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Metabolism Clinical and Experimental 55 (2006) 143–150

Metabolism
Clinical and Experimental

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Insulin resistance in hypertension quantified by oral glucose tolerance test: comparison of methods

Francesco Di Nardo[a], Fabrizio Casagrande[a], Massimo Boemi[b], Paolo Fumelli[b], Pierpaolo Morosini[c], Roberto Burattini[a,*]

[a]*Department of Electromagnetics and Bioengineering, Polytechnic University of Marche, 60131 Ancona, Italy*
[b]*Metabolic Disease and Diabetes Unit, INRCA-IRCCS, 60131 Ancona, Italy*
[c]*Unit of Internal Medicine, "C. G. Mazzoni" General Hospital, 63100 Ascoli Piceno, Italy*

Received 1 February 2005; accepted 29 August 2005

Abstract

Four methods reported in the literature for evaluation of insulin sensitivity indexes from oral glucose tolerance tests (OGTTs) were analyzed and compared in order to test their ability to discriminate the insulin-resistant state in hypertension. To this aim, 15 normoglycemic subjects, not affected by metabolic syndrome, underwent a 22-sample, 300-minute OGTT. Eight subjects were normotensive (mean age, 47.0 ± 4.2 years) and 7 were hypertensive (mean age, 53.6 ± 1.6 years). The following insulin sensitivity indexes were computed and compared: (*a*) 2 indexes, $IS_{IE22/300}$ and $IS_{IE8/180}$, provided by an integral equation (IE) method applied to the full OGTT and to a reduced 8-sample, 180-minute data subset, respectively; (*b*) 2 indexes, $IS_{OGIS180}$ and $IS_{OGIS120}$, computed by the oral glucose, insulin sensitivity (OGIS) method, which only requires 3 blood samples taken within 180 and 120 minutes, respectively; (*c*) an index, IS_{ISI}, which considers fasting and mean insulinemia and glycemia measured during a 5-sample, 120-minute OGTT; and (*d*) an index, IS_{MCR}, which considers body mass index and requires 2 blood samples taken within 120 minutes. Except the $IS_{OGIS180}$, all other indexes were able to detect a significant reduction (unpaired Student *t* test, $P < .05$) of insulin sensitivity in our hypertensive group compared with the normotensive group. Failure of $IS_{OGIS180}$ was explained by the fact that this index did not capture the information portrayed by the peak of insulinemia in hypertensive patients, which occurred around the 90th minute. Intraclass correlation coefficients higher than 0.89 demonstrated a substantial agreement between $IS_{IE22/300}$ and $IS_{IE8/180}$ indexes. These are the only indexes characterized by units of measure consistent with the definition of insulin sensitivity as the ability of insulin to enhance glucose effectiveness.

1. Introduction

Insulin resistance has been increasingly recognized as a component of the hypertensive state. Knowledge of the etiology of insulin resistance and its link with hypertension, however, is far from complete [1-14]. An improvement of knowledge can be determined by clinical applications of methods that allow quantification of indexes of insulin sensitivity in hypertensive patients to be compared with normotensive subjects. Reliability of euglycemic-hyperinsulinemic clamp technique and minimal model analysis of frequently sampled intravenous glucose tolerance test (FSIGTT) data [15-17] is widely accepted. Based on these methods, a significant reduction of insulin sensitivity was observed in previous studies, including our own, in young [1], middle-aged [8], and elderly [7,14] nonobese and nondiabetic hypertensive patients compared with age-matched groups of control subjects. Unfortunately, the applicability of these methods for clinical practice and epidemiological purposes is hampered by the time, cost, invasiveness, and skill required.

Over the last decade, easier methods have been developed to overcome this difficulty, which produce indexes based on various weighted combinations of glucose and insulin at fasting [18-21] or during oral glucose tolerance test (OGTT) or meal glucose tolerance test (MGTT) [22-29]. Indexes relying upon single fasting blood samples, such as those derived from homeostasis model assessment (HOMA), quantitative insulin sensitivity check index (QUICKI), and its revised version (R-QUICKI), are attractive for their simplicity [18-21]. However, it has been

* Corresponding author. Fax: +39 071 220 4224.
E-mail address: r.burattini@univpm.it (R. Burattini).

0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.08.006

shown [30] that these are relatively crude methods for the quantification of insulin resistance in comparison to the minimal model analysis of FSIGTT data and are therefore of limited value for the assessment of the metabolic status of an individual patient. Indexes derived from OGTT or MGTT are better suited for epidemiological studies. In subjects presenting varying degrees of glucose tolerance, Matsuda and DeFronzo [22] found that an index that considers insulin and glucose concentration data both in the basal state (fasting glucose and fasting insulin concentrations) and after the ingestion of a glucose load (mean glucose and mean insulin concentrations during an OGTT) provides reasonable approximation of whole-body insulin sensitivity (this commonly referred to as insulin sensitivity index, or ISI, method). A different empirical method is based on the assessment of insulin sensitivity as the metabolic clearance rate (MCR) of glucose (MCR method) approximated by an empirical equation that accounts for body mass index (BMI) and insulinemia and glycemia data measured during an OGTT [24]. In contrast to these empirical approaches, alternative methods such as the oral glucose, insulin sensitivity (OGIS) method [25] and the integral equation (IE) method [23] have been proposed with indexes of insulin sensitivity from model-derived formulas.

A comparative analysis of ISI, MCR, OGIS, and IE methods was performed in the present study in order to test the ability of related indexes of insulin sensitivity to discriminate the insulin-resistant state in nondiabetic hypertensive patients, who are not even affected by metabolic syndrome (MS) [31].

2. Materials and methods

2.1. Patients

This study included 15 volunteer subjects of white ethnicity who were recruited at the Metabolic Disease and Diabetes Unit of the INRCA-IRCCS, Ancona, Italy, and the Unit of Internal Medicine, "C. G. Mazzoni" General Hospital, Ascoli Piceno, Italy. Subjects were divided into 2 groups: a group of 8 normotensive subjects (N group) and a group of 7 hypertensive patients (H group). Mean age and male-female ratio for each group are given in Table 1 together with clinical data.

Special care was exercised in recruiting subjects to avoid MS as a confounder in evaluating alterations of insulin sensitivity in hypertension. The MS was defined according to the Adult Treatment Panel III (ATP III) criteria [31], that is, presence of 3 or more of the following criteria: fasting glucose of 6.1 mmol/L or higher (110 mg/dL); waist circumference of more than 102 cm in men and more than 88 cm in women; triglycerides of 1.695 mmol/L or higher (150 mg/dL); high-density lipoprotein cholesterol of less than 1.036 mmol/L (40 mg/dL) in men and less than 1.295 mmol/L (50 mg/dL) in women; and blood pressure of 130/85 mm Hg or higher. Subjects were excluded from participation if they had a history of diabetes mellitus and had a fasting glycemia of 110 mg/dL or higher. Subjects included in the N group had seated diastolic and systolic blood pressure levels of 85 mm Hg or less, and 130 mm Hg or less, respectively, and showed no more than 2 of the remaining 3 ATP III criteria. Besides hypertension, the normoglycemic patients included in the H group showed the presence of no more than one of the remaining three ATP III criteria.

Table 1
Characteristics of normotensive (N group) and hypertensive (H group) subjects in basal state

Variable	N group (n = 8)	H group (n = 7)
Male/female	4/4	4/3
Age (y)	47.0 ± 4.2	53.6 ± 1.6
SBP (mm Hg)	125 ± 2	136 ± 4
DBP (mm Hg)	78 ± 2	91 ± 2*
BMI (kg/m^2)	24.9 ± 1.1	25.4 ± 0.9
Waist circumference (cm)	88.1 ± 4.1	87.6 ± 5.3
Triglycerides (mg/dL)	75 ± 17	156 ± 21*
Cholesterol (mg/dL)	193 ± 7	215 ± 11
High-density lipoprotein cholesterol (mg/dL)	54.0 ± 4.8	54.1 ± 4.7
Glycemia (mg/dL)	80.6 ± 3.9	91.0 ± 4.2
Insulinemia (μU/mL)	5.3 ± 0.6	10.4 ± 1.2*

Values are means ± SE. BMI is defined as the ratio of body weight to the square of height.

* The H group showed a significant ($P < .05$) increase in DBP (Wilcoxon rank sum test) as well as in triglycerides and insulinemia (unpaired Student t test). SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

All hypertensive patients were under antihypertensive drug therapy with calcium channel blockers or angiotensin-converting enzyme inhibitors for more than 1 year. Based on previous studies, this antihypertensive drug therapy appears metabolically neutral or to induce a small improvement in insulin sensitivity only in the absence of familial predisposition to hypertension [2,8,9,11,32-34]. A previous application of minimal model and FSIGTT in hypertensive patients, before and after antihypertensive treatment, showed no significant change in glucose effectiveness (GE) [8]. Based on these previous reports, risks of suspension of therapy were avoided in this study.

2.2. Oral glucose tolerance test protocol

Each subject underwent an OGTT. All gave informed consent to the procedures approved by the Ethics Committee. Starting time was 8:30 AM after overnight fast. A 75-g glucose load was administered, and 22 blood samples were taken: one fasting blood sample was taken immediately before ($t = 0$) glucose administration. Twenty-one additional samples were taken at minutes 10, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 255, 270, 285, and 300 after glucose administration [26].

Blood was promptly centrifuged, and glycemia was immediately measured by the glucose oxidase method with an automated glucose analyzer. The remaining plasma was

stored at −20°C for later insulin dosage. Insulinemia was determined by commercially available radioimmunoassays (Biodata, Guidonia Montecelio, Rome, Italy). The sensitivity and intra- and interassay precision of the insulin were 1 μU/mL, 5.4% ± 1.0%, and 5.5% ± 1.2%, respectively. The cross-reactivity for human proinsulin was 14%.

2.3. Methods for quantification of insulin sensitivity

2.3.1. Integral equation method

For the measurement of insulin sensitivity from oral glucose tests, the classic minimal model of glucose kinetics was coupled by Caumo et al [23] with an equation describing the rate of appearance of glucose into the systemic circulation after glucose ingestion. This approach yielded the following expression for an index, IS_{IE} [23]:

$$IS_{IE} = \frac{f \cdot \frac{D}{W} \cdot \frac{AUC[\Delta G(t)/G(t)]}{AUC[\Delta G(t)]} - GE \cdot AUC[\Delta G(t)/G(t)]}{AUC[\Delta I(t)]} \quad (1)$$

In this equation, AUC denotes the area under the curves of glycemia, $G(t)$ (mg/dL), and insulinemia, $I(t)$ (μU/mL), in the time frame from $t = 0$ to the end of test ($t = T$); $\Delta G(t)$ and $\Delta I(t)$ are glycemia and insulinemia above basal, respectively; GE is the glucose effectiveness ($min^{-1} \cdot dL \cdot kg^{-1}$), given by the product of fractional GE, S_G (min^{-1}), and glucose distribution volume, V (dL/kg); D/W is the dose of ingested glucose per unit of body weight (mg/kg); f is the fraction of ingested glucose that actually appears in the systemic circulation (ie, survives gastrointestinal absorption and one-pass hepatic uptake). The unit of measure for IS_{IE} is $min^{-1} \cdot dL \cdot kg^{-1}/(\mu U/mL)$.

When glycemia falls below the basal level during the test, a modified version of Eq 1 is needed [23].

$$IS_{IE} = \frac{f \cdot \frac{D}{W} \cdot \frac{AUC[\Delta G(t)/G(t)]_0^{t_0} - AUC[\Delta G(t)/G(t)]_{t_0}^{T}}{AUC[\Delta G(t)]_0^{t_0} - AUC[\Delta G(t)]_{t_0}^{T}} - GE \cdot AUC[\Delta G(t)/G(t)]}{AUC[\Delta I(t)]} \quad (2)$$

The AUC of $\Delta G(t)/G(t)$ and $\Delta G(t)$ are separately calculated in the intervals $0 - t_0$ and $t_0 - T$. The negative AUC calculated in the interval $t_0 - T$ is subtracted from the positive AUC calculated in the interval $0 - t_0$.

Population values of f and GE are to be filled into Eqs 1 and 2 to calculate IS_{IE} [23]. In accordance with estimates reported by others from model-independent, dual-tracer OGTT studies (Table 4.1 in Reference [35]), computations in our N group were performed after assuming $f = 0.87$ and $GE = 3.7 \times 10^{-2}$ $min^{-1} \cdot dL \cdot kg^{-1}$ (this was obtained from the product of $S_G = 0.028$ min^{-1} and $V = 1.34$ dL/kg). When insulin sensitivity of our H group was computed, the question arose as to whether it was worth to use, for this group, the same values of f and GE as those assumed for the N group. With respect to the choice of GE, we showed recently that, in the absence of MS, age is a primary predictor of deterioration in GE, independent of hypertension [14]. Based on this finding, and taking into account that age was not significantly different between our N and H groups, we assumed the same value of GE for both groups. With respect to the choice of f, we used for the H group the same value (0.87) used for the N group, thus making the assumption of no alteration of gastrointestinal function in hypertension.

In accordance with Breda et al [26], all 22 blood samples of our 300-minute OGTT were used to compute insulin sensitivity from Eqs 1 and 2. This protocol was denoted as the $OGTT_{22/300}$ and the related index was denoted as $IS_{IE22/300}$.

Eqs 1 and 2 were applied to an 8-sample, 180-minute reduced data subset with blood samples taken at minutes 0, 10, 20, 30, 60, 90, 120, and 180 ($OGTT_{8/180}$) as proposed in Reference [35] to test the reproducibility of insulin sensitivity estimates provided by the IE method from a reduced OGTT protocol (more suitable for population studies). The index of insulin sensitivity obtained from this $OGTT_{8/180}$ protocol was denoted $IS_{IE8/180}$.

2.3.2. Oral glucose, insulin sensitivity method

This is an alternative model-based method, which was derived from the glucose clamp [25]. It provides an index, $IS_{OGIS180}$ (the subscript 180 refers to the time length of the oral protocol), which is computed from only 3 measurements of glycemia and insulinemia data according to the following equations [25]

$$IS_{OGIS180} = \frac{1}{2}\left[B + \sqrt{B^2 + 4p_5p_6(G_{120} - G_{CLAMP})CL_{OGTT}}\right] \quad (3)$$

where CL_{OGTT} and B are expressed as:

$$CL_{OGTT} = p_4 \frac{\frac{p_1D_0 - V\frac{[G_{180} - G_{120}]}{60}}{G_{120}} + \frac{p_3}{G_0}}{I_{120} - I_0 + p_2} \quad (4)$$

$$B = [p_5(G_{120} - G_{CLAMP}) + 1]CL_{OGTT} \quad (5)$$

In these equations, G and I are glucose and insulin concentrations, respectively, with subscripts representing sampling time instants (minutes 0, 120, and 180). D_0 is the oral glucose dose per unit of body surface area (g/m^2). V represents the total glucose distribution volume (mL/kg). G_{CLAMP} is the clamp glucose concentration (normally, 90 mg/dL). The values for the parameters p_1, p_2, p_3, p_4, p_5, and p_6 are reported in Table 2.

According to Mari et al [25], Eqs 3-5, which were set up for a 180-minute oral protocol, can be adapted to a 120-minute protocol by replacing G_{180}, G_{120}, and I_{120} with G_{120}, G_{90}, and I_{90}, respectively. The related index, $IS_{OGIS120}$, was also evaluated in the present study. The unit of measure for both $IS_{OGIS180}$ and $IS_{OGIS120}$ is $mL \cdot min^{-1} \cdot m^{-2}$.

The procedure for computation of both $IS_{OGIS180}$ and $IS_{OGIS120}$ was downloaded from the Web page http://www.ladseb.pd.cnr.it/bioing/ogis/home.html.

Table 2
Parameters of the equations that allow calculation of $IS_{OGIS180}$ and $IS_{OGIS120}$ indexes

Parameters	$IS_{OGIS180}$	$IS_{OGIS120}$
p_1 (min^{-1})	289	650
p_2 (μU/mL)	270	325
p_3 (10^3 mg · min^{-1} · m^{-2})	14.0	81.3
p_4 (μU/mL)	440	132
p_5 (10^{-6} mL/mg)	637	652
p_6 (mL · min^{-1} · m^{-2})	117	173

p_1, p_2, p_3, p_4, p_5, and p_6 are the parameters that appear in Eqs 3-5 of the OGIS method. Values are taken from Mari et al [25].

2.3.3. Insulin sensitivity index method

This is an empirical method (denoted as ISI) that produces an index, IS_{ISI}, calculated by means of the following equation [22]

$$IS_{ISI} = \frac{10^4}{\sqrt{G_0 I_0 G_m I_m}} \quad (6)$$

where G_0 and I_0 are fasting glycemia and insulinemia ($t = 0$). G_m and I_m are mean glycemia and insulinemia taken during an OGTT. Although this formula can be used with any OGTT, it was validated [22] with a 0-, 30-, 60-, 90-, and 120-minute protocol. This sampling schedule was considered in the present study. The unit of measure of insulinemia and glycemia has not been considered relevant in the original article because they only influence the scale of the IS_{ISI} index, and a factor 10^4 was introduced to correct the scale. If this factor (whose units of measure are not clearly defined in the literature) is assumed as dimensionless, and units of measure of insulinemia and glycemia are μU/mL and mg/dL, respectively, the IS_{ISI} is expressed as dL · mg^{-1} · μU^{-1} · mL.

2.3.4. Metabolic clearance rate method

The MCR is an empirical method that provides an index, IS_{MCR}, defined as [24]:

$$IS_{MCR} = 18.8 - 0.271BMI - 0.0052I_{120} - 0.27G_{90} \quad (7)$$

where BMI (kg/m^2) is the body mass index, I_{120} is insulinemia (pmol/L) at 120th minute, and G_{90} is glycemia (mmol/L) at 90th minute. This equation was assessed in relation to the MCR of glucose calculated as the average glucose infusion rate during the last hour of the euglycemic clamp [24]. Referring to Fig. 1 in Reference [24], IS_{MCR} values are expressed in mg · min^{-1} · kg^{-1}.

2.4. Statistical analysis

All data and results are given as means ± SE. Lilliefors [36] test (suitable for small samples) was used to evaluate the hypothesis that each data vector or parameter vector had a normal distribution with unspecified mean and variance, against the alternative that these vectors do not have normal distribution (significance was set at 5% level). For normally distributed samples, 2-tailed nonpaired Student t test was applied to analyze the differences between N and H groups. A P value of less than .05 was considered as statistically significant. Wilcoxon rank sum test was used to compare samples that were not normally distributed (significance was set at 5% level).

Intraclass correlation coefficient [37] was used to evaluate (a) the agreement of paired sample sets, $OGTT_{22/300}$ and $OGTT_{8/180}$, in providing $IS_{IE22/300}$ and $IS_{IE8/180}$ estimates, respectively, and (b) the agreement of $OGIS_{180}$ and $OGIS_{120}$ in providing $IS_{OGIS180}$ and $IS_{OGIS180}$ estimates, respectively.

3. Results

3.1. Patient characteristics

Average clinical data that characterize our N and H groups are presented in Table 1. The H group showed significantly higher diastolic blood pressure, triglycerides, and insulinemia. Fasting insulinemia, however, was within normality even in hypertensive patients, with values never exceeding 20 μU/mL. No significant differences were observed in all other variables.

Time course of mean glycemia, $G(t)$, and insulinemia, $I(t)$, levels as measured in the N and H groups, throughout the entire OGTT, are shown in Fig. 1A and B, respectively.

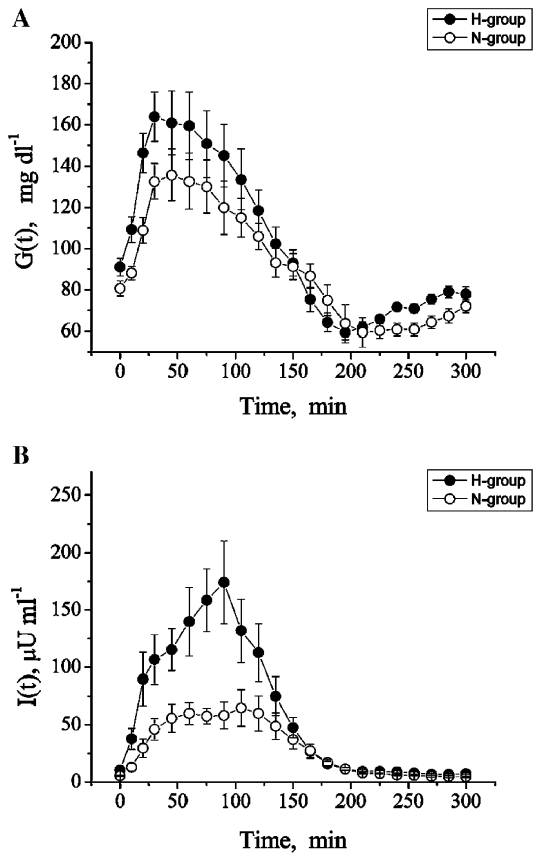

Fig. 1. Glycemia (A) and insulinemia (B) as a function of time during our $OGTT_{22/300}$ protocol in the N group (open circles) and the H group (closed circles). Values are expressed as means ± SE of 7 hypertensive and 8 normotensive subjects.

Both glycemia and insulinemia were higher in the H group with significant ($P < .05$) differences in $G(t)$, in the time frame from 10 to 30 minutes, and in $I(t)$, in the time frame from 0 to 105 minutes. The peak value of $I(t)$ occurred around the 90th minute (I_{90}) and was almost 3 times higher ($P < .01$) in the H group (174 ± 36 μU/mL) than in the N group (58.1 ± 11.6 μU/mL). The corresponding G_{90} value of glycemia was 21% higher in the H group than in the N group, but the difference was not statistically significant.

3.2. Estimates of insulin sensitivity

Means ± SE of insulin sensitivity provided by the IE method for the full $OGTT_{22/300}$ protocol and for the reduced $OGTT_{8/180}$ protocol are reported in Table 3 together with mean values of insulin sensitivity provided by the $OGIS_{180}$, $OGIS_{120}$, ISI, and MCR methods.

Intraclass correlation coefficient between $IS_{IE8/180}$ and $IS_{IE22/300}$ was 0.89 in the N group and 0.94 in the H group. The plot of $IS_{IE8/180}$ vs $IS_{IE22/300}$ values (Fig. 2) indicates that all subjects (normotensive and hypertensive) lie near the line of unity. Insulin sensitivity expressed by the $IS_{IE22/300}$ and $IS_{IE8/180}$ indexes showed 60% ($P < .01$) and 57% ($P < .05$) reduction, respectively, in hypertension. These results indicate a substantial agreement of the 2 paired methods in estimating insulin sensitivity and discriminating the insulin-resistant state in hypertension.

Intraclass correlation coefficient between $IS_{OGIS180}$ and $IS_{OGIS120}$ was 0.84 in the N group and reduced to 0.60 in the H group. This reduction indicates a substantial disagreement between the 2 methods in evaluating insulin sensitivity in hypertension. This disagreement finds a confirmation in that the $IS_{OGIS120}$ index was able to detect a significant (26%, $P < .005$) reduction of insulin sensitivity in hypertensive patients, whereas no significant reduction was detected by the $IS_{OGIS180}$.

Both the empirical IS_{ISI} and IS_{MCR} indexes detected a significant reduction of insulin sensitivity in hypertension (Table 3). On average, the magnitude of this reduction was approximately 61% ($P < .001$) for the IS_{ISI} and 26% ($P < .05$) for the IS_{MCR}.

Table 3
Estimates of indexes of insulin sensitivity from OGTT protocols

	N group (n = 8)	H group (n = 7)	Unpaired Student *t* test (*P*)
IE method			
$IS_{IE22/300}$	10.9 ± 1.6	4.4 ± 1.3	.01
$IS_{IE8/180}$	12.7 ± 2.0	5.4 ± 1.5	.05
OGIS method			
$IS_{OGIS180}$	464 ± 23	412 ± 33	NS
$IS_{OGIS120}$	460 ± 19	339 ± 28	.005
ISI method			
IS_{ISI}	7.7 ± 0.9	3.0 ± 0.3	.001
MCR method			
IS_{MCR}	8.4 ± 0.3	6.2 ± 0.9	.05

Values are means ± SE of N and H groups. $IS_{IE22/300}$ and $IS_{IE8/180}$ (10^{-4} min^{-1} · dL · kg^{-1}/[μU/mL]) are indexes obtained from the IE method [23] applied to the full $OGTT_{22/300}$ protocol and to the reduced $OGTT_{8/180}$ data subset, respectively. $IS_{OGIS180}$ and $IS_{OGIS120}$ (mL · min^{-1} · m^{-2}) are indexes obtained from OGIS methods [25], which require 3 blood samples from a 180- and a 120-minute OGTT, respectively. IS_{ISI} (dL · mg^{-1} · μU^{-1} · mL) is an empirical index that considers [22] fasting glucose and insulin concentrations together with mean glucose and mean insulin concentrations during a 120-minute OGTT. IS_{MCR} (mg · min^{-1} · kg^{-1}) is an index computed by an empirical equation that accounts for BMI, insulinemia at the 120th minute and glycemia at the 90th minute after ingestion of a glucose load [24].

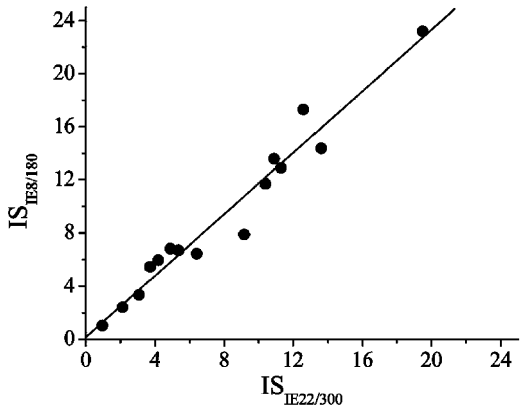

Fig. 2. Plot of $IS_{IE8/180}$ vs $IS_{IE22/300}$ values (10^{-4} min^{-1} · dL · kg^{-1}/[μU/mL]) of insulin sensitivity estimated in our normotensive and hypertensive subjects. Regression analysis yielded a slope of 1.18 and an intercept of -0.12×10^{-4} min^{-1} · dL · kg^{-1}/(μU/mL), with a correlation coefficient of 0.98.

4. Discussion

The insulin-resistant state in hypertension is a time-honored notion [1-14]. Quantification of insulin sensitivity by well-accepted techniques, such as the euglycemic-hyperinsulinemic clamp and the minimal model analysis of FSIGTT data, in groups of nonobese and nondiabetic hypertensive patients, compared with groups of control subjects matched for age and BMI, showed significant reductions in hypertension ranging from 26% to 70% [1,7,8,14].

Based on these previous findings, we tested, in the present study, the ability of OGTT-based methods (IE, OGIS, ISI, and MCR) to detect the expected significant reduction of insulin sensitivity in hypertensive patients (H group) compared with a group of normotensive subjects (N group). To avoid the confounding effects of MS, defined according to ATP III criteria [31], as a state associated with excess risk of diabetes and cardiovascular disease, we exercised special care in recruiting subjects. All participants were normoglycemic and did not show the presence of more than 2 of the remaining 4 ATP III criteria [31]. Our selection criteria reduced the number of patients eligible for the study, but strengthened the reliability of results in terms of the link between reduced insulin sensitivity and hypertension.

According to the literature [14,38,39], insulin action measured with either a FSIGTT or an euglycemic-hyperinsulinemic clamp does not depend on age in healthy

subjects when adjusted for BMI or waist-to-hip ratio. Obesity is known to affect insulin sensitivity beyond hypertension [4-6,12]. The effects of this potential confounding factor were minimized by our selection criteria, such that the mean BMI was approximately 25 kg/m^2 (Table 1) with no significant difference between N and H groups.

Application of the IE method to our N group yielded mean $IS_{IE22/300}$ and $IS_{IE8/180}$ values (Table 3), which fall between the mean value of 8.3×10^{-4} min^{-1} · dL · kg^{-1}/(μU/mL) found by Breda et al [26] in 7 healthy subjects from an OGTT and the mean values of $13.6 \pm 3.9 \times 10^{-4}$ min^{-1} · dL · kg^{-1}/(μU/mL) and $12.9 \pm 3.5 \times 10^{-4}$ min^{-1} · dL · kg^{-1}/(μU/mL) reported by Caumo et al [23] from 240- and 180-minute MGTT, respectively.

The novel application of the IE method to the H group, performed in the present study, demonstrated that this method allows discrimination of a significant defect in insulin action associated with hypertension. This is true with using either the $IS_{IE22/300}$ or the $IS_{IE8/180}$ index. Both these indexes showed a significant reduction by almost 60% in the H group (Table 3) with respect to the N group and met the expectation based on previous reports [1,7,8,14]. The substantial agreement between the $IS_{IE22/300}$ and $IS_{IE8/180}$ samples is supported by intraclass correlation coefficients not lower than 0.89 and the regression coefficient of 0.98 found from plotting all $IS_{IE8/180}$ values (from N and H groups) vs the corresponding $IS_{IE22/300}$ values (Fig. 2). The reproducibility of insulin sensitivity estimates from only 8 glucose and insulin samples in the time frame from 0 to 180 minutes makes the IE method appealing for clinical practice and epidemiological purposes. By definition, the IE method requires that glucose and insulin concentrations have returned to basal levels at the end of the test to correctly calculate the areas under the curves. Fig. 1A and B shows that this restriction (which may render the method problematic to use in subjects with impaired glucose tolerance or impaired insulin secretion) is satisfied in our normotensive and hypertensive subjects.

Glucose uptake is dependent upon insulin-independent as well as insulin-dependent mechanisms. In the IE method, the insulin-independent component is quantified by GE. This parameter, as well as the fraction, f, of ingested glucose that actually appears in the systemic circulation, cannot be determined on individual basis. Both them need to be given an assumed value. The fact that these parameters are derived from the formulation of the original minimal model of glucose kinetics and its subsequent adaptation to the OGTT [23] leads to the determination of suitable values [23,26,27,35]. In the present study, we assumed GE = 3.7×10^{-2} min^{-1} · dL · kg^{-1} and $f = 0.87$ in accordance with a recent findings from dual-tracer OGTT studies [35].

The same GE value was assumed for both our N and H groups because we showed previously that GE is independent of hypertension [14]. However, because of age-related reduction of GE [14], the population value of GE derived in Reference [35] from subjects with mean age of 41 years might be overestimated for our N and H groups with mean age of approximately 50 years. We therefore tested the sensitivity of IS_{IE} indexes to a 15% reduction of GE in both N and H groups. A 6% increase in normotensive subjects and a 7% increase in hypertensive patients were found, which did not affect substantially the alterations of insulin sensitivity between normal and hypertensive state.

The value of $f = 0.87$ was assumed for both our N and H groups. Although we may be confident that this value holds for healthy subjects [35], it cannot be excluded that hypertensive patients are characterized by having a different value. Especially an increase of f in the H group would result in higher values of IS_{IE} indexes, which might affect the statistical significance of their difference with respect to the N group. We then tested this critical condition by increasing f up to 0.96 in the H group. Mean $IS_{IE22/300}$ and $IS_{IE8/180}$ were $5.1 \pm 1.4 \times 10^{-4}$ min^{-1} · dL · kg^{-1}/(μU/mL) and $6.2 \pm 1.6 \times 10^{-4}$ min^{-1} · dL · kg^{-1}/(μU/mL), respectively, and showed significant 53% ($P < .05$) and 51% ($P < .05$) reductions, respectively, compared with the corresponding values determined in the N group with $f = 0.87$ (Table 3). Thus, the ability of these indexes to discriminate a significant reduction of insulin sensitivity in hypertension was preserved.

With respect to the OGIS method, no statistically significant reduction in the $IS_{OGIS180}$ index found in our H group compared with the N group (Table 3) questions the applicability of this index to hypertensive patients. Because the $IS_{OGIS180}$ uses G_{180}, G_{120}, and I_{120} measurements, a possible cause of failure is that the I_{120} value of insulinemia is used while the peak happens earlier, around the 90th minute. A hyperinsulinemic response in hypertensive patients, with normal beta-cell sensitivity, is functional to the normalization of the glycemic response in the presence of reduced tissue insulin sensitivity [13]. Missing the information portrayed by the peak of insulin response may affect the ability of the $IS_{OGIS180}$ to detect a significant reduction of insulin sensitivity in hypertension. A confirmation of this hypothesis is found in that the $IS_{OGIS120}$ index, which uses the I_{90} value, rather than the I_{120}, captures the insulin peak and yields a significant reduction ($P < .005$) of insulin sensitivity in hypertension. Thus, the version of the OGIS method, which produces the $IS_{OGIS120}$ index, applies to nondiabetic hypertensive patients. A limitation may be found in that the OGIS requires the assumption of as many as 6 parameters (p_i, i =1,...,6 in Table 1), and it is not known if they hold for both normal and hypertensive states.

In contrast to the model-based IE and OGIS methods, the ISI and MCR methods consist of purely empiric formulas. Especially, the ISI method does not require the definition of any kind of coefficient other than the 10^4 scale factor (Eq 6), which does not appear essential, not even being clearly defined in terms of units of measure. This method is based on the simple concept that the higher the fasting and

postperturbation glucose and insulin concentrations, within 120 minutes, the lower is insulin sensitivity. Sufficient information on the deviation of insulin and glucose concentrations from fasting values, which appears important for the characterization of the dynamics of glucose-insulin system, is captured by the average values of G_m and I_m computed over 5 glucose and insulin samples. Application of this method to our subject groups yielded 61% ($P < .001$) reduction of insulin sensitivity in hypertension (Table 3), which is comparable with reported findings [1,7,8,14] and with the almost 60% reduction detected here by the IE method from full and reduced OGTT.

The MCR method combines BMI, I_{120}, and G_{90}, thus missing the information portrayed by the I_{90} insulinemic peak in hypertensive patients. It has been reported by others that this method may yield negative values of insulin sensitivity, which are meaningless [40]. A negative value was also observed by us in a hypertensive patient with MS, not included in our H group, who had BMI = 27.7 kg/m^2, I_{120} = 286 μU/mL, and G_{90} = 196 mg/dL. Negative values of IS_{MCR} in the presence of hyperinsulinemia may be because the coefficients characterizing Eq 7 empirically determined from fitting to a data set [24] have limited validity in hypertension. Values of insulin sensitivity produced by the other methods ($IS_{IE8/180}$ = 0.5 × 10^{-4} · min^{-1} · dL · kg^{-1}/[μU/mL], $IS_{OGIS120}$ = 213 mL · min^{-1} · m^{-2}, and IS_{ISI} = 1.0 dL · mg^{-1} · μU^{-1} · mL) fell in a very low range of insulin sensitivity, as expected.

5. Conclusions

Our results indicate that the $IS_{IE22/300}$, $IS_{IE8/180}$, $IS_{OGIS120, ISISI}$, and IS_{MCR} indexes are able to discriminate a defect of insulin action in hypertension. However, the IS_{MCR} showed a limitation in that it may yield meaningless negative values of insulin sensitivity in circumstances characterized by relatively high values of BMI and hyperinsulinemic response to an oral glucose load. The IS_{ISI} empiric index has the advantage of simplicity. In absolute terms, if the 10^4 factor was released from Eq 6, the order of magnitude of IS_{ISI} would be the same as that of the $IS_{IE22/300}$ and $IS_{IE8/180}$ indexes (Table 3), but with different units or measure. These IE-based indexes have the advantage of being derived from the minimal model of glucose kinetics by adding a parsimonious parametric representation of splanchnic glucose absorption [23]. The consequent physiological characterization of the GE and f parameters leads the assumption of suitable values for normotensive and hypertensive subjects and is liable to future improvement. The $IS_{IE8/180}$ index is appealing because it requires only 8 glucose and insulin samples in 180 minutes. The plot of IS_{ISI} vs $IS_{IE8/180}$ displayed in Fig. 3 indicates that they are measuring similar trends in the alteration of insulin sensitivity with hypertension, in the absence of MS. If we convey on the concept that units of measure are important, we can conclude that only the IE method produces an index characterized by units of measure consistent with the definition of insulin sensitivity as the ability of insulin to enhance GE [16].

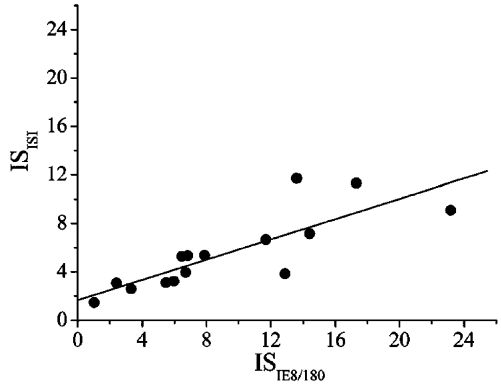

Fig. 3. Plot of IS_{ISI} (dL · mg^{-1} · μU^{-1} · mL) vs $IS_{IE8/180}$ (10^{-4} min^{-1} · dL · kg^{-1}/[μU/mL]) values of insulin sensitivity estimated in our normotensive and hypertensive subjects. Regression analysis indicated a slope of 0.42 × 10^4 min · kg · mg^{-1} and an intercept of 1.65 dL · mg^{-1} · μU^{-1} · mL, with a correlation coefficient of 0.82.

Acknowledgment

This work was supported in part by the Italian Ministry of Instruction, University and Research (PRIN-COFIN 2004 grant to R Burattini).

References

[1] Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, et al. Insulin resistance in essential hypertension. N Engl J Med 1987;317:350-7.
[2] Shen DC, Shieh SM, Fuh MM, Wu DA, Chen YD, Reaven GM. Resistance to insulin-stimulated-glucose uptake in patients with hypertension. J Clin Endocrinol Metab 1988;66:580-3.
[3] Swislocki AL, Hoffman BB, Reaven GM. Insulin resistance, glucose intolerance and hyperinsulinemia in patients with hypertension. Am J Hypertens 1989;2:419-23.
[4] Pollare T, Lithell H, Berne C. Insulin resistance is a characteristic feature of primary hypertension independent of obesity. Metabolism 1990;39:167-74.
[5] Ferrari P, Weidmann P. Insulin, insulin sensitivity and hypertension. J Hypertens 1990;8:491-500.
[6] DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991; 14:173-94.
[7] Supiano MA, Hogikyan RV, Morrow LA, Ortiz-Alonso FJ, Herman WH, Bergman RN, et al. Hypertension and insulin resistance: role of sympathetic nervous system activity. Am J Physiol 1992;263: E935-42.
[8] Kautzky-Willer A, Pacini G, Weissel M, Capek M, Ludvik B, Prager R. Elevated hepatic insulin extraction in essential hypertension. Hypertension 1993;21:646-53.
[9] Corry DB, Tuck ML. Glucose and insulin metabolism in hypertension. Am J Nephrol 1996;16:223-36.
[10] Natalucci S, Boemi M, Fumelli P, Testa I, Fumelli D, Burattini R. One- and two-compartment minimal models detect similar alterations

of glucose metabolism indexes in hypertension. Metabolism 2000;49:1529-36.
[11] Grandi AM, Zanzi P, Broggi R, Fachinetti A, Guasti L, Ceriani L, et al. Longitudinal changes of insulin sensitivity in essential hypertension: influence of blood pressure control and familial predisposition to hypertension. J Clin Endocrinol Metab 2001;86: 3027-31.
[12] Masuo K, Mikami H, Ogihara T, Tuck ML. Weight reduction and pharmacologic treatment in obese hypertensives. Am J Hypertens 2001;14:530-8.
[13] Natalucci S, Boemi M, Fumelli D, Fumelli P, Burattini R. Interaction between glucose metabolism and endogenous insulin release in hypertension. Metabolism 2002;51:297-303.
[14] Burattini R, Di Nardo F, Boemi M, Fumelli P. Alteration of insulin sensitivity and glucose effectiveness with age and hypertension. Am J Hypertens 2005 [in press].
[15] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214-23.
[16] Bergman RN, Ider YZ, Bowden CR, Cobelli C. Quantitative estimation of insulin sensitivity. Am J Physiol 1979;236:E667-77.
[17] Saad MF, Anderson RL, Laws A, Watanabe RM, Kades WW, Chen YD, et al. A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. Insulin Resistance Atherosclerosis Study. Diabetes 1994;43:1114-21.
[18] Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. Diabetes Care 1997;20:1087-92.
[19] Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000;23:57-63.
[20] Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402-10.
[21] Perseghin G, Caumo A, Caloni M, Testolin G, Luzi L. Incorporation of the fasting plasma FFA concentration in to QUICKI improves its association with insulin sensitivity in nonobese individuals. J Clin Endocrinol Metab 2001;86:4776-81.
[22] Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462-70.
[23] Caumo A, Bergman RN, Cobelli C. Insulin sensitivity from meal tolerance tests in normal subjects: a minimal model index. J Clin Endocrinol Metab 2000;85:4396-402.
[24] Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Jarvinen H, Van Haeften T, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 2000; 23:295-301.
[25] Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 2001;24:539-48.
[26] Breda E, Cavaghan MK, Toffolo G, Polonsky KS, Cobelli C. Oral glucose tolerance test minimal model indexes of β-cell function and insulin sensitivity. Diabetes 2001;50:150-8.
[27] Dalla Man C, Caumo A, Cobelli C. The oral glucose minimal model: estimation of insulin sensitivity from a meal test. IEEE Trans Biomed Eng 2002;49:419-29.
[28] Castillo MJ. Simple assessment of insulin sensitivity and secretion. Int Diabetes Monit 2002;14:1-7.
[29] Di Nardo F, Casagrande F, Carle F, Boemi M, Fumelli P, Morosini P, et al. Evaluation of insulin sensitivity from a minimal model adapted to oral glucose tests. In: Ursino M, Brebbia CA, Pontrelli G, Magosso E, editors. Modelling in Medicine and Biology, vol 6. Southampton, UK: WIT Press. p. 67-76.
[30] Vaccaro O, Masulli M, Cuomo V, Rivellese AA, Uusitupa M, Vessby B, et al. Comparative evaluation of simple indices of insulin resistance. Metabolism 2004;53:1522-6.
[31] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
[32] Berne C, Pollare T, Lithell H. Effects of antihypertensives on insulin sensitivity with special reference to ACE inhibitors. Diabetes Care 1991;14(Suppl 4):39-47.
[33] Santoro D, Natali A, Palombo C, Brandi LS, Piatti M, Ghione S, et al. Effects of chronic angiotensin converting enzyme inhibition on glucose tolerance and insulin sensitivity in essential hypertension. Hypertension 1992;20:181-91.
[34] Sowers JR. Effects of calcium antagonists on insulin sensitivity and others metabolic parameters. Am J Cardiol 1997;79:24-8.
[35] Dalla Man C. Oral Glucose minimal models: formulation, validation and use. PhD thesis, University of Padua, Italy; 2004.
[36] Lilliefors HW. On the Kolmogorov-Smirnov test for normality with men and variance unknown. J Am Stat Assoc 1967;62: 399-402.
[37] Fleiss JL. Statistical methods for rates and proportion. Wiley: New York; 1980. p. 212-36.
[38] Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G, Smith U. Insulin action and age. European Group for the Study of Insulin Resistance (EGIR). Diabetes 1996;45:947-53.
[39] Basu R, Breda E, Oberg AL, Powell CC, Dalla Man C, Basu A, et al. Mechanisms of age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. Diabetes 2003;52:1738-48.
[40] Pacini G, Mari A. Methods for clinical assessment of insulin sensitivity and β-cell function. Best Pract Res Clin Endocrinol Metab 2003;17:305-22.