]	4.1 $\pm$ 1.6*
Insulinogenic Index	OGTT	1.38 $\pm$ 1.37	1.1 $\pm$ 0.8
	MTT	2.0 $\pm$ 1.9	1.7 $\pm$ 1.1 [†]
Disposition Index	OGTT	5.3 $\pm$ 5.5	3.4 $\pm$ 3.3
	MTT	10.5 $\pm$ 11.2 [†]	6.1 $\pm$ 4.1 [†]
PG- AUC, (mg/dL $\times$ min)	OGTT	16,117.0 $\pm$ 2,350.3	17,147.6 $\pm$ 3,154.7
	MTT	14,107.5 $\pm$ 1,999.8 ^{†††}	15,367.8 $\pm$ 24,222.8* [†]
Insulin-AUC, (μ U/mL $\times$ min)	OGTT	8,434.1 $\pm$ 4,562.6	9,168.1 $\pm$ 4,216.8
	MTT	6,505.9 $\pm$ 3,073.1	7,717.6 $\pm$ 3,336.2

Δ TG was serum TG levels at 120 min after the MTT above the baseline TG values.

Data are means $\pm$ SD and median (Q1–Q3).

Statistical significance of changes in TG levels was analyzed using Wilcoxon rank-sum test. The other parameters were analyzed by a paired *t*-test.

**** $p < 0.001$, *** $p < 0.01$, ** $p < 0.02$, * $p < 0.05$ as compared with that of the lower TG group (TG <142 mg/dL).

†††† $p < 0.001$, ††† $p < 0.01$, †† $p < 0.02$, † $p < 0.05$ compared with that of OGTT data.

PG/insulin-AUC, plasma glucose/Insulin–area under the curve; HOMA-R, homeostatic model assessment of insulin resistance; s-BP/d-BP, systolic/diastolic blood pressure

Fig. 4 The correlations of the 2h-TG levels or $\log_{10}$ 2h-TG levels after the MTT with the baseline TG levels (A), HOMA-R (B), and Matsuda index after the MTT (C).

HOMA-R, lower Matsuda index, and higher PG-AUC without any differences in the insulinogenic index or disposition index compared to the data of the lower 2h-TG group, respectively. There were also significant correlations between the 2h-TG levels after the MTT and the baseline TG levels ($r = 0.93$, $p < 0.001$) (Fig. 4A). The $\log_{10}$ 2h-TG levels after the MTT were each significantly correlated with either HOMA-R (Fig. 4B, $r = 0.41$, $p = 0.002$) or the Matsuda index (Fig. 4C, $r = -0.56$, $p < 0.001$) after the MTT. However, the 2h-TG levels were not correlated with the insulinogenic index or the disposition index (data not shown).

Discussion

We introduced a new MTT to evaluate impaired glucose tolerance in morbidly obese patients who were on a waiting list for bariatric surgery. This specific type of test meal contains the same amount of glucose used in the standard 75 g OGTT, along with a high fat content (28.5 g butter fat), and a low protein content (8 g). Thus, we speculated that, compared to the OGTT, a high fat meal might modulate an incretin-induced insulin secretion as well as delayed gastric emptying, resulting in decreased PG levels [17–19]. However, before clinically applying this meal test for the evaluation of glucose intolerance in morbidly obese patients, we needed to confirm the concordance rate in the classification of glucose intolerance compared to the values determined by the standard 75 g OGTT.

The concordance rate of the glucose intolerance staging calculated from the MTT data compared with that of the OGTT

We first observed that the 2h-PG values after the MTT were not significantly different from those of the 75 g OGTT (Table 2), indicating that the diagnosis of glucose

intolerance based on the MTT might be comparable to that based on the 75 g OGTT. This result was confirmed in the NGT patients defined by the OGTT data such that 82.5% of the NGT patients were classified as meeting the NGT criteria and 17.5% of the NGT patients were shifted to IGT based on their MTT data (Fig. 1A and Table 3). These results suggested that the MTT data in the patients with NGT had high reliability to be substituted for the 75 g OGTT. In addition, the NGT patients showed significant linear correlations in the insulinogenic index (Fig. 3A), Matsuda index (Fig. 3D), and disposition index (Fig. 3G) between the MTT and OGTT. On the other hand, as shown in Table 3, severity of glucose intolerance measured by the MTT in both patients with IGT and DM was milder than that of the OGTT. These differences between the MTT and OGTT might be associated with an effect of the fat in the new test meal.

Postprandial increases in GIP levels and the glucose disposition rate after the MTT

In the present series of morbidly obese patients, the GLP-1 levels were significantly increased or tended to be increased above the baseline levels after both the MTT and OGTT without significant differences between the two tests. Previously, an increase in GIP secretion after the OGTT was reported to be positively associated with body mass index in Japanese subjects with NGT [27]. We observed that the postprandial GIP levels were significantly increased after the MTT and OGTT, which was suggested by previous data on a fat-containing meal [28, 29]. We also found that the postprandial GIP levels were significantly higher after the MTT compared with the OGTT in morbidly obese patients irrespective of whether or not the patients were glucose intolerant. Thus, the lower postprandial PG values after the MTT compared with the OGTT might be associated with fat-induced GIP secretion. It has already been reported that a

high fat diet containing saturated fatty acid stimulates postprandial insulin release *via* increased GIP secretion [30]. We used 28.5 g of butter fat (43.3% of total energy), 75 g of glucose-containing carbohydrate and a low protein content in the new MTT meal. However, in the present study, we could not observe any increases in insulin secretion, despite the marked increase in GIP levels between 30 min and 120 min after the MTT. The increased GIP levels thus appeared to be related to the lower glucose levels rather than a stimulation of insulin secretion after a single administration of a high fat meal. However, GIP has been reported to show no influence on gastric emptying in humans [31]. In the present study, GLP-1 was also not a candidate of incretin to stimulate glucose utilization after the MTT, since plasma GLP-1 levels were similar between the MTT and OGTT. Furthermore, in the present study, we measured only inactive forms of GLP-1 levels, and did not measure active forms of GLP-1 in either the OGTT or MTT studies. However, plasma concentrations of inactive GLP-1 forms were nearly equal to the total GLP-1, where active forms were rapidly metabolized into inactive forms at the present blood sampling. Although delayed gastric emptying might be a cause of the present results, we did not measure the gastric emptying rate or plasma levels of the other gut hormones controlling gastric emptying after a fat and glucose intake in the present study [32]. We observed an MTT-induced improvement of glucose excursion in both NGT and IGT patients, which was associated with an enhancement of the disposition index after the MTT compared to the OGTT (Table 4). These results might be associated with increased plasma GIP and GLP-1 levels after the MTT in NGT and IGT patients, although we only measured inactive forms of plasma GLP-1 (equal to total GLP-1) and total GIP levels, which measured both active and inactive forms of GIP in the present study. Although total incretin measurements reflected the changes in active forms of those incretin hormones, it should be kept in mind that the relative proportion of active GIP levels might be modified by the DPP-4 activity [33]. As another explanation, it has been reported that a novel dual GIP and GLP-1 receptor agonist, tirzepatide, exerts substantially greater glucose-lowering effects compared to a selective GLP-1 receptor agonist alone due to improvements of both insulin sensitivity and glucose-dependent insulin secretion [34]. However, it is also careful to evaluate that those results are obtained under chronic treatment with a pharmacological dose of tirzepatide, which may be much higher than the physiological plasma GIP/GLP-1 levels. Therefore, it may be inappropriate to link the dual GIP/GLP-1 receptor agonist data to the present results. In addition, both glucose uptake studies and insulin signal-

ing in human adipocytes have indicated that GIP is an insulin-sensitizer incretin. However, none of the physiological effects of GIP are detected in human fat cells obtained from obese patients because of the reduced GIP receptor levels [35]. In any case, the increases in both GIP and GLP-1 levels after the MTT could not exclude the possible link to a significant reduction in PG without significant increases in insulin levels after the MTT compared with the OGTT data.

Although the plasma GLP-1 and GIP levels were measured in only 37 patients in the present study, the glucose tolerance and various parameters of insulin secretion and insulin sensitivity calculated from the OGTT and MTT data were similar to the results of the full-set analyses, as shown in Supplementary Fig. 1 and Supplementary Table 1.

Postprandial hypoglycemia is a clinical problem in morbidly obese patients undergoing bariatric surgery

Postprandial hypoglycemia with neuroglycopenia after bariatric surgery is now increasingly recognized as a complication to be avoided [13, 14]. Possible causes of this troublesome complication include late dumping syndrome, nesidioblastosis, and (rarely) insulinoma [36]. The incidence, severity, treatment including nutritional intervention, and outcomes of the hypoglycemia in patients after bariatric surgery have been reported in large numbers of patients [37–40]. Glucose intolerance in morbidly obese patients with or without T2DM has been evaluated by using the standard 75 g OGTT. This test has sometimes shown postprandial hyperinsulinemia-related hypoglycemia among morbidly obese patients who undergo bariatric surgery [13–15]. This hypoglycemia is potentially linked to altered glucose-induced incretin secretion and islet hyperfunction. Optimal fat intake may be useful to prevent postprandial hyperinsulinemic hypoglycemia after bariatric surgery due to increased secretion of GLP-1, GIP, and the other gut hormones, which is related to delayed gastric emptying and appropriate insulin secretion in response to the meal loading. Further studies will be required to determine how best to prevent the postprandial hyperinsulinemia-related hypoglycemia during the glucose tolerance test using this new MTT in morbidly obese patients undergone bariatric surgery in the future.

Postprandial hypertriglyceridemia and insulin resistance: evaluation using the MTT

Our present analyses revealed an association of insulin resistance and postprandial hypertriglyceridemia after the MTT in morbidly obese patients. Previously, it has been reported that morbidly obese patients with postprandial hypertriglyceridemia are associated with insulin

resistance and their dyslipidemia is well controlled after bariatric surgery [41, 42]. However, the postprandial TG levels are not increased above the baseline levels after the standard OGTT [27]. In the present study, we used the new MTT containing 75 g of glucose and 28.5 g of butter fat and measured the postprandial TG levels. We found that the Log_{10} 2h-TG values were correlated well with HOMA-R ($r = 0.41$, $p < 0.01$), and the Matsuda index ($r = -0.56$, $p < 0.001$) estimated by MTT data, which indicated that the 2h-TG values after the MTT were significantly associated with indices of insulin resistance. In addition, the increment of 2h-TG levels above baseline values was significantly higher in the higher 2h-TG group than in the lower 2h-TG group (Table 6). In the present study, under the fasting condition, 13 patients (23%) showed a higher baseline TG level (≥ 150 mg/dL) and 44 patients (77%) showed a lower baseline TG level. These results thus demonstrated that morbidly obese patients did not always show the higher baseline TG levels, and measurement of postprandial TG levels induced by the MTT might be a better predictor of improved insulin resistance than measurement of fasting TG levels.

Study limitations

Our evaluation of insulin sensitivity and insulin secretion using clinical parameters, *i.e.*, the insulinogenic index, Matsuda index, and disposition index, should be considered a study limitation, since the 2h-PG values after 75 g OGTT are known to exhibit high intra-individual variability and even higher within-subject variability; in addition, the measurement of the insulinogenic index has also been reported to be highly variable [43, 44]. In addition, for our evaluation of the secretion and the sensitivity of insulin, we used the same formulae that were developed for analysis of the standard OGTT data. Nonetheless, our findings in the present study revealed that this test meal was applicable to estimate improvements of the glucose intolerance, the acute response of insulin secretion, insulin resistance, gut hormone responses to macronutrients, and the *in vivo* glucose disposition rate at the condition linking closer to daily dietary habits in morbidly obese patients in comparison to the standard OGTT. The normalization of these parameters might be good indicators for the treatment of metabolic abnormalities in morbidly obese

patients after bariatric surgery in the future.

Other study limitations were as follows. The DM patients used various oral glucose-lowering drugs; for example, 17 patients used metformin with GLP-1 receptor agonists (9 patients), DPP-4 inhibitors (7 patients), SGLT2 inhibitors (6 patients), thiazolidinedione (2 patients), and/or sulfonylurea (1 patient). Although these drugs could have modified the results of our analyses, we did not evaluate their impact in the present study. In addition, we studied postprandial increases in TG levels at 2 h after MTT, although it might be better to take these measurements at a later time after an intake of a fat-containing meal. However, many studies have evaluated glucose and lipid excursions 2 or 3 h after a meal challenge at the same time in patients with morbid obesity with or without T2DM [41, 45].

In conclusion, the results of the present investigation revealed that a new MTT using a 75 g glucose-containing carbohydrate, high fat, and low protein meal was useful to evaluate improvements in impaired glucose and lipid metabolism simultaneously, as well as various indices determining glucose and triglyceride excursions—*i.e.*, impaired insulin sensitivity, abnormal insulin and gut hormone secretions—in morbidly obese patients undergone bariatric surgery.

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Disclosure

Kashiwagi A. has acted as a medical consultant for Sunstar, Inc. and has received consulting fees. The remaining authors, YY, YO, MK, YT, CA, OS, JIK, MW, MI, TT, AH, TK, and AS, have nothing to disclose.

Internal Ethics Guideline

The institutional Review board at the Kusatsu General Hospital approved the study protocol (date 2017/3/24, No. 2017-0317-05).

References

1. Karuranga S, Malanda B, Saeedi P, Salpea P (eds) (2019) IDF Diabetes Atlas (9th). Internal Diabetes Federation, Brussels, Belgium: 1–161.
2. Tanaka H, Kokubo Y (2005) Epidemiology of obesity in Japan. *JMAJ* 48: 34–41.
3. Sasaki A, Wakabayashi G, Yonei Y (2014) Current status

- of bariatric surgery in Japan and effectiveness in obesity and diabetes. *J Gastroenterol* 49: 57–63.
4. WHO Expert Consultation (2004) Appropriate body mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 363: 157–163.
  5. Schauer PR, Mingrone G, Ikramuddin S, Wolfe B (2016) Clinical outcomes of metabolic surgery: efficacy of glycemic control, weight loss, and remission of diabetes. *Diabetes Care* 39: 902–911.
  6. Cummings DE, Rubino F (2018) Metabolic surgery for the treatment of type 2 diabetes in obese individuals. *Diabetologia* 61: 257–264.
  7. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Aminian A, *et al.* (2017) Bariatric surgery versus intensive medical therapy for diabetes-5-year outcomes. *N Engl J Med* 376: 641–651.
  8. Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, *et al.* (2015) Bariatric-metabolic surgery versus conventional medical treatment in obese patients with type 2 diabetes: 5 year-follow-up of an open-label, single-centre, randomised controlled trial. *Lancet* 386: 964–973.
  9. Pournaras DJ, Osborne A, Hawkins SC, Vincent RP, Mahon D, *et al.* (2010) Remission of type 2 diabetes after gastric bypass and banding: mechanisms and 2 year-outcomes. *Ann Surg* 252: 966–971.
  10. Osland E, Yunus RM, Khan S, Memon B, Memon MA (2017) Diabetes improvement and resolution following laparoscopic vertical sleeve gastrectomy (LVSG) versus laparoscopic Roux-en-Y gastric bypass (LRYGB) procedures: a systematic review of randomized controlled trials. *Surg Endosc* 31: 1952–1963.
  11. Rosenthal R, Li X, Samuel S, Martinez P, Zheng C (2009) Effect of sleeve gastrectomy on patients with diabetes mellitus. *Surg Obes Relat Dis* 5: 429–434.
  12. Ugi S, Morino K, Yamaguchi T, Yamamoto H, Kaida S, *et al.* (2019) Preserving β -cell function is the major determinant of diabetes remission following laparoscopic sleeve gastrectomy in Japanese obese diabetic patients. *Endocr J* 66: 817–826.
  13. Eisenberg D, Azagury DE, Ghiassi S, Grover BT, Kim JJ (2017) ASMBS position statement on postprandial hyperinsulinemic hypoglycemia after bariatric surgery. *Surg Obes Relat Dis* 13: 371–378.
  14. Malik S, Mitchell JE, Steffen K, Engel S, Wiisanen R, *et al.* (2016) Recognition and management of hyperinsulinemic hypoglycemia after bariatric surgery. *Obes Res Clin Pract* 10: 1–14.
  15. Lee CJ, Clark JM, Schweitzer M, Magnuson T, Steele K, *et al.* (2015) Prevalence of and risk factors for hypoglycemic symptoms after gastric bypass and sleeve gastrectomy. *Obesity (Silver Spring)* 23: 1079–1084.
  16. Harano Y, Miyawaki T, Nabiki J, Shibachi M, Adachi T, *et al.* (2006) Development of cookie test for the simultaneous determination of glucose intolerance, hyperinsulinemia, insulin resistance and postprandial dyslipidemia. *Endocr J* 53: 173–180.
  17. Morgan LM, Hampton SM, Tredger JA, Cramb R, Marks V (1988) Modification of gastric inhibitory polypeptide (GIP) secretion in man by a high-fat diet. *Br J Nutr* 59: 373–380.
  18. Trico D, Natali A (2017) Modulation of postprandial glycemic responses by noncarbohydrate nutrients provides novel approaches to the prevention and treatment of type 2 diabetes. *Am J Clin Nutr* 106: 701–702.
  19. Gentilcore D, Chaikomin R, Jones KL, Russo A, Feinle-Bisset C, *et al.* (2006) Effects of fat on gastric emptying of and the glycemic, insulin, and incretin responses to a carbohydrate meal in type 2 diabetes. *J Clin Endocrinol Metab* 91: 2062–2067.
  20. Tewari N, Awad S, Macdonald IA, Lobo DN (2018) A comparison of three methods to assess body composition. *Nutrition* 47: 1–5.
  21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, *et al.* (1985) Homeostasis model assessment: insulin resistance and β cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412–419.
  22. Ahuja V, Kadowaki T, Evans RW, Kadota A, Okamura T, *et al.* (2015) Comparison of HOMA-IR, HOMA- β % and disposition index between US white men and Japanese men in Japan: the ERA JUMP study. *Diabetologia* 58: 265–271.
  23. Matsuda M, DeFronzo RA (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22: 1462–1470.
  24. Takahara M, Katakami N, Kaneto H, Noguchi M, Shimomura I (2013) Distribution of Matsuda index in Japanese healthy subjects. *J Diabetes Investig* 4: 369–371.
  25. Lorenzo C, Wagenknecht LE, Rewers MJ, Karter AJ, Bergman RN (2011) Disposition index, glucose effectiveness, and conversion to type 2 diabetes: the Insulin Resistance Atherosclerosis Study (IRAS). *Diabetes Care* 33: 2098–2103.
  26. Seino Y, Nanjo K, Tajima N, Kadowaki T, Kashiwagi A, *et al.* (2010) Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Investig* 1: 213–228.
  27. Harada N, Hamasaki A, Yamane S, Muraoka A, Joo E, *et al.* (2011) Plasma gastric inhibitory polypeptide and glucagon-like peptide-1 levels after glucose loading are associated with different factors in Japanese subjects. *J Diabetes Investig* 2: 193–199.
  28. Yamane S, Harada N, Hamasaki A, Muraoka A, Joo E, *et al.* (2012) Effects of glucose and meal ingestion on incretin secretion in Japanese subjects with normal glucose tolerance. *J Diabetes Investig* 3: 80–85.
  29. Oda H, Hiramatsu T, Mori A, Sako T (2020) Influence of various fat sources on the glucose, insulin, and incretin concentration in healthy cats. *J Pet Anim Nutr* 23: 59–67.
  30. Itoh K, Moriguchi R, Yamada Y, Fujita M, Yamato T, *et al.* (2014) High saturated fatty acid intake induces insulin secretion by elevating gastric inhibitory polypeptide levels

- in healthy individuals. *Nutr Res* 34: 653–660.
31. Meier IJ, Goetze O, Anstipp J, Hagemann D, Holst IJ, *et al.* (2004) Gastric inhibitory polypeptide does not inhibit gastric emptying in humans. *Am J Physiol Metab* 286: E621–E625.
  32. Boyd KA, O'donovan DG, Doran S, Wishart J, Chapman IM, *et al.* (2003) High-fat diet effects on gut motility, hormone, and appetite responses to duodenal lipid in healthy men. *Am J Physiol Gastrointest Liver Physiol* 284: G188–G196.
  33. Yanagimachi T, Fujita Y, Takeda Y, Honjo J, Yokoyama H, *et al.* (2020) Receptor-mediated bioassay reflects dynamic change of glucose-dependent insulinotropic polypeptide by dipeptidyl peptidase 4 inhibitor treatment in subjects with type 2 diabetes. *Front Endocrinol (Lausanne)* 11: 214.
  34. Thomas MK, Nikooinnejad A, Bray R, Cui X, Wilson J, *et al.* (2021) Dual GIP and GLP-1 receptor agonist tirzepatide improves beta-cell function and insulin sensitivity in type 2 diabetes. *J Clin Endocrinol Metab* 106: 388–396.
  35. Ceperuelo-Mallafre V, Duran X, Pachón G, Roche K, Garrido-Sánchez L, *et al.* (2014) Disruption of GIP/GIPR axis in human adipose tissue is linked to obesity and insulin resistance. *J Clin Endocrinol Metab* 99: E908–E919.
  36. Moreira RO, Moreira RB, Machado NA, Goncalves TB, Coutinho WF (2008) Post-prandial hypoglycemia after bariatric surgery: pharmacological treatment with verapamil and acarbose. *Obes Surg* 18: 1618–1621.
  37. Hanipah ZN, Punchai S, Birriel TJ, Lansang MC, Kashyap SR, *et al.* (2018) Clinical features of symptomatic hypoglycemia observed after bariatric surgery. *Surg Obes Relat Dis* 14: 1335–1339.
  38. Kellogg TA, Bantle JP, Leslie DB, Redmond JB, Slusarek B, *et al.* (2008) Postgastric bypass hyperinsulinemic hypoglycemia syndrome: characterization and response to a modified diet. *Surg Obes Relat Dis* 4: 492–499.
  39. Goldfine AB, Mun EC, Devine E, Bernier R, Baz Hecht M, *et al.* (2007) Patients with neuroglycopenia after gastric bypass surgery have exaggerated incretin and insulin secretory responses to a mixed meal. *J Clin Endocrinol Metab* 92: 4678–4685.
  40. Suhl E, Anderson-Haynes SE, Mulla C, Patti ME (2017) Medical nutrition therapy for post-bariatric hypoglycemia: practical insights. *Surg Obes Relat Dis* 13: 888–896.
  41. Tinahones FJ, Queipo-Ortuño MI, Clemente-Postigo M, Fernnandez-García D, Mingrone G, *et al.* (2013) Postprandial hypertriglyceridemia predicts improvement in insulin resistance in obese patients after bariatric surgery. *Surg Obes Relat Dis* 9: 213–218.
  42. Climent E, Benaiges D, Goday A, Villatoro M, Julià H, *et al.* (2020) Morbid obesity and dyslipidemia: The impact of bariatric surgery. *Clin Investig Arterioscler* 32: 79–86.
  43. Singal P, Janghorbani M, Schuette SA, Chisholm R, Mather KJ (2010) Intra-variability of CO₂ breath isotope enrichment compared to blood glucose in the oral glucose tolerance test. *Diabetes Technol Ther* 12: 947–953.
  44. Utzschneider KM, Prigeon RL, Tong J, Gerchman F, Carr DB, *et al.* (2007) Within-subject variability of measures of beta cell function derived from a 2h OGTT: implications for research studies. *Diabetologia* 50: 2516–2525.
  45. Riboldi BP, Luft VC, de Castilhos CD, de Cardoso LO, Schmidt MI, *et al.* (2015) Glucose and triglyceride excursions following a standardized meal in individuals with diabetes: ELSA-Brasil study. *Cardiovasc Diabetol* 14: 21.