

# Evaluation of insulin sensitivity and glucose effectiveness during a standardized breakfast test: comparison with the minimal model analysis of an intravenous glucose tolerance test

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## Abstract

There is a need for reliable measurements of insulin sensitivity (SI) simpler than the euglycemic hyperinsulinemic clamp or the intravenous glucose tolerance test (IVGTT), which could be used when the simpler surrogates based on fasting insulin ( $I_b$ ) and glucose ( $G_b$ ) lose their validity. Several evaluations of SI derived from oral glucose tolerance test (OGTT) or its physiologic form, the standardized breakfast test (SBT), have been proposed. We aimed at determining which SBT-derived measurements of SI give the best prediction of the values obtained with the minimal model analysis of an IVGTT. Twenty-eight subjects (23 females and 5 males; age,  $44.3 \pm 0.6$  years) with a wide range of glucose tolerance randomly underwent a hyperglucidic SBT and an IVGTT with minimal model analysis. Correlations of 35 indices (converted if appropriated into similar units) with IVGTT-derived SI were calculated, and the accuracy of the empiric formulas obtained with the 11 best predictions were evaluated with Bland-Altman plots. Subjects covered all the spectrum of SI between 0.19 and  $21.3 \text{ min}^{-1}/(\mu\text{U} \cdot \text{mL}^{-1}) \times 10^{-4}$ . Eight procedures yielded satisfactory predictions of minimal model SI: (1) SI (from Matsuda's composite index) =  $-1.24 + 65/(I_b G_b I_m G_m)^{-0.5}$ ; (2) SI =  $1.89 + 2690/(I_b G_b I_m G_m)$ ; (3) SI (from Bennett's index) =  $-2.93 + 5.16/(\log I_b \times \log G_b)$ ; (4) SI (from Sluiter's index) =  $0.2 + 2400/(I_p G_p)$ ; (5) SI =  $-8.54 + 38.4/(\text{Belfiore's ISI index})$ ; (6) SI (from Cederholm's formula) =  $76/(G_m \log I_m)$ ; (7) SI =  $0.248 + 0.947/G_b I_m$ ; (8) SI (from Mari's "oral glucose insulin sensitivity" index) = oral glucose insulin sensitivity/ $I_p$ ; (9) Caumo's model. Glucose effectiveness  $S_g$  can also be accurately predicted by the following formula:  $S_g = 2.921e^{-0.185(\frac{G_{60}}{G_b} - \frac{G_b}{I_p})}$  ( $I_p$  = insulin peak;  $G_p$  = glucose peak;  $I_a$  = insulin area;  $G_a$  = glucose area;  $G_{60}$  = glycemia at 60 minutes). The hyperglucidic SBT can provide accurate evaluations of SI and  $S_g$ , either by elaborated models or by simple empiric formulas.

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## 1. Introduction

Because of the importance of the concept of insulin resistance, standardized guidelines for the routine diagnosis of this situation have been developed. They consist of a "metabolic score" based on simple clinical symptoms. However, because of its cost and complexity, the routine diagnosis of insulin resistance is not usually included in this evaluation. It would nevertheless be interesting to accurately measure insulin sensitivity (SI) in some cases for either research or for specific clinical purposes. Unfortunately, until now, the 2 most widely accepted methods for quantifying SI, the euglycemic hyperinsulinemic clamp and the intravenous glucose tolerance test (IVGTT) with minimal model

analysis, are 2 rather sophisticated methods [1]. They are well adapted for physiologic or pathophysiologic investigations performed on limited numbers of individuals, but can hardly be proposed for routine assessment of insulin resistance in clinical practice because of their invasiveness, duration, and cost of reagents. Besides these "gold standard" approaches, a large body of literature has been generated over the last past years about simplified measurements using baseline values of insulin and glucose. The most widely used of these indices are the "homeostasis model assessment insulin resistance index" (HOMA-IR) [2] and the "quick insulin sensitivity index" (QUICKI) [3], which is a reciprocal logarithmic transformation of the HOMA-IR. Although they are actually based on different theoretical assumptions, they all basically rely on the same fundamental concept: when SI decreases, there is a compensatory rise in insulin [4] and, to a lesser extent, in glucose. Therefore, in all situations where

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insulin is able to mirror insulin resistance, these indices have been repeatedly demonstrated to be quite precise predictors of the values of SI that could be obtained with the clamp or the minimal model [5]. By contrast, there are some situations in which this feedback relationship between insulin release and SI is disturbed, and in these cases, the validity of these surrogates has been seriously challenged. This is the case in diabetes when fasting glycemia exceeds 126 mg/dL [6], in patients who have reactive hypoglycemia [7,8], in athletes [7,9,10], and in adolescents from families at risk for diabetes [11]. In fact, all these limits of validity are situations where it would sometimes be interesting to assess SI, to discuss whether insulin sensitizers are the logical treatment or not. Therefore, beside the gold standards (clamp, minimal model) and the surrogates (HOMA, QUICKI, etc), there is room for an alternative approach, which is easier to perform than the former in clinical practice and still valid when surrogates lose their accuracy [11].

Promising approaches have been provided by the mathematical analysis of the classic oral glucose tolerance test (OGTT) [12,13]. Among the various indices of SI derived from this test, some have been shown to exhibit a satisfactory accuracy [14,15], so that the OGTT, although less used for this purpose, is likely to provide a fair evaluation of SI.

Actually, in most studies, various “indices” of SI (or insulin resistance) are proposed, but there is generally no attempt to convert these indices into physiologic units, that is, a flow rate of glucose disappearance per unit of insulinemia above baseline. However, such a conversion can be done and helps to standardize the results and to compare more closely the various methods.

Rather than the classic OGTT, we chose to investigate for this purpose the standardized breakfast test (SBT), which is a “physiologic” variant of OGTT [16] offering several advantages: (a) lack of artifactual postload hypoglycemia, thus making this test suitable for the study of postprandial hypoglycemia [17], a situation which is frequently due to high values of SI [8,18,19], but also to hyperinsulinism in a context of insulin resistance [8]; (b) use of a physiologic stimulus triggering a cephalic phase proportional to palatability scores [20]; (c) possibility, according to previous reports, to measure SI with a modified algorithm based on the minimal model [21] as well as glucose effectiveness [22] and insulin secretion [23].

Therefore, we aimed at comparing SBT-derived measurements of SI and Sg (expressed in standardized physiologic units) with reference measurement obtained with the minimal model analysis of an IVGTT and to determine which SBT-derived indices correlate the best with those IVGTT-derived measurements.

## 2. Research design and methods

### 2.1. Subjects

Twenty-eight subjects (23 females and 5 males; age,  $44.3 \pm 0.6$  years) with a wide range of body mass index

(BMI) values randomly underwent an SBT and IVGTT designed for minimal model analysis. Subjects with type 2 diabetes mellitus, glucose intolerance, or postprandial reactive hypoglycemia, as well as highly trained athletes, were not excluded to represent all the range of SI and glucose tolerance as shown in Table 1. Based on the guidelines from the 2001 National Cholesterol Education Program Adult Treatment Panel (NCEP-ATPIII), any 3 of the following traits in the same individual meet the criteria for the metabolic syndrome: (a) abdominal obesity, a waist circumference of more than 102 cm (40 in) in men and more than 88 cm (35 in) in women; (b) serum triglycerides of 150 mg/dL or higher; (c) high-density lipoprotein cholesterol of 40 mg/dL or lower in men and 50 mg/dL or lower in women; (d) blood pressure of 130/85 or more; and (e) fasting blood glucose of 110 mg/dL or higher. Individuals were given a score ranging between 0 and 5 according to this list of symptoms. In this study, we thus used this score to stratify the group of subjects into 4 subgroups.

All patients received detailed printed and oral information and gave their informed consent. The protocol was approved by the local ethical committee according to the French regulation (law of March 5, 2002, No. 2002-1138, describing the rights of patients and the quality of the French health care system, and modifying the “Huriet-Sérusclat” law [No. 88-1138] that regulates biomedical research protocols).

### 2.2. Intravenous glucose tolerance test with minimal model analysis

Although no alimentary restriction was imposed, subjects were asked to fast for 12 hours before the beginning of the test at 9:00 AM. A cannula was placed in the cephalic vein at the level of the cubital fossa for blood sampling at various times, whereas glucose injection was performed in the contralateral cephalic vein. Glucose (0.5 g/kg, solution at 30%) was slowly injected during 3 minutes. Insulin (0.02 U/kg body weight, ie, 1 or 2 units) was injected intravenously immediately after 19 minutes. Blood samples were drawn twice before the glucose bolus and at 1, 3, 4, 8, 10, 15, 19, 20, 22, 30, 41, 70, 90, and 180 minutes after the onset of the glucose injection [24–26].

Table 1  
Characteristics of the patients included in the study

	Mean $\pm$ SEM	Range (extreme values)
Sex ratio (M/F)	5/23	
Age (y)	$44.33 \pm 3.30$	17–73
Weight (kg)	$85.65 \pm 1.89$	65–117.4
Height (cm)	$163.33 \pm 0.98$	151–172
BMI (kg/m <sup>2</sup> )	$32.24 \pm 0.90$	25.1–50.1
Fasting blood glucose (mmol $\cdot$ L <sup>-1</sup> )	$5.71 \pm 0.09$	3.3–15.5
Fasting serum insulin ( $\mu$ U $\cdot$ mL <sup>-1</sup> )	$11.63 \pm 0.23$	1–25
SI (min <sup>-1</sup> /[ $\mu$ U $\cdot$ mL <sup>-1</sup> ] $\times 10^{-4}$ )	$4.8 \pm 0.93$	0.19–21.3
Sg (min <sup>-1</sup> $\times 10^{-2}$ )	$2.28 \pm 0.22$	0.47–4.32

### 2.3. Standardized breakfast test

As for the IVGTT, subjects had been asked to fast for 12 hours before commencement of the standardized breakfast, which was composed of bread (80 g), butter (10 g), jam (20 g), skimmed concentrated milk (80 mL) (Gloria SA, Paris, France), sugar (10 g), and powder coffee (2.5 g). The breakfast thus comprised 2070 kJ with 9.1% proteins, 27.5% lipids, and 63.4% carbohydrates. The average time for consuming the meal was 6 minutes. Blood samples were taken twice before the meal and at 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes after the start of the meal. This test, which has been designed to detect postprandial reactive hypoglycemia, elicits the same glycaemic response as the conventional OGTT [8,17]. For the purpose of the study, we used only the periods 0 to 180 minutes for the calculation of SI.

### 2.4. Laboratory measurements

All samples were analyzed for plasma insulin by a radioimmunoassay (kit SB-INSI-5 from the international CIS) and plasma glucose with a Beckman glucose analyzer II (Fullerton, CA). The within-assay coefficient of variation for insulin was determined by repetitive measurements of the same sample and was between 8.6% (low values) and 9.7% (high values). The between-assay coefficient of variation for insulin was between 12.5% (low values) and 14.4% (high values). The sensitivity (lowest detectable value) was 2  $\mu$ U/mL.

#### 2.4.1. Calculation of SI and glucose effectiveness from IVGTT data

Minimal model analysis of IVGTT according to Bergman et al [1,27,28] was done with the software TISPAG from the Department of Physiology of the University of Montpellier I, France [24–26], which uses a nonlinear least square estimation. This program gave the values of SI and glucose effectiveness (Sg). SI and Sg are calculated from the following equations:

$$G(t)/t = -[p1 + X(t)]G(t) + p1G_b$$

$$G(0) = G_0$$

$$X(t)/t = -p2X(t) + p3[I(t) - I_b]$$

$$X(0) = 0$$

where  $G(t)$  and  $I(t)$  are plasma glucose and insulin concentrations;  $X(t)$  is the insulin in a compartment remote from plasma (“insulin action”); and  $p1$  to  $p3$  are model parameters.  $G_0$  is the glucose concentration that one would obtain immediately after injection, if there was instantaneous mixing in the extracellular fluid compartment.  $G_b$  and  $I_b$  are basal values of glucose and insulin. Parameter  $p1$  represents Sg, that is, the fractional disappearance rate of glucose, independent of any insulin response, and  $p3$  and  $p2$  determine the kinetics of insulin transport, respectively, into

and out of the remote insulin compartment where insulin action is expressed. Insulin sensitivity is an index of the influence of plasma insulin to change glucose’s own effect on glucose concentration. Thus, SI is equal to  $-p3/p2$ .

#### 2.4.2. Measurements of insulin sensitivity during the breakfast test

We used only values of glucose and insulin obtained between 0 and 180 minutes. Three kinds of indices from fasting and postload glucose and insulin values (see Table 2) were calculated: surrogates from basal glucose and insulin values, empirical indices developed for the OGTT, and more complexes indices given by a physiologic model of glucose disposal after a glucose load. In each case, we first calculated raw correlation values between them and SI-derived minimal model (Table 2). As shown on Table 2, most indices were given by a simple formula, but some of them needed a slightly more sophisticated calculation or the use of software. We gave special attention to these less simplistic indices that are underlain by physiologic concepts rather than a purely empirical approach. The HOMA-%SI was obtained with the software HOMA-2 kindly provided by Jonathan Levy [5]. The OGIS [12], an estimate of the glucose clearance during a hyperinsulinemic euglycemic glucose clamp (expressed in mL/min per square meter of body surface area) is easily obtained from a Microsoft Excel workbook that can be downloaded at (<http://www.isib.cnr.it/bioing/ogis/webogis/ogis.html>). The model of Caumo [21] extends Bergman’s minimal model computation to the analysis of an SBT and provides an evaluation of SI that can actually be calculated with a Microsoft Excel workbook according to the formulas published by its authors in their original article [21]. Although simpler than all these indices, Belfiore’s [13] models are also based on an elaborate modeling concept aiming at a quantitative measurement of SI from the insulin and glucose levels recorded during an OGTT. The first formula:  $ISI(gly) = 2/[(INSp \times GLYp) + 1]$ , where  $ISI(gly)$  indicates the SI index toward glycemia with  $INSp$  and  $GLYp$  indicating insulinemic and glycemic areas, respectively, during OGTT. If this test uses the “areas” during OGTT, it is called  $ISI(gly)$ -a. By using basal levels, the same formulas give the SI in the basal state or  $ISI(gly)$ -b. Both the basal levels and areas are expressed by taking the “mean normal value” as 1 (ie, by dividing the observed value by the mean normal value). In healthy subjects,  $ISI(gly)$  is always 1, with maximal variations among patients between 0 and 2.  $ISI(gly)$  can be calculated with an Excel page downloadable from <http://users.iol.it/francesco.belfiore/index.htm>.

Concerning all these indices, 2 important methodological remarks are needed. First, it is clear that the physiologic significance of all indices is not similar and that an index of insulin resistance should be expected to correlate better with  $1/SI$  than with SI itself, because insulin resistance is the reciprocal of SI ( $IR = 1/SI$ ), that is, the relationship between insulin resistance and SI is not linear but curvilinear. Thus,

given the fact that the linear correlation coefficient between  $x$  and  $1/x$  is equal to  $-0.45$ , comparing together indices of IR and indices of SI may be misleading. To overcome this important problem, we correlated all indices to both SI and  $1/\text{SI}$  (see Table 2). In addition, some indices evaluate a CHO

oxidation flow rate, that is, an insulin-mediated glucose uptake (IMGU) expressed in  $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ , whereas others give a dose-response relationship (SI) expressed in  $\text{min}^{-1}/(\mu\text{U} \cdot \text{mL}^{-1}) \times 10^{-4}$ . Correspondence between SI and IMGU is calculated as classically reported [47] with the

Table 2

Raw regression coefficients between SBT measurements of SI (or resistance) and SI or resistance ( $1/\text{SI}$ ) measured with the IVGTT

Simple measure (abbreviation) (reference)	Formula	Linear correlation (Pearson $r$ coefficient) with SI from IVGTT	Linear correlation (Pearson $r$ coefficient) with $1/\text{SI}$ from IVGTT
Matsuda composite index [29]	$\text{SI}_{\text{comp}} = 10^4/(I_b G_b I_m G_m)^{0.5}$	$r = 0.656, P = .00015$	$r = -0.333, P = .08$
$1/(I_b G_b I_m G_m)$ [22,30]	$\text{SI} = 10^4/(I_b G_b I_m G_m)$	$r = 0.650, P = .00018$	$r = -0.250, P = .199$
Bennett BFS [31]	$1/(\log I_b \times \log G_b)$	$r = 0.620, P = .00055$	$r = -0.575, P = .001$
Sluiter's index [32]	"A" = $10^4/(I_{\text{max}} G_{\text{max}})$	$r = 0.575, P = .0014$	$r = 0.141, P = .474$
Belfiore (area) ( $\text{SI}_{\text{gly}_a}$ ) [13]	$\text{SI}_{\text{gly}_a} = 2/[(I_a/mI_a + G_a/mG_a) + 1]$	$r = 0.5468, P = .0026$	$r = -0.546, P = .002$
Cederholm [33]	$\text{SI} = \text{constant}/(G_m \log I_m)$	$r = 0.527, P = 0.003$	$r = 0.578, P = .0013$
$1/G_b I_m$	$1/G_b I_m$	$r = 0.525, P = .004$	$r = -0.396, P = .037$
$1/I_m G_m$	$1/I_m G_m$	$r = 0.501, P = .007$	$r = -0.386, P = .04$
Gutt $\text{SI}_{0,120}$ ( $\text{SI}_{0,120}$ ) [34]	$(m/\text{MPG}) / \log \text{MSI}$	$r = 0.486, P = .009$	$r = -0.577, P = .001$
Mari's $\text{Cl}_{\text{OGTT}}$ [12] corrected for insulin peak response	$\text{Cl}_{\text{OGTT}}$ (from model)/ $I_p$	$r = 0.458, P = .0142$	$r = -0.202, P = .302$
Mari's OGIS [12] corrected for insulin peak response	$\text{OGIS}$ (from model)/ $I_p$	$r = 0.4543, P = .015$	$r = -0.141, P = .475$
$\log \text{HOMA}$ [35]	$\log (I_b G_b/22.5)$	$r = -0.448, P = .167$	$r = -0.252, P = .195$
$\text{SI} = 40/I_b$ [36,37]	$\text{SI} = 40/I_b$	$r = 0.446, P = .017$	$r = 0.660, P = .00013$
Caumo [21]	Minimal model calculation	$r = 0.442, P = .019$	$r = -0.268, P = .167$
Mari's $\text{Cl}_{\text{OGTT}}$ [12] corrected for insulin mean response	$\text{Cl}_{\text{OGTT}}$ (from model)/ $I_m$	$r = 0.4411, P = .0188$	$r = -0.193, P = .326$
Mari's OGIS [12] corrected for insulin mean response	$\text{OGIS}$ (from model)/ $I_m$	$r = 0.4322, P = .0216$	$r = -0.231, P = .236$
HOMA-IR [2,5]	$I_b G_b/22.5$	$r = -0.426, P = .02$	$r = 0.298, P = .123$
Soonthornpun [38]	$\text{SI} = [1.9/6 \times \text{weight (kg)} \times G_b + 520 - 1.9/18 \times \text{weight (kg)} \times G_a - \text{urinary glucose (mmol/1.8)}]/(I_a (\text{pmol/h} \cdot \text{L}) \times \text{weight (kg)})]$	$r = 0.396, P = .037$	$r = -0.086, P = .66$
Stumvoll [39]	$0.156 - 0.0000459 \times I_2 - 0.0000321 \times I_b - 0.00541 \times G_b$	$r = 0.373, P = .05$	$r = -0.683, P = .00006$
Avignon's $\text{SI}_{2h}$ [40]	$\text{SI}_{2h} = 10^8 / 1120 (\mu\text{U/mL}) \times \text{G120 (mg/dL)} \times V_D$ , where $V_D = 150 \text{ mL/kg body weight}$	$r = 0.360, P = .06$	$r = 0.124, P = .53$
$\text{SI}_{2h} = 1000/I_{2h}$ [30]	$10000/(I_{120} \times G_{120})$	$r = 0.352, P = .07$	$r = -0.319, P = .098$
$1/I_m$	$1/I_m$	$r = 0.3401, P = .08$	$r = -0.0725, P = .714$
QUICKI [3]	$1/(\log I_b + \log G_b)$	$r = 0.336, P = .055$	$r = -0.4478, P = .012$
Cheng [41]	Log summed postload insulin	$r = -0.3306, P = .0858$	$r = 0.0484, P = .8068$
Avignon's index SIM [40]	$[(w \times \text{Si}_b) + \text{Si}_{2h}]/2$	$r = 0.320, P = .097$	$r = -0.576, P = .0013$
Belfiore $\text{SI}_{\text{gly}_b}$ [13,42]	$2/[(I_{b/N} \times G_{b/N}) + 1]$	$r = 0.317, P = .10$	$r = .064, P = .746$
ISI Stumvoll [43]	$\text{ISI} (\mu\text{mol L kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}) = 0.226 - 0.0032 \text{ BMI} - 0.0000645 I_{120} - 0.0037 G_{90}$	$r = 0.310, P = .11$	$r = -0.303, P = .117$
$1/I_b I_m$	$1/I_b I_m$	$r = 0.292, P = .132$	$r = 0.178, P = .365$
MCR Stumvoll [43]	$\text{MCR (mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}) = 18.8 - 0.271 \text{ BMI} - 0.0052 I_{120} - 0.27 G_{90}$	$r = 0.230, P = .238$	$r = -0.225, P = .250$
Cederholm-Wibell [44]	$M = 79000/(120 \times \text{weight [kg]}) + (0.33 \times [G_b - G_{120}])$	$r = 0.221, P = 0.258$	$r = -0.032, P = .87$
$\text{Cl}_{\text{OGTT}}$ (Mari) [12]	Given by modeling	$r = 0.205, P = .296$	$r = -0.216, P = .556$
Jensen [45]	$\text{SI}_{30} = (I_{30} - I_0)/(G_{30} - G_0)$	$r = 0.141, P = .476$	$r = 0.0139, P = .9439$
Fasting glucose to insulin ratio $G_b/I_b$ [46]	$G_b/I_b$	$r = -0.113, P = .568$	$r = -0.191, P = 0.330$
OGIS (Mari) [12]	Given by modeling	$r = 0.113, P = .568$	$r = -0.116, P = .330$
HOMA-%SI [5]	Given by modeling	$r = 0.052, P = .780$	$r = 0.296, P = .126$

$m = [75000 \text{ mg} + (\text{fasting glucose} - 2\text{-hour glucose}) \times 0.19 \times \text{body weight}]/120 \text{ minutes}$ ;  $w = \text{mean Si}_{2h}/\text{mean Si}_b$ ;  $\text{Si}_b = 10^8/(\text{fasting insulin } [\mu\text{U/mL}] \times \text{fasting glucose } [\text{mg/dL}] \times V_D)$ ;  $\text{Si}_{2h} = 10^8/(2\text{-hour insulin } [\mu\text{U/mL}] \times 2\text{-hour glucose } [\text{mg/dL}] \times V_D)$ , where  $V_D = 150 \text{ mL/kg body weight}$  [40].  $G_b$  indicates baseline glucose value  $G$  (mmol/L);  $I_b$ , baseline insulin value  $I$  ( $\mu\text{U/mL}$ );  $G_m$ , mean of 180-minute glycemia value after glucose charge;  $I_m$ , mean of 180-minute insulin value after glucose charge;  $G_p$ , the high glycemia of the test;  $I_p$ , the highest insulin value during the test; MPG, mean of fasting and 2-hour glucose concentrations (mg/dL); MSI, mean of fasting and 2-hour insulin concentrations (mU/L);  $G_0$ , fasting glucose;  $G_{120}$ , 2-hour glucose;  $G_a$ , glucose area;  $I_a$ , insulin area;  $I_0$ , fasting insulin;  $I_{120}$ , 2-hour insulin;  $I_a$ , insulin area;  $mG_0$ , mean fasting glucose;  $mG_a$ , mean glucose area;  $mI_0$ , mean fasting insulin;  $mI_a$ , mean insulin area; CHO, carbohydrate.



formula  $SI = \text{IMGU}/(G_{200}I_{200}V_D)$ , where  $G_{200}I_{200}$  are blood glucose and serum insulin during IVGTT at values of blood glucose closer to 200 mg/dL, and  $V_D$  the glucose disposition volume assumed here to be 0.16 L/kg of body weight [47].

Accordingly, after calculation of the regression parameters of the index (or of its reciprocal) with SI, we converted all SBT-derived indices into physiologic units of SI similar to those given by the minimal model ( $\text{min}^{-1}/[\mu\text{U/mL}] \times 10^{-4}$ ) and then compared each set of results with IVGTT measurements with Bland-Altman plots as described below.

#### 2.4.3. Measurements of glucose effectiveness

In a preceding study [22], we reported that glucose effectiveness ( $S_g$ ) was well (but nonlinearly) correlated with the difference between glycemia at 60 minutes after the meal and fasting glycemia ( $G_{60} - G_0$ ) and could thus be predicted with the empirical formula  $S_g = 2.921e^{-0.185(G_{60} - G_0)}$ . We thus also included this empirical formula in the validation study.

#### 2.5. Statistics

Results are presented as mean  $\pm$  SEM. To compare all these evaluations of SI, we used 2 methods in addition to the classic correlation analysis with Spearman coefficients. First, a jackknife procedure [41] was used to compare correlation coefficients. We randomly selected 50% of the subjects and calculated the absolute value of the  $r$  coefficient for each SBT index with IVGTT measurement of SI. This procedure was repeated 100 times. The mean values of  $r$  that were obtained were thus compared with a 1-way analysis of variance. Later, the accuracy of each prediction of SI vs its minimal model evaluation was tested on Bland-Altman plots with the software Method Validator (copyright 1997, Ph Marquis, Metz). The Bland-Altman plot consists of an x-axis showing the mean of the results of the 2 methods (assumed true value) and a y-axis, which represents the absolute difference between the 2 methods (evaluation of the measurement error). The plot includes the

line for the mean difference and the experimentally observed  $2\sigma$  limits of the differences between the 2 methods (95% limits of agreement). The mean difference indicates that there is a general trend to overestimate or to underestimate the parameter. The 95% limits of agreement should be interpreted in comparison with a clinically acceptable difference between the 2 methods. If these 95% limits of agreement are clinically acceptable, methods could be used interchangeably [48–50]. Sensitivity of a method for detecting insulin resistance was calculated as the number of truly positive subjects divided by the sum of true positive and false negative, that sum representing the total number of insulin-resistant patients in the sample of subjects. The specificity was calculated as the number of truly negative subjects divided by the sum of false positive and true negative. The positive predictive value was calculated as the number of truly positive subjects divided by the sum of true positive and false positive. The negative predictive value was calculated as the number of truly negative subjects divided by the sum of true negative and false-negative ones. All these 4 indices were expressed as percentages.

### 3. Results

#### 3.1. Comparison of various indices with minimal model SI

Raw correlations of all indices with the value of SI given by the minimal model and its reciprocal ( $1/\text{SI}$ ) are shown in Table 2. Matsuda's [29] composite index given by the formula  $\text{ISI}_{\text{comp}} = 10^4/(I_b G_b I_m G_m)^{0.5}$  gives the highest  $r$  value, but interestingly a simpler anonymous index [22,30] close to it [ $\text{SI} = 10^4/(I_b G_b I_m G_m)$ ] gives an almost similar correlation without the need of calculating a square root. Surprisingly, a very simplistic index based on fasting glucose and insulin such as HOMA-IR or QUICKI, Bennett' [31] BFS ( $\text{BFS} = 1/[\log I_b \times \log G_b]$ ) appears in this study to be also well correlated with SI. Several very simple indices like Sluiter's [32] index "A" =  $10^4/(I_p G_p)$ , SI from Cederholm's [33] index =  $\text{constant}/(G_m \log I_m)$ , the

Table 3  
Prediction of minimal model-derived SI values from the SBT with various formulas

Raw linear correlation (Pearson $r$ coefficient) with SI from IVGTT	Average linear correlation (Pearson $r$ coefficient) after jackknife procedure	Name of the method	Empiric formula for calculating from the original index values of SI expressed in $\text{min}^{-1}/(\mu\text{U/mL}) \times 10^{-4}$	Bland-Altman difference plot	
				Mean difference	95% Limits of agreement
0.656	0.700	Matsuda	$\text{SI} = -1.24 + 65/(I_b G_b I_m G_m)^{-0.5}$	0.0063	−1.43 to 1.44
0.650	0.734	$1/(I_b G_b I_m G_m)$	$\text{SI} = 1.89 + 2690/(I_b G_b I_m G_m)$	0.0047	−1.44 to 1.45
0.620	0.647	Bennett	$\text{SI} = -2.93 + 5.16/(\log I_b \times \log G_b)$	0.0088	−1.52 to 1.54
0.575	0.673	Sluiter	$\text{SI} = 0.2 + 2400/(I_p G_p)$	−0.0091	−1.57 to 1.50
0.547	0.604	Belfiore area	$\text{SI} = -8.54 + 38.4/[(I_a/mI_a + G_a/mG_a) + 1]$	−0.0243	−1.62 to 1.58
0.527	0.575	Cederholm	$\text{SI} = 76/(G_m \log I_m)$	0.0707	−1.61 to 1.60
0.525	0.618	$1/G_b I_m$	$\text{SI} = 0.248 + 0.947/G_b I_m$	0.00145	−1.62 to 1.62
0.454	0.503	OGIS (Mari)	$\text{SI} = \text{OGIS}/I_p$	−0.727	−2.45 to 0.994
0.442	0.441	Caumo	SI given by Caumo's model	0.711	−1.28 to 2.7
0.396	0.464	Soonthornpun	$\text{SI} = 0.626 \times (\text{Soonthornpun's ISI}) + 0.707$	−1.71	−3.61 to 0.063
0.360	0.405	Avignon's $\text{SI}_2$	$\text{SI} = 2.812 + 67 \times 10^5/(I_{120} G_{120} V_D)$	0.0136	−1.76 to 1.79

The accuracy of each formula is checked with Bland-Altman plots.

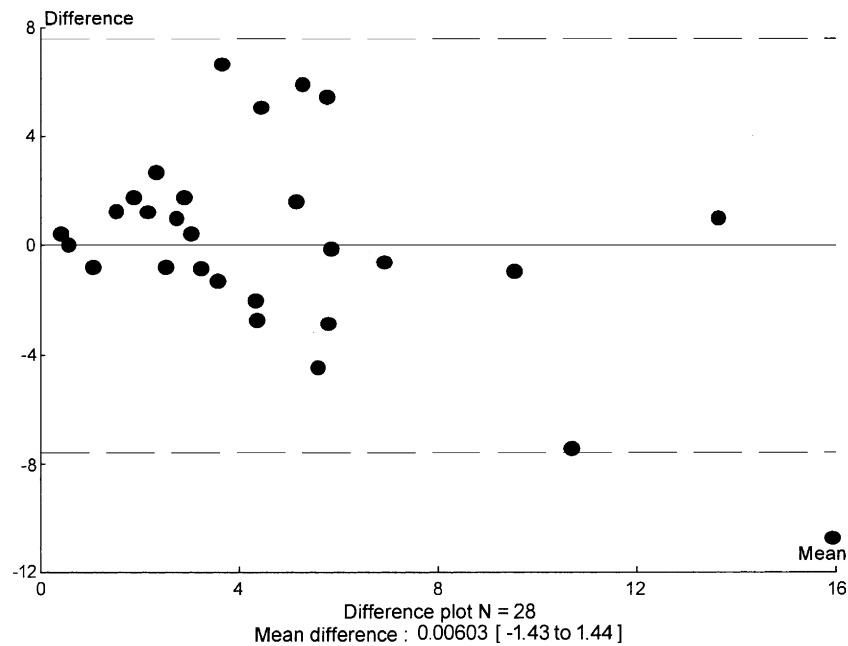


Fig. 1. Bland-Altman plot showing the concordance between SI measured with the minimal model analysis of an IVGTT (reference method) and minimal model analysis of the SBT with an empirical formula using Matsuda's composite index:  $SI = -1.24 + 65/(I_b G_b I_m G_m)^{-0.5}$ .

anonymous ratios  $1/G_b I_m$  and  $1/I_m G_m$ , and Gutt's [34]  $ISI_{0,120} = (m/MPG)/\log MSI$  are also rather well correlated to SI. Another interesting predictor is given by one of Bel-fiore's indices [13], the  $ISI_{gly\_a} = 2/[(I_a/mI_a + G_a/mG_a) + 1]$ , which is based on the ratio between postload areas of glucose and insulin and mean normal values of these areas. As expected, Caumo's minimal model analysis of the SBT is also a good predictor of SI, even if the comparison of raw correlation coefficients does not select it in the first place. Mari's [12] OGIS also gives interesting correlations, but this

issue is more complicated because OGIS results can be expressed as a glucose clearance  $Cl_{OGTT}$  and as an OGIS index, which predicts from this  $Cl_{OGTT}$  the glucose clearance that would be given by a euglycemic clamp. Not surprisingly, the raw linear correlations of both  $Cl_{OGTT}$  and OGIS with SI are poor ( $r = 0.205$ ,  $P = .296$  and  $r = 0.113$ ,  $P = .568$ , respectively; see Table 2). However, these clearances need to be corrected for insulin response to be expressed with units comparable to those of SI. We thus calculated the ratios between both OGIS and  $Cl_{OGTT}$  to  $I_a$ ,

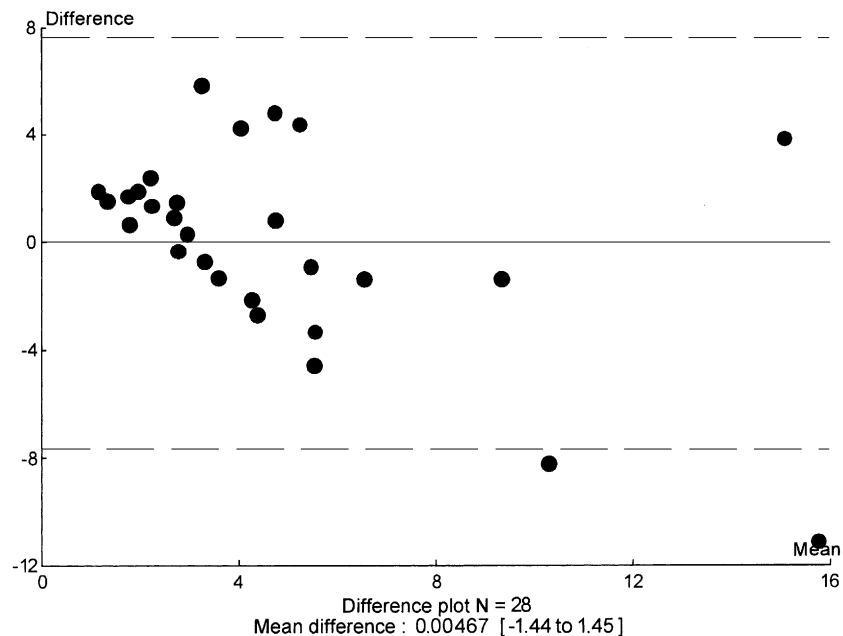


Fig. 2. Bland-Altman plot showing the concordance between SI measured with the minimal model analysis of an IVGTT (reference method) and minimal model analysis of the SBT with the empirical formula  $SI = 1.89 + 2690/(I_b G_b I_m G_m)$ .

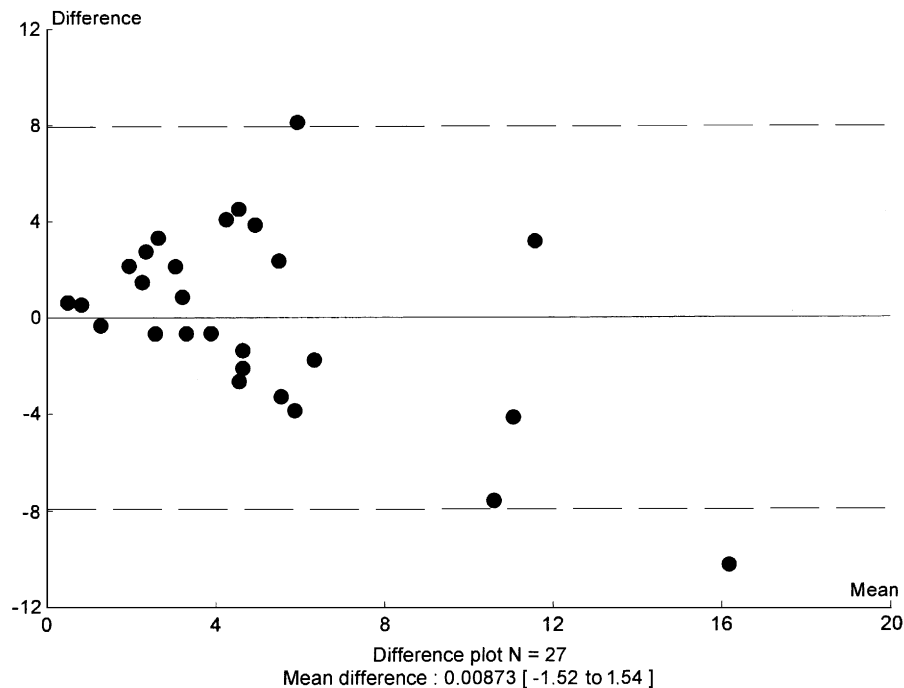


Fig. 3. Bland-Altman plot showing the concordance between SI measured with the minimal model analysis of an IVGTT (reference method) and an empirical formula using Bennett's index:  $SI = -2.93 + 5.16/(\log I_b \times \log G_b)$ .

$I_m$ , and  $I_p$ . For OGIS, the  $r$  coefficients were 0.3696, 0.4394, and 0.4543, respectively, whereas for  $C1_{OGTT}$  they were 0.4394, 0.4411, and 0.458. If  $r$  coefficients are used to evaluate accuracy, the best predictor was corrected  $C1_{OGTT}$  rather than corrected OGIS, and the best way to express insulin response for this correction was  $I_p$  rather than  $I_a$  or  $I_m$ . Other indices gave less interesting correlations.

From these first findings we elaborated predictive equations designed to calculate the SI value that would be given by the IVGTT. These are shown in Table 3. According to the ranking of  $r$  values, Matsuda's composite index, the

index  $1/(I_b G_b I_m G_m)$ , Bennett's index, Sluiter's index, Belfiore area, Cederholm's index,  $1/G_b I_m$ , OGIS (Mari) corrected for insulin peak, and Caumo's minimal model analysis correlate with SI with  $r$  coefficients ranging between 0.656 and 0.442. For Belfiore's index  $\{ISI_{gly-a} = 2/[(I_a/mI_a + G_a/mG_a) + 1]\}$ , 2 values for  $mI_a$  and  $mG_a$ , representing normal postload areas of insulin and glucose, are needed. We used here the mean areas obtained in our experimental sample of 28 patients, that is,  $mI_a = 141$  and  $mG_a = 22.4$ . We also studied formulas derived from Soonthornpun's [38] model and from Avignon's [40] model

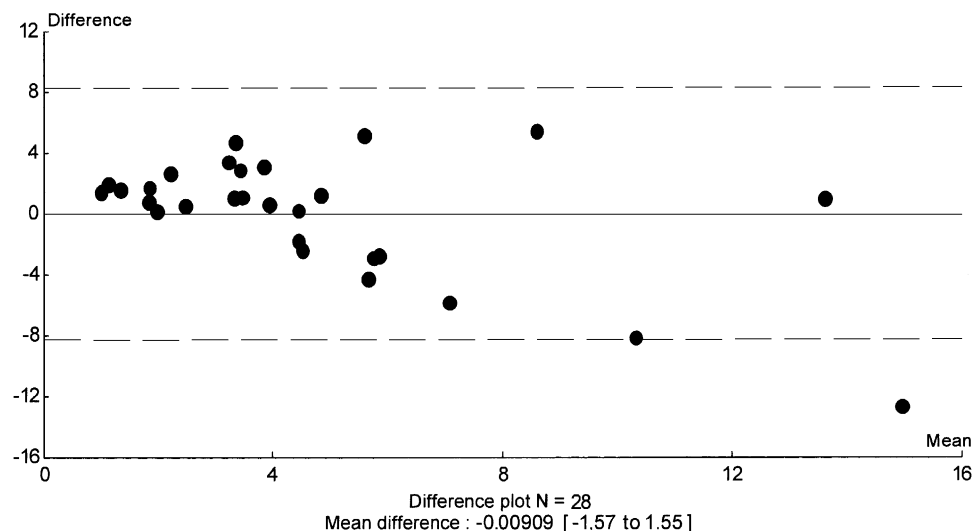


Fig. 4. Bland-Altman plot showing the concordance between SI measured with the minimal model analysis of an IVGTT (reference method) and an empirical formula using Sluiter's index to analyze the SBT:  $SI = 0.2 + 2400/(I_p G_p)$ .

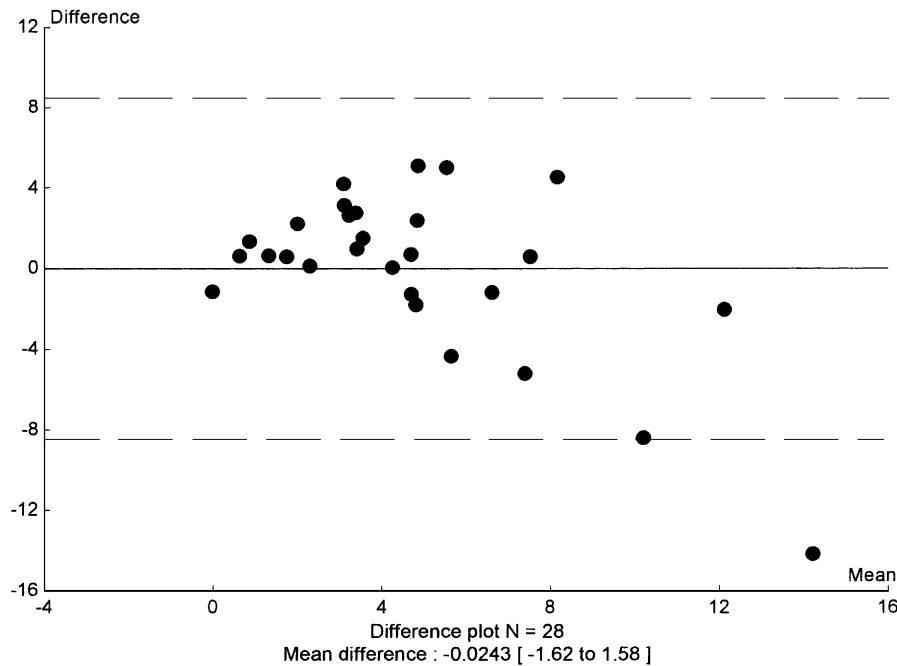


Fig. 5. Bland-Altman plot showing the concordance between SI measured with the minimal model analysis of an IVGTT (reference method) and the analysis of the SBT with Belfiore's index using postload areas under the curve  $\{SI = -8.54 + 38.4/[(I_a/mI_a + G_a/mG_a) + 1]\}$ .

whose  $r$  values are lower, below 0.4. To compare all these evaluations of SI, we used 2 methods. First, a jackknife procedure, as explained above, was used to compare the correlation coefficients. Later, the accuracy of each prediction of SI vs its minimal model evaluation was tested on Bland-Altman plots. The mean  $r$  values given by the jackknife procedure and parameters of the Bland-Altman plots are shown in Table 3. Actually, the jackknife rankings

appear in almost the same order as the raw correlation coefficients, and this calculation is unable to select by ANOVA a method significantly superior to the others. Figs. 1–9 show the Bland-Altman plots for the 9 best indices. On the whole, the 95% limits of agreement seem to be clinically acceptable and discrepancies mostly appear in the upper range of SI, above a value of  $10 \text{ min}^{-1}/(\mu\text{U} \cdot \text{mL}^{-1}) \times 10^{-4}$ , which is approximately the limit of the

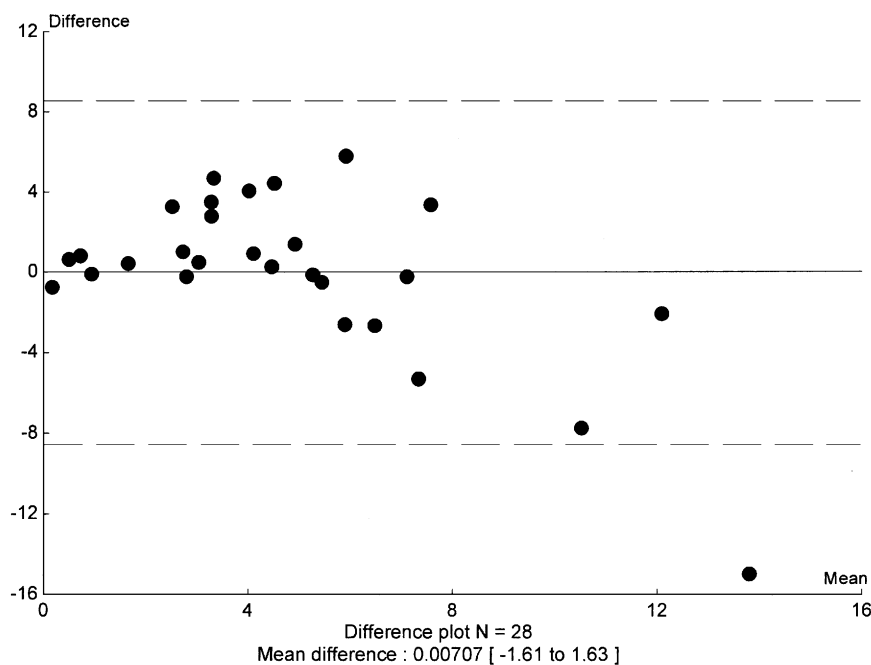


Fig. 6. Bland-Altman plot showing the concordance between SI measured with the minimal model analysis of an IVGTT (reference method) and the analysis of the SBT with an empirical formula adapted from the Cederholm's index:  $SI = 76/(G_m \log I_m)$ .



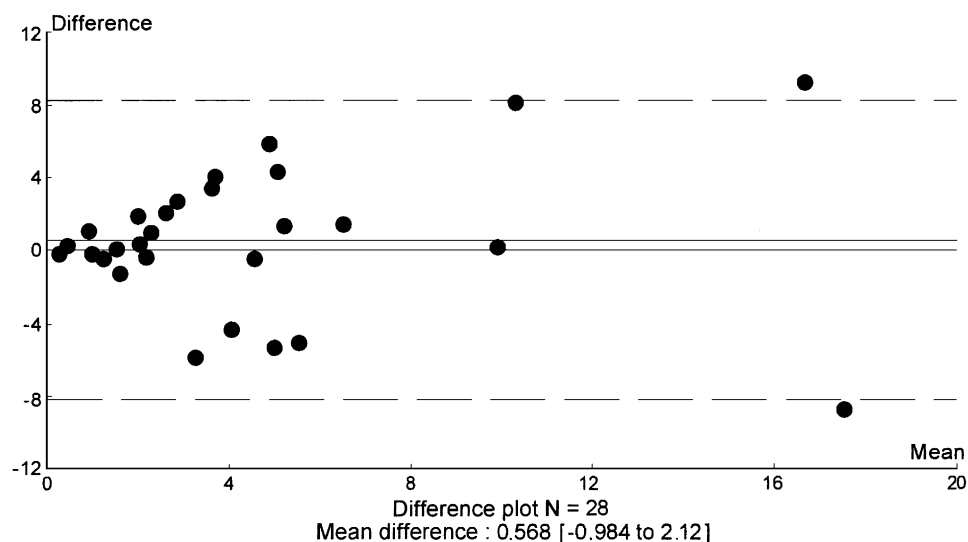


Fig. 7. Bland-Altman plot showing the concordance between SI measured with the minimal model analysis of an IVGTT (reference method) and the analysis of the SBT with an empirical formula  $SI = 0.248 + 0.947/G_b I_m$ , which can in fact be simplified into the approximation  $SI = 1/G_b I_m$ .

upper quartile in our laboratory. This is particularly true for Mari's OGIS (Fig. 8) and Caumo's model (Fig. 9). If only values lower than  $10 \text{ min}^{-1}/(\mu\text{U} \cdot \text{mL}^{-1}) \times 10^{-4}$  are studied, the accuracy of Caumo's model becomes quite fair with a mean difference of 0.685 (95% limits of agreement ranging between  $-0.577$  and  $1.95$ ). By contrast, if the same thing is done for Mari's OGIS (Fig. 8), the mean difference becomes 1.64 and the 95% limits of agreement range between 0.39 and 2.95, so that the accuracy is not improved. Fig. 10 shows the Bland-Altman plot of the prediction of glucose effectiveness with the empirical formula  $S_g = 2.921e^{-0.185(\frac{G}{60} - \frac{G}{b})}$ .

### 3.2. Analysis according to the NCEP-ATPIII score

Patients were classified according to the NCEP-ATPIII score. According to this score, 11 patients were found to

exhibit the metabolic syndrome (score of  $\geq 3$ ; 2 patients with a score of 3, 6 patients with a score of 4, and 3 patients with a score of 5). By contrast, the 17 other patients with a score lower than 3 did not exhibit the syndrome. Four patients had a score equal to 0, 9 had a score equal to 1, and 4 had a score equal to 2. This score was not normally distributed (Kolmogorov-Smirnov test  $P = .0006$ ). It appeared to be negatively correlated with SI (Spearman rank order correlation  $r = -0.61$ ,  $P = .001$ ). The subgroup with a score higher than 3 had a lower SI ( $2.2 \pm 0.106$  vs  $6.24 \pm 0.15$ ,  $P < .001$ ). In the low-score group there were 2 values of SI within the lower quartile ( $<1.1$ ), that is, 11% and 22% ( $n = 4$ ) of values of SI within the upper quartile ( $>9.5$ ). By contrast in the high-score group, there were 4 subjects with low SI, that is, 40%. Surrogates correlate with the score as follows (ranking according to the raw

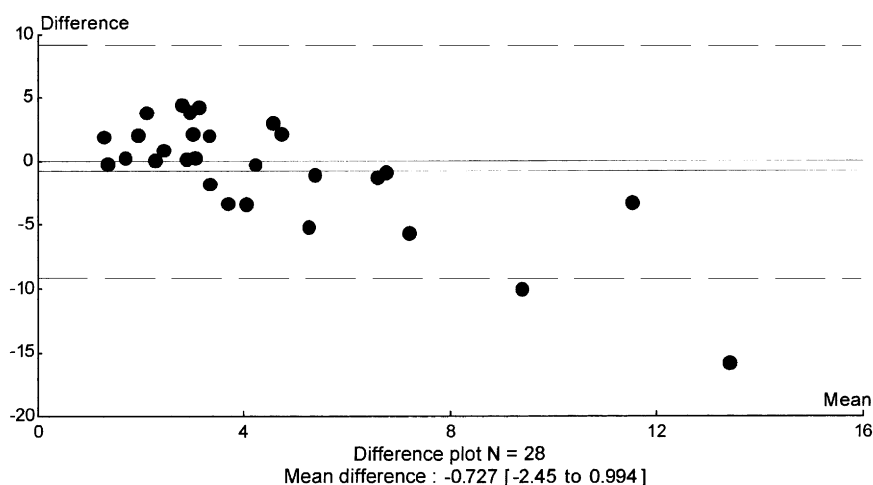


Fig. 8. Bland-Altman plot showing the concordance between SI measured with the minimal model analysis of an IVGTT (reference method) and analysis of the SBT with Mari's OGIS model corrected by insulin peak response ( $SI = OGIS/I_p$ ). There is a trend toward underestimation for high values of SI, and if only values less than  $10 \text{ min}^{-1}/(\mu\text{U} \cdot \text{mL}^{-1}) \times 10^{-4}$  are analyzed, the mean difference becomes 1.64 and the 95% limits of agreement become  $-0.39$  to  $2.95$ .

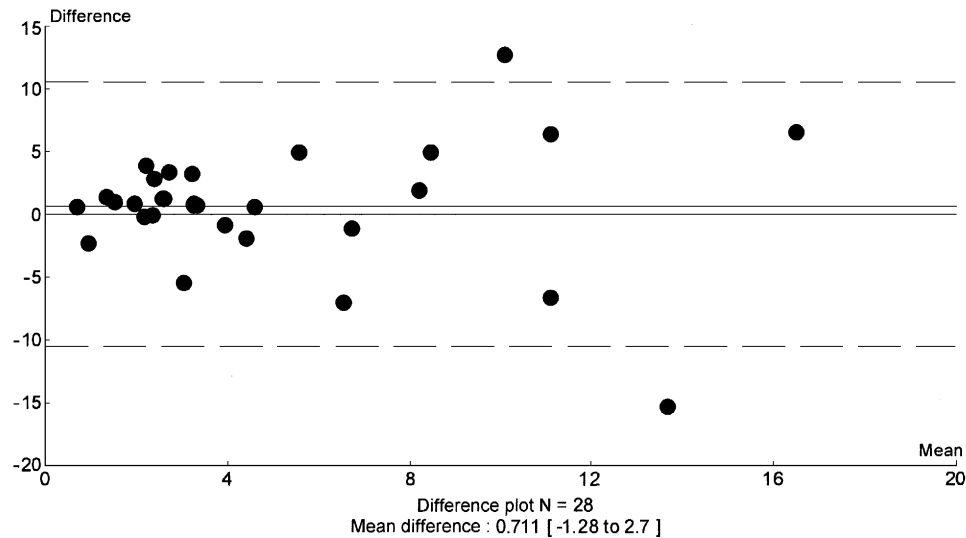


Fig. 9. Bland-Altman plot showing the concordance between SI measured with the minimal model analysis of an IVGTT (reference method) and minimal model analysis of the SBT with Caumo's model (without any correction). Discrepancies exceeding the 95% limits of agreement are shown only for high values of SI ( $>10 \text{ min}^{-1}/\mu\text{U} \cdot \text{mL}^{-1} \times 10^{-4}$ ), and if only values less than  $10 \text{ min}^{-1}/(\mu\text{U} \cdot \text{mL}^{-1}) \times 10^{-4}$  are analyzed, the mean difference becomes 0.685 and the 95% limits of agreement become  $-0.577$  to  $1.95$ .

$r$  value):  $\text{SI} = 40/I_b$  ( $r = -0.738$ ); Cederholm's index ( $r = -0.734$ ); Gutt's index ( $r = -0.691$ ); Belfiore area ( $r = -0.597$ ); Bennett's index ( $r = -0.58900$ ); Sluiter's index ( $r = -0.563$ ); HOMA-IR ( $r = 0.591$ );  $\text{SI} = -1/I_m G_m$  ( $r = 0.53458$ ); Cederholm-Wibell's [44] index ( $r = -0.521$ );  $\text{SI} = 1/G_b I_m$  ( $r = -0.496$ );  $1/\text{QUICKI}$  ( $r = 0.488$ ); reciprocal of Stumvoll's [43] index ( $r = -0.468$ ); Avignon's  $\text{SI}_{2h}$  ( $r = -0.468$ ). Correlations between the NCEP ATPIII score and other indices do not reach significance (QUICKI,  $1/\text{HOMA}$ ;  $G_b/I_b$ ;  $1/I_m$ ;  $I_b/G_b$ , etc).

In the sample of subjects studied here, the sensitivity of the NCEP-ATPIII score for diagnosing an insulin resistance evidenced with IVGTT-derived minimal model (ie,  $\text{SI} < 1.1 \text{ min}^{-1}/[\mu\text{U} \cdot \text{mL}^{-1}] \times 10^{-4}$ ) was 66.7%, the specificity 72.7%, the positive predictive value 40%, and the negative predictive value 88.9%.

The 28 subjects of the study were then divided into 2 subgroups according to the NCEP-ATPIII score. In subjects with a score of 3 or higher (ie, with a diagnosis of metabolic syndrome), surrogates rank as follows (ranking according to the raw  $r$  value):  $1/I_b I_m$  ( $r = 0.689$ ,  $P = .0016$ ); QUICKI ( $r = 0.669$ ); Matsuda ( $r = 0.666$ ); Belfiore's  $\text{ISI}_{\text{gly}_b}$  ( $r = 0.660$ ); Bennett ( $r = 0.659$ ); Avignon's  $\text{SI}_m$  ( $r = 0.655$ );  $\text{SI} = 1/I_b G_m I_m G_m$  ( $r = 0.647$ );  $\log \text{HOMA-IR}$  ( $r = -0.647$ ); HOMA-IR ( $r = -0.577$ );  $G_b/I_b$  ( $r = 0.564$ ); Mari's  $\text{CI}_{\text{OGTT}}/I_p$  ( $r = 0.503$ ). Other indices are not significantly correlated to SI in this subgroup. If subjects with a score of higher than 3 are studied, we find a quite different ranking:  $1/I_m G_m$  ( $r = 0.909$ ); OGIS ( $r = 0.905$ ); Belfiore area ( $r = 0.831$ ); Sluiter ( $r = 0.829$ );  $1/G_b I_m$  ( $r = 0.816$ ); Cederholm ( $r = 0.765$ );  $\text{SI} = 40/I_b$  ( $r = 0.639$ ). Other indices are not significantly

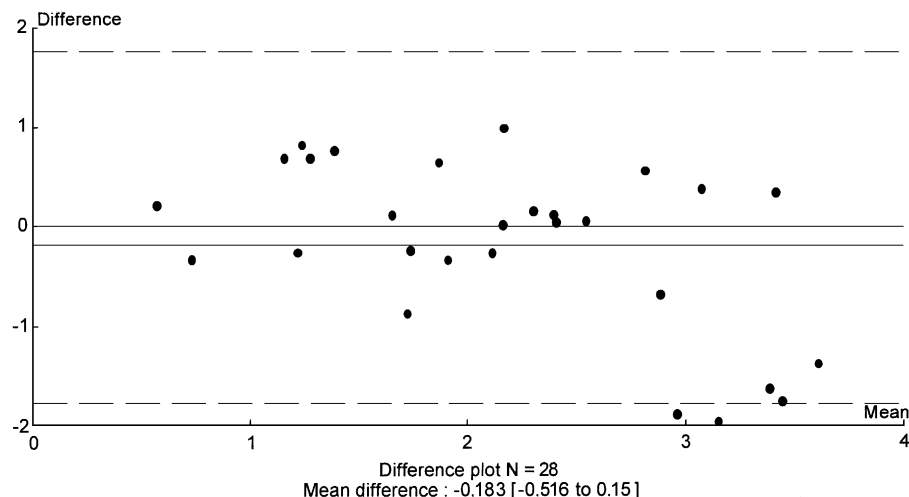


Fig. 10. Bland-Altman plot showing the concordance between Sg measured with the minimal model analysis of an IVGTT (reference method) and Sg obtained from the SBT with the empirical formula  $\text{Sg} = 2.921e^{-0.185(\frac{G}{60} - \frac{G}{b})}$ .

correlated to SI in this subgroup. Below 3, both postload ( $n = 5$ ) and fasting surrogates ( $n = 6$ ) are correlated to SI, whereas above this value only one surrogate ( $SI = 40/I_b$ ) is correlated to SI, other indices being SBT postload ones. The poor  $r$  coefficients in these subgroups for several surrogates selected by the analysis on the whole group are because of the narrow range of SI in the insulin-resistant subgroup, in which SI is low in all but 1 patient. The Bland-Altman plots for these surrogates in subgroups (data not shown) do not modify the overall conclusions presented above, and the 8 procedures found to yield satisfactory predictions of minimal model SI still exhibit a satisfactory reliability when tested with this procedure in subgroups.

Similarly, we classified our patients according to glucose tolerance. It was normal in 19 subjects and pathologic in the 9 others: 5 with type 2 diabetes mellitus, 3 with impaired glucose tolerance (IGT), and 1 with impaired fasting glucose (IFG). Insulin sensitivity was higher in the group with normal glucose tolerance than the group with abnormal glucose tolerance ( $6.09 \pm 1.23$  vs  $2.07 \pm 0.24$ ). As performed above for the NCEP-ATPIII score, we analyzed separately the correlations between SI and SBT-based predictions in each subgroup. In the IGT/diabetes subgroup, no fasting index remained correlated to SI. Only post-SBT ones remained correlated (Sluiter, Belfiore, Cederholm,  $1/G_b I_m$ ,  $1/I_m G_m$ ). Interestingly, in both subgroups, Caumo's index remains satisfactory. This index remains correlated in both the normal glucose tolerance (NGT) ( $r = 0.508$ ) and the IGT/diabetes ( $r = 0.790$ ) subgroup and exhibits on Bland-Altman plots a satisfactory concordance with the minimal model analysis of the IVGTT.

Because the study was not initially designed to study this issue in subgroups, those results are not developed here thoroughly.

#### 4. Discussion

This study shows that there are several accurate methods for evaluating SI from a SBT. Several previously reported empirical formulas designed for evaluating SI from an OGTT can, if one applies appropriate coefficients, correctly predict from the SBT the value of SI that would be given by the minimal model analysis of an IVGTT. As shown on Table 3, Matsuda's composite index, the ratio  $1/(I_b G_b I_m G_m)$ , both Bennett's and Sluiter's indices, Belfiore area, Cederholm's index, the ratio  $1/G_b I_m$ , the OGIS, and Caumo's model are fair predictors of SI.

The goal of this study was to validate simple and reliable procedures for the measurement of SI suitable for clinical research, but also, if necessary, for assessing easily and safely this physiologic function in patient care. One can object that we already have for this purpose the NCEP-ATPIII score and the simple surrogates (HOMA, QUICKI,  $SI = 40/I_b$ , etc) based on fasting insulin and/or glucose. Concerning the NCEP-ATPIII score it is interesting to notice that it is quite well (negatively) correlated to SI, further demonstrating its

clinical relevance. However, this clinical score is by no means a measurement of insulin's effect on glucose disposal and is not designed to replace it. Our calculation of the accuracy of the NCEP-ATPIII score for diagnosing an insulin resistance evidenced with IVGTT-derived minimal model (sensitivity 66.7%, specificity 72.7%, positive predictive value 40%, and negative predictive value 88.9%) shows that there are too much discrepancies and that this score is not a reliable predictor of this biologic variable. An alternative usual tool for this diagnosis is the use of simple surrogates. However, they appear to be valid as far as insulin secretion is able to mirror insulin resistance, whereas they become meaningless when this homeostatic loop is disturbed. Our finding that in the subgroup of subjects with a NCEP score higher than 3, those surrogates are no longer correlated with SI while they are well correlated to it in subjects with a score of less than 3 is in agreement with this statement. Clearly, as demonstrated by a huge body of literature, if one needs an evaluation of SI in subjects whose glucoregulation is likely to be disturbed, surrogates can be misleading and it is better to use a dynamic measurement such as those investigated here.

We chose to investigate the SBT rather than the OGTT for the following reasons. Because recent guidelines [51] state that "the OGTT is not recommended for routine clinical use," the SBT becomes an interesting alternative to the standard OGTT for an in-depth assessment of glucoregulation in specific situations because it gives a more physiologic description of the body's response to an oral carbohydrate load [16], avoiding an important artifact that is the occurrence of frequent postload hypoglycemia, which have no clinical relevance [8,52]. Furthermore, the development of mathematical procedures that can provide during this SBT the same information as given with the IVGTT or the glucose clamp further increases its interest. With such developments, the SBT can represent a simple procedure, less unpleasant for the patient than any other assessment of glucose metabolism (including the standard OGTT), and providing both a physiologic picture of glucoregulation and a sophisticated analysis of it in terms of SI, glucose effectiveness, and insulin secretion.

There are several published protocols for SBTs, and our SBT [4] is slightly different from the one that has been studied and modeled by Caumo [21] and whose accuracy has been recently demonstrated by a validation study against both the IVGTT and the glucose clamp [53]. Our results indicate, as shown above, that the minimal model analysis of this SBT according to Caumo gives without the need of any correction values almost similar to those given by the IVGTT despite a few discrepancies in the higher range of SI. Therefore, slight differences in the meal composition do not seem to impair the validity of this analysis.

In this study we used a sample of subjects designed for representing all the spectrum of SI values and glucose tolerance because, in previous reports, we pointed out that simple indices of SI provided by fasting values of glucose and insulin are accurate only within defined limits of

validity and may be misleading in many conditions that disturb the feedback loop linking SI to insulin release. Both diabetic subjects and subjects with high values of SI are included in our sample of subjects, so that our conclusions are likely to be valid for those populations also. By contrast, a specific study remains to be done in adolescents [11]. In addition, although the full range of SI values is covered by the current study, our subjects are all overweight in terms of BMI because the BMIs of subjects range between 25.1 to 50.1 kg/m<sup>2</sup>. Therefore, it will be probably useful to extend this validation study to subjects whose BMI is lower than 25 kg/m<sup>2</sup> to fully ascertain that our conclusions remain true in that range of BMI.

To select the best method, we tried to rank them with several procedures, but this ranking should be considered with caution. It is clear that raw *r* values can be misleading and are surely not the best way to compare 2 separate measurements of the same parameter [48]. Thus, we added to this approach 2 other procedures. The jackknife procedure aims at predicting the *r* value that would be obtained on a more general population, by modifying randomly a large number of times the composition of the sample. As shown above, the jackknife does not markedly alter the ranking of the methods provided by the raw *r* coefficients, suggesting that this simple procedure did not markedly bias the classification. More interestingly, the Bland-Altman plot, which has been designed to compare methods and to avoid the pitfalls of classic correlation analysis [50,53], indicates a satisfactory concordance between these formulas and the IVGTT results because the mean difference is always close to 0 and the 95% limits of agreement are lower than  $2 \text{ min}^{-1}/(\mu\text{U} \cdot \text{mL}^{-1}) \times 10^{-4}$ , keeping in mind that the reproducibility of the minimal model analysis of the IVGTT in subjects tested twice is 30% [54]. Thus, those 95% limits of agreement are lower than the interday variability and seem to be quite acceptable.

If study subjects are divided into 2 subgroups, most correlations lose their significance because of the low number of subjects. In addition, SI varies in a narrow range in the subgroup with a NCEP-ATPIII score higher than 3 and behaves rather as a constant than as a variable, thus impeding the relevance of the correlation analysis. Actually, when tested with the Bland-Altman procedure, the surrogates selected with the overall analysis still appear to predict satisfactorily SI according to the criteria discussed above. Thus, this separate analysis does not modify our overall conclusions about the best indices that can be derived from the SBT.

By contrast, it is interesting to notice that in subjects with a NCEP-ATPIII score lower than 3 (ie, not insulin-resistant), both fasting and postload measurements are correlated to SI, whereas in subjects with a score of 3 or more, only postload measurements remain correlated to SI. As already discussed above, this finding further supports our purpose to develop indices based on postload measurements for patients whose glucoregulation may be disturbed. On the whole, the results

of this study are in agreement with the assumption that the indices selected by our analysis are valid within all the range of SI, regardless of the glucoregulatory status of patients. Only situations of profound insulinopenia such as type 1 diabetes mellitus may be inappropriate for the use of the SBT because a bolus of insulin would be needed, thus implying a specific protocol that has not yet been tested. Because the SBT procedure makes no assumption about the relationship between insulin release and SI, but does only analyze the actual postload kinetics of blood glucose and insulin, it is likely that in the situations where fasting surrogates are no longer valid (overt type 2 diabetes mellitus, reactive hypoglycemia, athletes, puberty, etc), the SBT could be a reliable assessment of SI. However, further specific analyses in such subgroups will be needed to ascertain this hypothesis.

Actually, the choice of the “best” solution for calculating SI during an SBT depends on several criteria. Elaborated models such as that of Caumo or Mari have the advantage of relying on a robust physiologic theoretical background. However, the best raw *r* coefficients (as well as jackknife *r* coefficients) are obtained in this sample for simpler approaches. The best ranked appears to be Matsuda’s composite index, which can thus be converted into physiologic units of SI by the means of a simple empirical formula. The simplicity of such an approach makes it very attractive. Interestingly, an even simpler “composite index” without square root,  $\text{SI} = 1.89 + 2690/(I_b G_b I_m G_m)$ , is as efficient as Matsuda’s formula to predict SI. Three other very simple formulas also yield a good agreement with the IVGTT: Sluiter’s index [ $\text{SI} = 0.2 + 2400/(I_p G_p)$ ], Cederholm’s index [ $\text{SI} = 76/(G_m \log I_m)$ ], and the anonymous formula  $\text{SI} = 0.248 + 0.947/G_b I_m$ , which can be in fact simplified by the approximation  $\text{SI} = 1/G_b I_m$ . Because of their simplicity, these methods are attractive.

Surprisingly, Bennett’s index [31] gives also in this study a fair prediction of SI [ $\text{SI} = -2.93 + 5.16/(\log I_b \times \log G_b)$ ], although this method uses only baseline insulin or glucose and is thus based on the same concepts than the HOMA-IR or the QUICKI. Actually, the good correlation of this index with SI is explained by its proportionality to SI in healthy subjects, whereas this proportionality is no longer found in subjects with a NCEP-ATPIII score of higher than 3, that is, patients in whom insulin resistance is likely to be found. This seems to indicate that this method suffers the same limits of validity than those surrogates, as previously discussed.

The more sophisticated calculations provided by Belfiore’s model, Mari’s OGIS, Caumo’s minimal model approach, and Soonthornpun’s formula require some comments. Although they do not appear first in the ranking with raw *r* coefficients, they have the advantage of being underlain by physiologic concepts and thus may be expected to be more robust than purely empirical approaches. However, each one has its specificity. Belfiore’s formula, which is the simpler of all these elaborated models, gives a good prediction of SI during the SBT with the



formula  $SI = -8.54 + 38.4/[(I_a/mI_a + G_a/mG_a) + 1]$ . This formula needs to include 2 parameters  $mI_a$  and  $mG_a$  that are the mean areas of postload insulin and glucose of a control population. In our study we simply used the mean values of all study subjects.

The calculation of OGIS [12] yields 2 indices of SI: the  $Cl_{OGTT}$ , which represents a model-derived calculation of glucose clearance during the OGTT, and OGIS, which predicts from  $Cl_{OGTT}$  the glucose clearance that would be given by a euglycemic clamp. Validity of OGIS has been demonstrated on a large population [15]. Not surprisingly, the raw linear correlations of both  $Cl_{OGTT}$  and OGIS with SI are poor (see Table 2) because SI and these clearances have not the same dimension. If these clearances are corrected for insulin response to be expressed in SI units, OGIS becomes a fair predictor of the value of SI that would be calculated during an IVGTT. Interestingly, this calculation is extremely simple because the best insulin value for this purpose appears to the maximal insulin value during the test ( $I_p$ ) rather than  $I_a$  or  $I_m$ , and the formula is very simple:  $SI = OGIS/I_p$ .

Concerning Caumo's model, it is surely the most sophisticated of those studied here, but can also be calculated on a simple Microsoft Excel workbook. Its apparently lower accuracy compared with Matsuda,  $1/(I_b G_b I_m G_m)$ , Bennett, Sluiter, Belfiore area, Cederholm,  $1/G_b I_m$ , and OGIS is actually due, as indicated above, to discrepancies in the upper range of the spectrum of SI values. If only values of SI below  $10 \text{ min}^{-1}/(\mu\text{U} \cdot \text{mL}^{-1}) \times 10^{-4}$  are considered, the accuracy of the method becomes quite satisfactory. Actually, in the recently published evaluation of this method, Steil et al [55] points out that values of SI given by this approach and those given by the minimal model analysis of the IVGTT are not similar because SBT gives values of SI 2-fold higher than IVGTT. In our study, this difference is not so important. If only values of SI lower than 10 are compared, SI values appear to be almost equivalent with both methods, without any correction. Possible explanations for this better concordance between IVGTT and SBT-derived values of SI are (a) a slight difference in the breakfast composition; (b) the fact that we only used the periods 0 to 180 minutes whereas Steil used 0 to 240 minutes; (c) the fact that Steil's study included apparently more subjects with very low values of SI (so called "SI zero values" [56]) that are apparently found only during the IVGTT and not during the SBT, at least in the sample studied by this author [55]. However, some more studies on this attractive analysis of the SBT are probably needed, as also indicated by Steil [55].

Finally, 2 well-known methods for calculating SI from the OGTT seem to give slightly less satisfactory results in the case of the SBT: Soonthornpun's formula and Avignon's  $SI_2$  index. The latter is not well correlated with IVGTT results, but the empirical formula converting it into SI units provides a fair prediction of IVGTT-derived SI when evaluated on Bland-Airman plots. These methods have both

been reported to work well with the OGTT. However, we think that, because of the good accuracy of the 8 others, our study does not give a strong support their choice for the calculation of SI during an SBT.

We also verified in this study the accuracy of a predictive formula for glucose effectiveness ( $S_g$ ) that we previously reported [22]. This formula was developed after we noticed that the rise in blood glucose during the first hour was correlated with  $S_g$ , this correlation exhibiting the shape of a negative exponential. Thus, empirically, we obtained the formula  $S_g = 2.921e^{-0.185(G_{60} - G_b)}$ . In this sample of subjects, it seems to provide a satisfactory evaluation of  $S_g$  as indicated by the Bland-Altman plot. However, further specific studies on this prediction of  $S_g$  during an SBT are probably required because this is only an empirical prediction with little theoretical background to support it.

In conclusion, this study indicates that 8 procedures can be used for calculating SI during an SBT interchangeably with the minimal model analysis of an IVGTT. All can be easily calculated on a desktop by implementing a set of formulas on a workbook. Two of these approaches (Caumo's model and Mari's OGIS) are based on sophisticated modeling, and the 6 others are extremely simple formulas using postload insulin and glucose. Four of those are adaptations with appropriate coefficients of already published formulas (Matsuda, Sluiter, Belfiore area, and Cederholm), but 2 other anonymous formulas give also good results:  $1/(I_b G_b I_m G_m)$  and  $1/G_b I_m$ . The simplest indices based on  $I_b$  and  $G_b$  are less accurate. Finally, the SBT can also provide an evaluation of glucose effectiveness with the formula  $S_g = 2.921e^{-0.185(G_{60} - G_b)}$ .

On the whole, the SBT appears to represent a global evaluation of glucoregulation, providing both a physiologic description of the body's response to a carbohydrate load (avoiding some artifacts of the standard OGTT such as the reactive hypoglycemia) and a precise analysis of this glucoregulation in terms of SI, insulin secretion, and glucose effectiveness. However, methodological studies on this latter aspect of SBTs remain scarce, and more research is probably needed in this field before this test could be considered as an equivalent of the gold standard glucose clamp or IVGTT. Nevertheless, our study, put together with several previous ones [12-15,21,29,32-34,38-44,55], shows that OGTT or SBTs give more reliable measurements of SI than indices based on fasting glucose and insulin and that they should probably be recommended for assessing SI in the numerous situations [7] where these indices lose their accuracy.

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This article is dedicated to the beloved memory of our late master André Orsetti (1936-1997) who has been fascinated during all his life by the perspective of a comprehensive assessment of glucoregulation by the means of a physiologic standardized breakfast test.



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