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ORIGINAL PAPER

Comparison of composite whole body insulin sensitivity index derived from mixed meal test and oral glucose tolerance test in insulin resistant obese subjects

Hadi Selimoglu · Cevdet Duran · Sinem Kiyici · Metin Guclu · Canan Ersoy · Guven Ozkaya · Erdinc Erturk · Ercan Tuncel · Sazi Imamoglu

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Abstract Apart from fasting blood glucose (FBG) and insulin (FBI), oral glucose tolerance test (OGTT) is also used in calculating insulin sensitivity. During OGTT, insulin secretion may not reflect normal physiological insulin secretion. Based on this idea, hepatic and whole body insulin sensitivity rates were tested during OGTT and mixed meal test (MMT) in obese subjects. Thirty-one women with Quantitative Insulin Sensitivity Check Index (QUICKI) values below 0.350 and body mass index (BMI) $\geq$30 were included into the study. OGTT with 75-g glucose and MMT 300 kcal were applied to all cases. Data obtained from OGTT and MMT were used in the assessment of insulin sensitivity with Hemostasis of Model Assessment-Insulin Resistance (HOMA-IR) and Matsuda's Composite Whole Body Insulin Sensitivity Index (Matsuda's ISI). Mean BMI, FBG, and FBI were 36.8 $\pm$ 3.9 kg/m^2, 100.5 $\pm$ 0.10 mg/dl, 16.2 $\pm$ 5.3 μg/ml, respectively. QUICKI was 0.31 $\pm$ 0.01 and HOMA-IR was 3.71 $\pm$ 0.88. Matsuda's ISI derived from OGTT was 6.96 $\pm$ 3.35 and from MMT was 11.32 $\pm$ 6.61. In analysis, it was demonstrated that there was a correlation between HOMA-IR, QUICKI, and Matsuda's ISIs derived from OGTT and MMT. Comparing the time periods separately, it was detected that despite similar increment in insulin levels, glucose levels were higher in OGTT than MMT at 15 and 30 min. Consequently, Matsuda's ISI was demonstrated to be effectively used with the data of MMT, as used with OGTT. Moreover, MMT was shown to be in parallel to physiologic insulin secretion and reflect pancreatic functions better compared to OGTT.

Keywords Insulin sensitivity · Mixed meal test · Oral glucose tolerance test · Obesity · Matsuda's Insulin Sensitivity Index

H. Selimoglu
Division of Endocrinology, Malatya State Hospital, Malatya, Turkey

C. Duran (✉)
Division of Endocrinology and Metabolism, Konya Education and Research Hospital, Meram Yeni Yol, 42100 Meram, Konya, Turkey
e-mail: drcduran@gmail.com

S. Kiyici · M. Guclu · C. Ersoy · E. Erturk · E. Tuncel · S. Imamoglu
Division of Endocrinology, Faculty of Medicine, Uludağ University, Bursa, Turkey

G. Ozkaya
Department of Biostatistic, Faculty of Medicine, Uludağ University, Bursa, Turkey

Introduction

The risk of type-2 diabetes and cardiovascular disease is three times higher in insulin-resistant subjects compared to healthy ones [1]. Insulin resistance (IR) is reported to be approximately 25% in healthy population, 60–75% in individuals with impaired glucose tolerance, and 85% in the patients with type-2 diabetes [2]. Beneficial effects of physical activity and weight loss on the prevention of development of type-2 diabetes are known. Besides drugs like metformin and thiazolidinediones decrease IR and the development of type-2 diabetes up to 50% [3, 4], which underlines the fact that individuals in higher risk groups should be detected earlier. Although, the resistance is encountered in most of the tissues, involved organs are mainly liver, muscles, and adipose tissue [5]. While physical activity reduces IR in muscles and metformin in liver, weight loss and thiozolidinediones reduce the

occurrence of diabetes by decreasing IR in both tissues as well as in adipose tissue. Therefore, if individuals with IR are detected via reliable and easily applicable testing methods for evaluating the degree and localization of IR, appropriate therapeutic approaches may reduce the risk of development of type-2 diabetes.

Among the methods, the gold standard is euglycemic hyperinsulinemic clamp calculating the effect of continuous insulin infusion on glucose utilization, while plasma glucose level is kept in a steady state by intravenous glucose infusion [6]. However, this technique is cumbersome and also time-consuming. Instead, there are several other methods calculating insulin sensitivity. Most of them calculate insulin sensitivity using fasting or postprandial serum glucose and insulin values [7, 8] or glucose and insulin values obtained during oral glucose tolerance test (OGTT) [9–11].

Due to easy clinical application, IR is mostly calculated by using a method called Hemostasis of Model Assessment-Insulin Resistance (HOMA-IR) mainly indicating hepatic IR [2, 8]. IR can be calculated by the mathematical formula of HOMA-IR by dividing the multiplication of fasting blood insulin (FBI) (μU/ml) and fasting blood glucose (FBG) (mmol/L) by 22.5. Elevated HOMA-IR levels account for low insulin sensitivity. In healthy subjects, mean HOMA-IR levels are below 2.7 [2], and this value may be witnessed differently in different populations. Quantitative Insulin Sensitivity Check Index (QUICKI) is another easy method used in the measurement of IR by using FBG and can be calculated by dividing 1 by the sum of the logarithms of FBI and FBG. The mean values for non-obese, obese, and diabetic subjects are 0.382, 0.331, and 0.304, respectively [7].

Although, euglycemic hyperinsulinemic clamp is the gold standard to calculate IR, whole body insulin sensitivity can also be calculated by using data derived from OGTT [9–11]. Matsuda's Insulin Sensitivity Index (Matsuda's ISI), showing hepatic and peripheric IR together, is calculated by the formula of $10000/(\text{FBG} \times \text{FBI} \times \text{mean glucose during OGTT} \times \text{mean insulin during OGTT})^{1/2}$ [9]. ISI calculated by Matsuda's index has been reported to be a significant indicator in the determination of the development of future diabetes risk [12]. OGTT should be used in the calculation of Matsuda's ISI and has been designed to classify normal, impaired, and diabetic tolerance [13]. OGTT is not a test performed under physiological conditions, as only pure glucose is given for the stimulation of insulin secretion. Therefore, insulin release from pancreas after OGTT may not reflect physiological insulin release. Insulin response occurring after oral meal intake may be affected by many enteric hormones, amino acids from foods, gastrointestinal motility, neural impulses during ingestion of foods, and gastric emptying time besides glucose obtained from carbohydrates [14–17]. After mixed meal test (MMT), while plasma glucose response increases less, insulin levels, especially first phase insulin release increase further, compared to OGTT [18]. Insulin response to glucose during OGTT may even vary from person to person [13]. Although, various MMTs composed of different food and containing different carbohydrates, fats, amino acids, along with the tests containing pure glucose are needed, these MMT tests have yet to be fully standardized. MMTs with different contents and calories were used in different studies [18–21]. Although, there are conflicting points concerning contents and calories MMT seems to be more physiological on insulin release from pancreas than OGTT.

To clarify the points discussed above, the aim of our study is to investigate the relationship between the changes in plasma glucose and insulin levels after OGTT and MMT in obese individuals, and hepatic and whole body IR calculated by HOMA, OUICKI, and Matsuda's ISI and compare them. Besides, we aimed to use the data obtained from MMT in Matsuda's ISI and compare the results obtained from OGTT in Matsuda's ISI which is the classical technique by equalizing the calories of OGTT and MMT, which will be the first in the literature.

Materials and methods

Females admitted to Endocrinology and Metabolism outpatient clinic of Uludağ University Medical School between March and July 2006 between the ages of 25–55 years, with a body mass index (BMI) of $\geq$30 kg/m^2, having a QUICKI [7] value of $<$0.350 with an indication of OGTT were included in this study. Subjects with the history of known diabetes, cardiovascular, or renal disease, known or suspected pregnancy, previous use of drugs leading to IR and liver function tests $\geq$3 times higher than the normal limits were excluded from the study. The study protocol was approved by the local research ethics committee. The study was carried out in accordance with the declaration of Helsinki, and all the participants were given written informed consent.

Anthropometric measurements were carried out in all the subjects. BMI was calculated using weight/height^2 (kg/m^2) formula. Body fat percentage was measured on a daily calibrated scale and recorded (TANITA Body Fat Monitor, TANITA Corporation, Tokyo, Japan). All the subjects received a standard diet containing $\geq$150 g carbohydrate for 3 days. In the morning of day 4 at 9 o'clock, OGTT with 75 g of glucose (300 kcal) was performed, and the following day 5 at 9 o'clock MMT having almost equal amount of calories with OGTT (containing 297.4 kcal 60% obtained from carbohydrates, 20% from fat, and 20% from

proteins) prepared by the same dietician in our clinic, was performed. In the morning of days 4 and 5, totally six samples of blood at 0, 15, 30, 60, 90, and 120 min were drawn for the measurements of glucose and insulin.

Blood samples were drawn after 12 h of fasting and stored at −80°C after centrifugation. Insulin levels were measured by Chemiluminescence Enzyme Immunoassay method (Immulite 2000 analyser, Siemens Medical Solutions Diagnostic, Lon Angles, CA, USA, normal range (NR) 9.3–29.1 μIU/ml). Glucose measurements were carried out using enzymatic method by autoanalyser (Aeroset system Abbott, Abbott Laboratories, Diagnostic Division, IL, USA, NR 70–110 mg/dl). IR was calculated by the formulas of QUICKI [7] and HOMA [8].

Whole body insulin sensitivity measurements were calculated separately with the formula of Matsuda's ISI [10000/(FBG × FBI × mean glucose during OGTT × mean insulin during OGTT)$^{1/2}$] using the data derived from both OGTT [9] and MMT.

Statistical methods

SPSS for Windows 13.0 (Chicago, IL, USA) was used for statistical analysis. Relations between the variables were investigated by the Pearson correlation test and the variables were compared with the paired sample tests. Trapezoid method was used for calculations of area under the curve (AUC). Results are given as mean ± standard deviation. $P < 0.05$ was accepted as significant.

Results

Thirty-eight subjects with previously mentioned criteria were included in this study. At the end of the study, seven subjects were excluded due to hemolyzed blood samples or incompliance with MMT. Thirty-one subjects completed the study. BMI, waist circumference, waist–hip ratio, and body fat percentage were 36.8 ± 3.9 kg/m^2, 111.7 ± 9.2 cm, 0.96 ± 0.07, and 42.7 ± 4.1%, respectively. FBG and FBI levels were 100.5 ± 10 mg/dl and 16.2 ± 5.3 μIU/ml, respectively. Insulin sensitivity calculated by QUICKI was 0.31 ± 0.01 (Table 1). Blood glucose and insulin measurements of all the cases derived from both OGTT and MMT are shown in Figs. 1 and 2.

Considering the second-hour OGTT and MMT test results ≥200 mg/dl, impaired glucose tolerance was detected in 13 (41%) and diabetes in 2 (6%) cases during OGTT, whereas IGT was detected only in 8 cases (25%), and no diabetes in MMT. IGT was detected in 5 cases in both OGTT and MMT according to second-hour glucose levels. Upon the examination of blood glucose levels at any time following MMT, blood glucose levels were higher than 200 mg/dl in 10 patients and higher than 140 mg/dl in 19 patients. Two patients who were diagnosed as having diabetes after OGTT were also diagnosed as diabetic according to their blood glucose levels measured at any time during MMT. Likewise, among 13 patients diagnosed as having IGT following OGTT, 8 indicated blood glucose levels higher than 140 mg/dl measured at any time during MMT. Five patients diagnosed as having IGT following OGTT, were diagnosed as having diabetic values according to blood glucose levels measured at any time during MMT.

Mean HOMA-IR value of our subjects was 3.71 ± 0.88, and Matsuda's ISI derived from OGTT and MMT were 6.96 ± 3.35 and 11.32 ± 6.61, respectively (Table 1). HOMA-IR, QUICKI, and ISI calculated by Matsuda's

Table 1 Demographic, biochemical, and insulin resistance values of the patients

Age (year)	40.4 ± 8.0
Weight (kg)	92.1 ± 11.0
BMI (kg/m^2)	36.8 ± 3.9
WHR	0.96 ± 0.07
Body fat mass (%)	42.7 ± 4.1
FBG (mg/dl)	100.5 ± 10
FBI (μIU/ml)	16.2 ± 5.3
HOMA	3.71 ± 0.88
OUICKI	0.31 ± 0.01
Matsuda's ISI derived from OGTT	6.96 ± 3.35
Matsuda's ISI derived from MMT	11.32 ± 6.61

BMI body mass index, *WHR* waist/hip ratio, *FBG* fasting blood glucose, *FBI* fasting blood insulin, *HOMA* hemostasis of model assessment, *OUICKI* Quantitative Insulin Sensitivity Check Index, *ISI* Insulin Sensitivity Index, *OGTT* oral glucose tolerance test, *MMT* mixed meal test

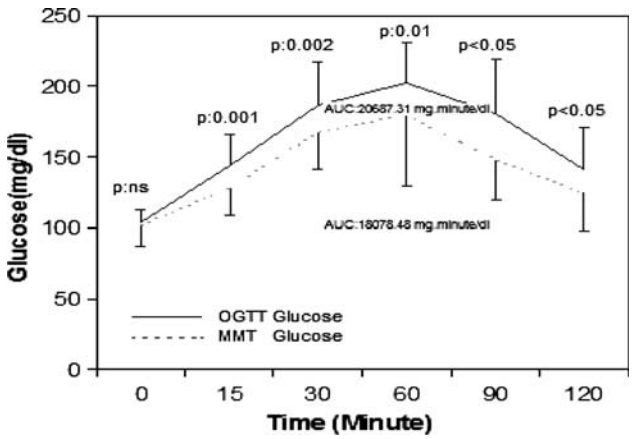

Fig. 1 AUCs of glucose levels (mg min/dl) calculated according to Trapezoid rules with those from OGTT and MMT. As a whole- and all-time periods, AUC–OGTT glucose > AUC–MMT glucose, $P < 0.05$ (*AUC* area under curve, *OGTT* oral glucose tolerance test, *MMT* mixed meal test)

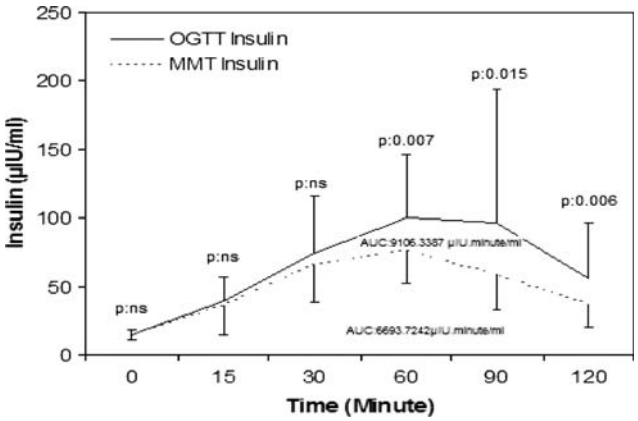

Fig. 2 AUCs of insulin levels (µIU min/ml) calculated according to Trapezoid rules with those from OGTT and MMT. As a whole and time periods at 30–60, 60–90, and 90–120 min, AUC–OGTT insulin > AUC–MMT insulin, $P < 0.05$ (*AUC* area under curve, *OGTT* oral glucose tolerance test, *MMT* mixed meal test, *ns* not significant)

Table 2 The correlation of samples matched with Matsuda's ISI derived from MMT to Matsuda's ISI derived from OGTT, HOMA, and QUICKI

	r	*P* value
HOMA vs. Matsuda MMT	−0.433	0.015
QUICKI vs. Matsuda MMT	0.374	0.038
Matsuda OGTT vs. Matsuda MMT	0.464	0.009

ISI Insulin Sensitivity Index, *HOMA* Hemostasis of Model Assessment, *QUICKI* Quantitative Insulin Sensitivity Check Index, *OGTT* oral glucose tolerance test, *MMT* mixed meal test

index derived from OGTT were found to be correlated to Matsuda's index derived from MMT (Table 2).

Glucose levels measured at 15, 30, 60, 90, and 120 min were higher in all the measurements during OGTT, whereas insulin levels were parallel to MMT at 15 and 30 min, but at 60, 90, and 120 min, it was also shown to be higher in OGTT (Table 1).

AUCs of glucose and insulin, calculated as a whole by trapezoid method, were higher in OGTT than MMT. When time periods were evaluated separately, OGTT showed higher glucose levels compared to MMT in all the time periods, whereas insulin levels were higher in time periods other than those between 0–15 and 15–30 min in OGTT, compared to MMT (Figs. 1, 2).

Discussion

In our study, it was shown that Matsuda's ISI derived from MMT is correlated with QUICKI, HOMA, and Matsuda's ISI derived from OGTT. Glucose levels were higher in OGTT than MMT both in all-time periods and in separately calculated periods. However, insulin levels were higher in OGTT than MMT, although, there was a similarity in time periods between 0–15 and 15–30 min.

Various formulas have been designed to calculate insulin sensitivity in daily practice. HOMA and QUICKI are two of most widely accepted formulas [7, 8]. FBG and FBI measurements are used in the calculation of insulin sensitivity by HOMA and QUICKI. FBG is normally regulated by endogenous glucose production from liver [22]. Consequently, calculations using fasting glucose measurements reflect hepatic IR other than peripheric insulin sensitivity. Matsuda and DeFronzo [9] stated that peripheric IR alone may be witnessed in the same subject with or without hepatic IR. In the pathophysiology of type-2 diabetes, it is well known that IR may play a significant role in peripheral tissues other than liver. Although, euglycemic hyperinsulinemic clamp technique was used as a reference in the calculation of the whole body insulin sensitivity in most of the studies, due to its impractical use [6], other formulas calculating whole body insulin sensitivity derived from OGTT were produced, and a correlation between these formulas and euglycemic hyperinsulinemic clamp technique was reported [9–11]. In the light of the information pooled from other studies, Matsuda's ISI [9], a technique used to calculate whole body IR by means of OGTT, was used instead of clamp techniques in our study, and it was shown that there was a significant correlation between Matsuda's ISI and HOMA and QUICKI showing IR in the liver. Glucose homeostasis is such a complicated event that many enteric hormones, neural factors, amino acids in food, gastrointestinal motility, and gastric emptying time play a significant role in insulin response after the ingestion of food in this process [14–17]. Insulin response to glucose may also change in the same subject during OGTT [13]. However, diabetes is today tried to be diagnosed only by determining whether insulin response to orally administered glucose is sufficient, by neglecting all the data mentioned above. Hormones secreted from gut-like glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) show their effects on carbohydrate metabolism in direct or indirect ways [14, 15]. Along with glucose-dependent insulin secretion, GLP-1 has an effect on the regulation of glucose homeostasis by inhibiting peptides like glucagon and pancreatic polypeptide and delaying gastric emptying. It was also shown that these peptides increase insulin-stimulated glucose utilization in muscle cells [23]. Besides, many amino acids in daily foods like arginine and leucine are also known to potentialize insulin secretion [16, 17]. Taking all these factors into consideration, different from other studies reported in the literature, MMT test with the same amount of calories was performed instead of OGTT in our study, and it was shown that the results obtained from Matsuda's ISI derived

from MMT were correlated with those derived from Matsuda's ISI from OGTT, QUICKI, and HOMA-IR.

Considering the measurement of time periods as a whole and separately, glucose levels were higher in OGTT than MMT. However, insulin levels measured between 0–15 and 15–30 min were similar, although, higher in OGTT, compared to MMT in whole-time periods and when time periods calculated at 30–60, 60–90, and 90–120 min were taken into consideration separately. Despite the existence of higher glucose levels in time periods calculated between 0–15 and 15–30 min, the determination of the similar insulin response in these time periods was considered to originate from positive effects of the factors mentioned above on insulin secretion in MMT, compared to OGTT. The difference of the glucose levels may be attributed to the expected results of the carbohydrate amount and content used in both the tests. Though, there is no standard concerning carbohydrate contents used in MMT in other studies [18–21], in our study total calories used in MMT was equivalized to that in OGTT (297.4 vs. 300 kCal, respectively). Compared to OGTT (containing 75-g pure glucose, ~300 kcal), carbohydrate content of MMT (43.7 g, ~180 kcal) was lower in our study. On condition that both the tests containing different calories had been performed by the same carbohydrate contents, they would not have been compared under equal circumstances. The insignificant increase in the elevation of insulin secretion during OGTT, compared to MMT, taking difference of the glucose contents into account, may be explained by the existence of the agents other than glucose, such as insulin stimulating agents, leucine, and arginine potentializing insulin secretion in the content of MMT [20]. However, it is a fact that the gastric emptying of pure glucose dissolved in 300 ml of water into small intestine takes place faster than that of MMT, and it is also absorbed more quickly. Slow gastric emptying during MMT may have a positive effect on insulin secretion, and this effect may also be attributed to the increase in the release of incretin hormones, such as GIP and GLP-1. Considering the effect of these hormones on the first phase of insulin secretion, the existence of similar insulin responses in time intervals calculated between 0–15 and 15–30 min may be explained by the increasing effects of these hormones on MMT, compared to OGTT. The significance of the evaluation of the first phase in insulin secretion might be easily understood as first phase is initially deteriorated in type-2 diabetics. Eventually, this evaluation may be made only by the test performed with MMT. Among the subjects in our study, 12 IGT and 2 diabetic cases were detected during OGTT and only 8 IGT during MMT according to blood glucose levels measured at the second hour. Five cases were IGTs according to both tests concerning second hour. In the light of the literature, cases with symptoms are diagnosed as diabetes when blood glucose levels are measured as 200 mg/dl and over at any time [24]. Although, cases are diagnosed as diabetes after glucose levels have been found in OGTT as 200 mg/dl and over at the second hour, the same situation is not appropriate for MMT [24]. However, at any time, an individual is diagnosed as diabetes if glucose levels are randomly measured as 200 mg/dl and over with hyperglycemia symptoms [24]. Considering MMT, it can be speculated that a diagnosis with blood glucose levels at 200 mg/dl and over at any time during MMT should be evaluated as diabetes. In this study, 12 IGT and 2 diabetic cases were determined during OGTT, whereas 19 IGTs and 10 diabetics were determined at any time during MMT. As individuals are never fed only with glucose, the evaluation of glucose homeostasis using only pure glucose may be questioned and carefully evaluated.

Conclusion

In conclusion, it was demonstrated that in insulin-resistant obese females, Matsuda's ISI derived from MMT can be effectively used like Matsuda's ISI derived from OGTT, and indicates a similarity to the mostly accepted methods used in the determination of IR, such as HOMA and QUICKI. Moreover, MMT was shown to have more physiological effect on insulin secretion and might reflect pancreatic functions better than OGTT.

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