

Reproducibility of Fasting and OGTT-derived Insulin Resistance Indices in Normoglycemic Women

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ABSTRACT

OBJECTIVE

To determine the reproducibility of fasting and oral glucose tolerance test (OGTT) -derived insulin-resistance (IR) indices in obese and nonobese women.

METHODS

Twenty-one obese (BMI 37.7 ± 6.3 kg/m²) and 14 nonobese (BMI 21.5 ± 1.0 kg/m²) age-matched, healthy, premenopausal women were included in the study. An OGTT was performed twice, with a 1-week interval between tests. IR was calculated from both fasting and post-load glucose and insulin values, using some of the more well-known indices.

RESULTS

When the 2 groups were evaluated separately, all indices were found to be reproducible in obese subjects, but some indices were not reproducible in nonobese healthy controls. When results were analyzed in the study population as a whole, all indices were reproducible.

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RÉSUMÉ

OBJECTIF

Déterminer la reproductibilité des index d'insulinorésistance dérivés de la glycémie à jeun et du test de tolérance au glucose par voie orale (TTGO) chez des femmes obèses et non obèses.

MÉTHODES

Vingt-et-une femmes obèses (IMC $37,7 \pm 6,3$ kg/m²) et 14 femmes non obèses (IMC $21,5 \pm 1,0$ kg/m²) appariées selon l'âge, en santé et en préménopause ont pris part à l'étude. On a effectué deux TTGO, à une semaine d'intervalle. L'insulinorésistance a été calculée à partir de la glycémie à jeun, de la glycémie après l'ingestion de glucose et de l'insulinémie, au moyen de certains des index les mieux connus.

RÉSULTATS

Lorsque les 2 groupes ont été évalués séparément, on a constaté que tous les index étaient reproductibles chez les sujets obèses, mais que certains index n'étaient pas reproductibles chez les témoins non obèses en bonne santé. Lorsque les résultats ont été analysés pour l'ensemble de la population à l'étude, tous les index étaient reproductibles.

CONCLUSIONS

Au cours de l'étude, tous les index d'insulinorésistance ont été reproductibles chez les sujets obèses, mais certains index n'ont pas été reproductibles chez les femmes non obèses. Les raisons sont peut-être le petit nombre de sujets non obèses ou une variabilité accrue des valeurs en présence d'une faible insulinorésistance. La reproductibilité observée dans l'ensemble du groupe plaide en faveur de la première raison, mais pour confirmer ces résultats, il faudra mener des études auprès d'un plus grand nombre de sujets ayant divers degrés d'insulinorésistance.

CONCLUSIONS

In this study, although reproducibility was noted for all IR indices in obese subjects, reproducibility was not observed for some of the indices in nonobese women. Reasons for this finding may have been the small patient population of nonobese subjects or increased variability of the measures in low IR states. The observed reproducibility in the evaluation of the entire group supports the former reason, but studies with larger patient populations and different levels of IR are needed to confirm these results.

INTRODUCTION

Insulin resistance (IR) and its accompanying metabolic abnormalities are known to play an important role in the pathophysiology of type 2 diabetes mellitus and coronary heart disease. Many prospective studies have confirmed that lifestyle changes may prevent the development of type 2 diabetes; accordingly, an accurate and reproducible method for measurement of IR is important, both to assess response to interventions and to develop new therapeutic strategies.

The gold standard for measurement of IR is the euglycemic clamp technique (ECT), which was introduced by DeFronzo and colleagues in 1979 (1). However, this method is time-consuming, difficult, invasive and impractical for use in large patient groups or epidemiological studies. In recent years, different mathematical formulas for measuring IR have been developed, all with the dual aims of practicality and high correlation with the gold-standard technique (2-7). These mathematical formulas use serum glucose and insulin levels (7-10), either when patients are fasting or during an oral glucose tolerance test (OGTT), which is considered to be a good physiological imitator of meal stimulation (5,6,11,12).

Fasting serum glucose levels are the result of hepatic gluconeogenesis and basal pancreatic insulin release. In contrast, many mechanisms affect serum glucose levels after oral glucose stimulation, including differences in glucose absorption, gastrointestinal hormones, neural stimulation and pancreatic beta-cell response. For this reason, fasting indices and indices derived from oral glucose stimulation represent different but related aspects of glucose homeostasis.

Although there are many studies evaluating the correlation of indices of IR with gold-standard techniques, there are only a few evaluating their reproducibility (13,14). The main objective of this study was to evaluate the reproducibility of fasting and post-load IR index values in normotensive and normoglycemic obese and nonobese premenopausal, healthy women.

METHODS

Twenty-one obese and 14 nonobese healthy, premenopausal women were chosen for the study, the obese women from among those attending the outpatient clinic for obesity and the nonobese controls from among hospital staff and medical students.

The obese subjects had not been on a calorie-controlled diet for at least 6 months prior to the study and had not previously taken antiobesity medications. Patients with a history of diabetes in first-degree relatives; impaired glucose tolerance; diabetes; hypertension; hyperlipidemia; known pulmonary, cardiac, hepatic, renal or other chronic disease; menstrual problems; secondary obesity; and/or on medication that could affect glucose metabolism (including oral contraceptives) were not included in the study.

All patients' waist and hip circumferences, weight and height were measured. Subjects were defined as obese according to Garrow's criteria (body mass index [BMI] $>30 \text{ kg/m}^2$) (15). Subjects with BMI values $<25 \text{ kg/m}^2$ were considered lean. Body height was measured without shoes to the nearest 0.5 cm; body weight was measured with light clothes on, without shoes, to the nearest 0.1 kg. BMI was calculated as weight (kg)/height (m)². Waist circumference was measured midway between the lower rib margin and the iliac crest. Hip circumference was measured as the widest circumference measured over the great trochanters. Waist/hip ratio (WHR) was calculated using these measurements. To rule hypertension, 3 blood pressure measurements were taken in the outpatient clinic for each subject, and 1 week blood pressure follow-up charts were requested; hypertension was defined as blood pressure levels $>140/90 \text{ mm Hg}$. Body fat ratio and body fat mass were measured with bioimpedance meter (Omron, BF302, Portable Body Fat Analyzer, Hofddorp, Germany). Fasting serum total, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and very-low-density lipoprotein (VLDL) cholesterol; triglycerides; C-peptide; and uric acid levels were measured in routine blood chemistry.

All OGTTs were done at 8:30 AM after 10–12 h of fasting. A polyethylene catheter was placed into the antecubital vein before the test. Fasting samples were taken at -30 , -15 and 0 min . After an oral standard load of 75 g glucose, blood samples were taken again at 30 , 60 , 90 and 120 min . After centrifugation, serum samples were stored at -70°C . Glucose levels were measured using the glucose oxidase method (Roche Modular, Hitachi, Japan). Insulin levels were measured using chemiluminescence assay (Immulite 2000, DPC, USA). To investigate reproducibility, testing was

repeated after 1 week. Patients followed a carbohydrate-rich diet during this interval.

IR was calculated from fasting samples using the following indices:

- Fasting Belfiore index: $2 / [\text{insulin (pmol/L)} \times \text{glucose (mmol/L)} + 1]$ (6)
- HOMA index: $[\text{fasting glucose (mmol/L)} \times \text{fasting insulin (}\mu\text{U/mL)}] / 22.5$ (7)
- QUICKI index: $1 / [\log (\text{insulin (}\mu\text{U/mL)}) + \log (\text{glucose (mg/dL)})]$ (8)
- Raynaud index: $40 / \text{insulin (}\mu\text{U/mL)}$ (9)

Indices derived from the OGTT measurements were as follows:

- ISI-composite index: $10\,000 / \sqrt{[\text{fasting glucose (mg/dL)} \times \text{fasting insulin (}\mu\text{U/mL)} \times (\text{mean OGTT glucose} \times \text{mean OGTT insulin})]}$ (11)
- Drivsholm index: $[\text{AUC glucose (mg/dL)} / \text{AUC insulin (}\mu\text{U/mL)}]$ (5)
- Cederholm index: $[75.000 + (0 \text{ min glucose [mmol/L]}$

$- 120 \text{ min glucose} \times 1.15 \times 180 \times 0.19 \times \text{body weight}] / [120 \times \log (\text{mean insulin (}\mu\text{U/mL)}) \times \text{mean glucose}]$ (12)

- OGTT-derived Belfiore index: $2 / [\text{AUC insulin (pmol/L)} \times \text{AUC glucose (mmol/L)} + 1]$ (6)
- Gutt index: $\text{ISI}_{0,120} = \text{metabolic clearance rate}^* / \log \text{mean fasting and 2 h serum insulin (}\mu\text{U/mL)}$ (16)
 - *metabolic clearance rate = $m\ddagger / \text{mean fasting and 2-h plasma glucose (mg/dL)}$
 - $\ddagger m = [75\,000 \text{ mg} + (\text{fasting glucose} - 2 \text{ h glucose}) \times 0.19 \times \text{body weight}] / 120 \text{ min}$
- Stumvoll index: $[0.22 - (0.0032 \times \text{BMI}) - (0.0000645 \times 2 \text{ h insulin (pmol/L)}) - [0.0037 \times 90 \text{ min glucose (mmol/L)}]]$ (17)

In statistical analysis, for comparison of the basic characteristics of the 2 groups, an independent samples t-test was used. Reproducibility of the indices was analyzed using intraclass correlation coefficient methods. For intraclass correlation coefficients, correlation coefficient (r) and significance of

Table 1. Baseline characteristics

Characteristic	Obese, mean \pm SD (n=21)	Nonobese, mean \pm SD (n=14)	p value
Age, years	29.9 \pm 7.7	27.4 \pm 6.8	NS*
Weight, kg	93.7 \pm 13.9	55.6 \pm 4.3	<0.05
BMI, kg/m ²	37.7 \pm 6.3	21.5 \pm 1.0	<0.05
Waist circumference, cm	98.5 \pm 10.4	67.9 \pm 3.1	<0.05
Waist/hip ratio	0.79 \pm 0.03	0.72 \pm 0.04	<0.05
Body fat ratio, %	41.1 \pm 3.9	22.9 \pm 3.7	<0.05
Body fat mass, kg	38.5 \pm 9.1	12.7 \pm 2.2	<0.05
Systolic blood pressure, mm Hg	120.7 \pm 12.5	99.3 \pm 11.4	<0.05
Diastolic blood pressure, mm Hg	79.3 \pm 8.4	67.1 \pm 8.3	<0.05
Total cholesterol, mmol/L	4.6 \pm 0.4	4.4 \pm 0.6	NS*
HDL cholesterol, mmol/L	1.2 \pm 0.2	1.6 \pm 0.2	<0.05
LDL cholesterol, mmol/L	2.8 \pm 0.4	2.5 \pm 0.6	NS*
VLDL cholesterol, mmol/L	0.6 \pm 0.2	0.3 \pm 0.1	<0.05
Triglycerides, mmol/L	1.2 \pm 0.5	0.7 \pm 0.2	<0.05
C-peptide, nmol/L	1.4 \pm 0.5	0.8 \pm 0.5	<0.05
Uric acid, μ mol/L	279.6 \pm 47.6	202.2 \pm 41.6	<0.05
Fasting glucose, mmol/L	4.8 \pm 0.5	4.3 \pm 0.4	<0.05
2-h glucose, mmol/L	115.2 \pm 14.5	90.5 \pm 18.4	<0.05
Fasting insulin, pmol/L	101.4 \pm 50.7	22.2 \pm 9.7	<0.05
2-h insulin, pmol/L	48.6 \pm 28.7	22.1 \pm 12.9	<0.05

*NS: p>0.05

BMI = body mass index

HDL = high-density lipoprotein

LDL = low-density lipoprotein

VLDL = very-low-density lipoprotein

correlations (p) are given. SPSS version 13.0 (SPSS Inc., Chicago, Illinois, US) and MedCalc® for Windows v.7.6.0.0 (Mariakerke, Belgium) were used for statistical analysis. For AUC calculation, the trapezoidal rule was used (18).

RESULTS

The study population consisted of 21 obese and 14 nonobese age-matched, normotensive, normolipidemic, glucose-tolerant premenopausal women. Baseline characteristics of both groups are shown in Table 1. Weight, BMI, waist circumference, WHR, body fat ratio, body fat mass, systolic and diastolic blood pressure, fasting serum total and VLDL cholesterol, triglycerides, C-peptide, uric acid, glucose and insulin levels were significantly higher and HDL-cholesterol was lower in the obese group compared with the nonobese healthy controls.

The calculated IR index results for both groups are shown in Table 2. The first-week tests of the obese group have been compared with the first-week tests of the nonobese healthy controls. There was a significant difference between the 2 groups for all tests, and IR was significantly higher in the obese group. The same differences were noted for the comparisons of the second-week tests (data not shown).

To evaluate reproducibility between the first- and second-week tests, intraclass correlation coefficients were calculated as shown in Table 3. The intraclass correlation coefficients analyzed for obese subjects showed reproducibility for all tests with high r values. For nonobese subjects, the ISI-composite, fasting and OGTT-derived Belfiore, Gutt and Cederholm indices were not reproducible. When the group was analyzed as a whole, however, all tests were reproducible.

When the differences between the initial measures and the duplicate measures performed after a 1-week interval were compared between the 2 groups, no significant difference was noted. Thus, the differences between the 2 measurements were similar in both groups for all tests (Table 4). In calculating the differences between the initial and duplicate measures in both groups, some of the mean values have

a negative result, since the absolute value of the second test was subtracted from the absolute value of the first.

DISCUSSION

In this study, IR indices of obese and nonobese healthy women were calculated from fasting and OGTT-derived glucose and insulin levels, and their reproducibility was investigated. Obese individuals were found to be insulin resistant, and all indices were significantly higher in this group. Reproducibility was assessed by intraclass correlation coefficient analysis. ISI-composite, fasting and OGTT-derived Belfiore, Cederholm and Gutt indices were not reproducible when the nonobese group was analyzed separately.

Obese and nonobese subjects who were normotensive, normolipidemic and normoglycemic have been included in this study. Subjects with these characteristics were chosen for the following reasons. First, diabetes prevention programs are more likely to be successful at the stage of normal glucose tolerance, rather than in glucose intolerance or overt type 2 diabetes (19), so it may be more important to measure IR accurately in normoglycemic individuals. Second, because OGTT-derived measures were used, subjects with diabetes and glucose intolerance were excluded to minimize the possibility of insulin secretion defects.

This study showed that insulin-resistance indices were reproducible in obese subjects, but that some were not reproducible in nonobese subjects. The indices that were not reproducible were mainly derived from OGTT measurements, and there may be a few reasons for this. First, the sample size was small in the nonobese group, possibly reducing the statistical power. Second, the measurements may have had increased variability in low insulin-resistant states. However, the observed reproducibility of the indices when the entire group was evaluated as a whole supports the former hypothesis. Studies investigating the reproducibility of these indices have been performed before with affirmative results (13,14,20,21). Also, the nonsignificant differences

Table 2. Comparison of IR indices between obese subjects and nonobese healthy controls

Index	Obese, mean±SD	Nonobese, mean±SD	p-value
Belfiore (fasting) (pmol ⁻¹ .l.mmol ⁻¹ .l)	0.4±0.2	1.0±0.2	<0.05
HOMA (mmol.μU.mL ²)	3.0±1.6	0.6±0.3	<0.05
QUICKI	0.3±0.02	0.4±0.03	<0.05
Raynaud (μUI ¹ .1)	3.5±1.6	14.4±5.4	<0.05
ISI-composite (UI ⁻¹ .ml.mg ⁻¹ .ml)	4.7±2.5	16.1±5.9	<0.05
Drivsholm	2.7±1.4	4.4±1.8	<0.05
Cederholm (mg.l.l.mmol ⁻¹ .μUI ⁻¹ .min ⁻¹)	52.9±12.7	90.2±25.2	<0.05
OGTT-Belfiore (pmol ⁻¹ .l.h ⁻¹ .mmol ⁻¹ .l.h ⁻¹)	0.59±0.25	1.03±0.2	<0.05
Gutt	44.6±8.1	75.1±20	<0.05
Stumvoll	0.05±0.03	0.1±0.01	<0.05

between duplicate measurements when the 2 groups were compared may be regarded as supportive of the reproducibility of these indexes.

With respect to the OGTT-derived Belfiore index, it may be tempting to speculate that this condition may be due to the sensitivity of the OGTT-derived Belfiore index formula to minor changes in serum glucose and insulin levels during the OGTT, while the designs of other formulas lead to more stable results. The Belfiore index also differs from the other indices in that it includes mean normal AUC glucose and AUC insulin values, which are calculated from every laboratory's OGTT results. Previous studies from different laboratories have shown that these mean normal values vary to a wide degree (6); however, it remains to be determined to what degree this difference affects the formula's reproducibility.

What constitutes the most practical and trustworthy index for measurement of IR has been a matter of debate for some time. Although it is accepted as the gold standard, ECT, in addition to being labour-intensive, has a few differences and disadvantages compared with fasting and OGTT-derived indices. First, ECT essentially measures peripheral insulin sensitivity, whereas this remains undetermined for fasting and OGTT-derived indices. Second, ECT measures insulin-stimulated glucose disposal only at insulin levels in the non-physiological range (22), although measurement of insulin effect on glucose uptake in physiological states is more important. When considered in this way, fasting and OGTT-derived indices use more physiological values of glucose and insulin; however, although they are practical, different approaches are used to derive the equations, some empirical

and some based on models. The validity of these indices depends on the validity of the assumptions they are based on. Although it is not sufficient to judge the validity of a method based only on its correlation with the ECT (22,23), the objective of our study would have been better achieved if the ECT had also been used.

Glucose homeostasis depends on a complex metabolic process, and the relationship between glucose and insulin is oversimplified, no matter how many parameters are included in these indices. Nevertheless, these formulas have been and will continue to be used in large epidemiological studies to predict type 2 diabetes or for other reasons. As a result, although there are many possible indices for measurement of IR, the choices may be limited for insulin-sensitive subjects. Further studies with larger patient groups may better illuminate why reproducibility is lower in insulin-sensitive subjects compared with insulin resistant ones.

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Table 3. Intraclass correlation coefficients (r) of fasting and OGTT-derived indices

Index	Obese, r value	Nonobese, r value	All, r value
Belfiore (fasting)	0.941*	0.542†	0.880*
HOMA	0.958*	0.814*	0.974*
QUICKI	0.946*	0.783*	0.964*
Raynaud	0.923*	0.760*	0.940*
ISI-composite	0.934*	0.498†	0.904*
Drivsholm	0.850*	0.645*	0.847*
Cederholm	0.853*	0.456†	0.841*
OGTT-Belfiore	0.900*	0.589†	0.900*
Gutt	0.841*	-0.196†	0.474*
Stumvoll	0.961*	0.788*	0.985*

*p<0.05

†p>0.05

Table 4. Comparison of differences in tests performed with a 1-week interval

Index	Group	Mean	SD*
Belfiore (fasting)	Obese	0.003	0.082
	Nonobese	-0.149	0.352
HOMA	Obese	0.050	0.689
	Nonobese	0.078	0.317
QUICKI	Obese	0.000	0.012
	Nonobese	-0.004	0.028
Raynaud	Obese	0.009	0.897
	Nonobese	-0.919	4.626
ISI-composite	Obese	0.370	1.240
	Nonobese	0.841	6.569
Drivsholm	Obese	0.078	0.888
	Nonobese	0.273	1.742
Cederholm	Obese	3.875	9.161
	Nonobese	-5.931	24.17
OGTT-Belfiore	Obese	0.067	0.139
	Nonobese	-0.007	0.238
Gutt	Obese	-0.045	6.610
	Nonobese	6.572	55.45
Stumvoll	Obese	0.001	0.011
	Nonobese	-0.000	0.008

*p>0.05

AUTHOR DISCLOSURES

No duality of interest declared.

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