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Insulin action and secretion in hypertension in the absence of metabolic syndrome: model-based assessment from oral glucose tolerance test

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Abstract

Relationship between insulin action and secretion was analyzed in 10 hypertensive patients (H group; 5 male, 5 female; 56.9 ± 2.5 years) compared with 10 normotensive subjects (N group; 5 male, 5 female; 51.7 ± 3.7 years; $P > .05$) matched for age, sex, and body mass index. All participants (normoglycemic, nonobese, and not affected by metabolic syndrome) underwent a 5-hour, 22-sample, oral (75 g) glucose tolerance test. Insulin sensitivity was quantified by quantitative insulin sensitivity check index and an insulin sensitivity index computed by minimal-model–based "integral equation." β-Cell responsivity indexes (dynamic, Φ_d; static, Φ_s; and global, Φ_{oral}) were estimated by C-peptide oral minimal model. Compared with the N group, our H group featured no significant difference ($P > .05$) in fasting glycemia, significant ($P < .02$) increase in plasma insulin (93%) and C-peptide (53%) concentrations, and significant ($P < .01$) reduction in both quantitative insulin sensitivity check index (10%) and insulin sensitivity index (68%). No significant variations of mean Φ_d, Φ_s, and Φ_{oral} were observed across the 2 groups in response to glucose challenge. Thus, insulin sensitivity deterioration in hypertension was not mirrored by a reciprocal change in β-cell responsivity. Nevertheless, our H group featured a 143% ($P < .005$) increase in the area under the curve of circulating insulin and a 34% ($P < .01$) reduction in the ratio between the area under C-peptide curve and the area under the curve of circulating insulin. These results support the hypothesis that decreased insulin clearance in hypertensive patients, not affected by metabolic syndrome, is a further regulatory mechanism, in addition to increased insulin secretion, to compensate for insulin resistance.

1. Introduction

An association among insulin resistance (decreased sensitivity or responsiveness to the metabolic actions of insulin), hyperinsulinemia, and hypertension is generally recognized [1-16]. Demonstration that essential hypertension is an insulin-resistant state in its own right implied its involvement in the insulin resistance syndrome, later transmuted into the metabolic syndrome (MS) [1,2,17,18]. The degree to which glucose tolerance deteriorates in insulin-resistant individuals varies as a function of both the magnitude of the loss of in vivo insulin action and the capacity of the β-cell secretion to compensate for this effect. Thus, for a more appropriate characterization of the relative role of insulin action and secretion in hypertension, not only has effort to be made on quantification of insulin sensitivity indexes; but these should be contrasted with indexes of β-cell responsiveness [2,13,15,19-24].

Previous reports by others [6] and ourselves [13] have investigated the dynamic relationship between insulin action and secretion in hypertensive patients by means of minimal model interpretations of data from frequently sampled intravenous glucose tolerance test (IVGTT). Minimal model of glucose kinetics [25] was used to quantify insulin sensitivity, whereas an earlier version [6] and an improved version [13] of C-peptide minimal model [26-28] were used to assess insulin secretion. Clinical applications of IVGTT-based parameter estimation methods have a limitation in that intravenous glucose injection remains cumbersome because of the difficulty of venous administration and the nonphy-

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siologic condition after the rapid glucose and insulin perturbations. Oral glucose tolerance test (OGTT) is easier to perform and more physiologic than IVGTT for involvement of incretin effect, most of which is due to the gut-derived incretin hormones glucagon-like peptide–1 and glucose-dependent insulinotropic polypeptide [24,29,30]. Recently, it has been demonstrated that, by extending to OGTT the C-peptide minimal model developed for IVGTT, it is possible to quantify characteristic parameters of β-cell responsivity to glucose stimulus and predict insulin secretion rate in individuals with various degrees of glucose tolerance [21,24,31,32]. No applications to hypertensive patients, however, have been reported.

Assessment of the β-cell function in the face of insulin resistance in hypertension, by interpreting OGTT data with C-peptide oral minimal model, was the aim of the present study. The integral equation (IE) method, previously proposed by Caumo et al [33] and tested by ourselves [34] in hypertensive patients, was used to assess insulin sensitivity. The quantitative insulin sensitivity check index (QUICKI) was also taken into consideration [14,35] because it has been reported that, despite this index being based on mathematical transformation of fasting plasma glucose and insulin levels, it is a simple and robust tool that is useful for evaluating and following the insulin resistance of hypertensive patients even in studies with a small number of subjects [14].

The OGTT data used in the present study were taken from a group of hypertensive patients compared with a control group of normotensive subjects. Special care was exercised in recruiting the participants to drop off the effects of confounding factors such as sex, age, obesity, and MS defined according to the Adult Treatment Panel III (ATP III) criteria [18].

2. Materials and methods

2.1. Patients and protocol

Twenty volunteer subjects of white ethnicity were recruited at the Metabolic Disease and Diabetes Unit of the INRCA-IRCCS, Ancona, Italy, and the Unit of Internal Medicine, "CG Mazzoni" General Hospital, Ascoli Piceno, Italy. All participants gave their informed written consent to the procedures, which were approved by the Ethics Committee.

Recruitment criteria were set up to avoid obesity and MS and to drop off differences in sex and age. Controlling for these confounders limited the number of participants. Subjects were divided into a group of 10 (5 men and 5 women) normotensive subjects (N group) and a group of 10 (5 men and 5 women) hypertensive patients (H group). Body mass index (BMI; kg/m^2), not included in the ATP III criteria, was less than 29 in all participants. On average, BMI and age showed no significant difference between the H and N groups (Table 1). To control for MS [18], we only selected normotensive and hypertensive subjects who had no history of diabetes mellitus and had a fasting glycemia of less than 110 mg/dL (6.1 mmol/L). Subjects included in the N group had seated blood pressure levels not exceeding 130-85 mm Hg and no more than 2 of the remaining 3 ATP III criteria, namely, waist circumference greater than 102 cm in men and greater than 88 cm in women, triglycerides at least 150 mg/dL (1.695 mmol/L), and high-density lipoprotein cholesterol less than 40 mg/dL (1.04 mmol/L) in men and less than 50 mg/dL (1.29 mmol/L) in women. No more than 1 of these 3 criteria was allowed, besides hypertension, in normoglycemic H patients.

Table 1
Clinical data for the study subjects

Variable	N group (n = 10)	H group (n = 10)	Statistics
Male/female	5/5	5/5	NS
Age (y)	51.7 ± 3.7	56.9 ± 2.5	NS
SBP (mm Hg)	126 ± 2	132 ± 3	NS
DBP (mm Hg)	76.4 ± 1.3	88.5 ± 2.2	$P < .001$*
BMI (kg/m^2)	24.7 ± 0.9	25.9 ± 0.7	NS
Waist circumference (cm)	85.7 ± 3.3	87.9 ± 4.0	NS
Triglycerides (mg/dL)	66.2 ± 10.9	135 ± 18	$P < .005^{\dagger}$
Cholesterol (mg/dL)	201 ± 9	207 ± 11	NS
HDL cholesterol (mg/dL)	54.6 ± 3.6	51.8 ± 3.6	NS
Serum creatinine (mg/dL)	0.82 ± 0.06	0.81 ± 0.05	NS
Creatinine clearance (mL/min)	99.6 ± 8.4	98.5 ± 5.0	NS
Fasting glycemia (mmol/L)	4.64 ± 0.16	5.08 ± 0.17	NS
2-h glycemia (mmol/L)	6.36 ± 0.46	6.50 ± 0.45	NS
Fasting insulinemia (pmol/L)	27.7 ± 2.7	53.5 ± 7.0	$P < .005^{\dagger}$
2-h insulinemia (pmol/L)	240 ± 52	610 ± 113	$P < .005$*
Fasting C-peptide (pmol/L)	463 ± 46	708 ± 46	$P < .005^{\dagger}$
2-h C-peptide (pmol/L)	2367 ± 321	3601 ± 345	$P < .02^{\dagger}$

Values are means ± SE. *Body mass index* (BMI) is defined as the ratio of body weight to the square of height. Two-hour glycemia, 2-hour insulinemia, and 2-hour C-peptide are measurements performed 2 hours after 75-g oral glucose challenge. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; NS, not significant.

* Wilcoxon rank sum test.

† Unpaired Student *t* test.

All our H patients were under antihypertensive drug therapy for more than 1 year. Controversial results have been reported as to the effect of antihypertensive treatment on glucose metabolism [14,36-38]. In the Atherosclerosis Risk in Communities Study [36], subjects who were taking a thiazide diuretic, angiotensin-converting enzyme (ACE) inhibitor, or calcium-channel antagonist were not at greater risk for subsequent development of diabetes mellitus than their untreated counterparts. In contrast, diabetes mellitus was 28% more likely to develop in subjects taking a β-blocker than in those taking no medication. In the Antihypertensive Treatment and Lipid Profile in a North of Sweden Efficacy Evaluation study [37], antihypertensive treatment with a diuretic, if needed combined with a β-blocker, was associated with an aggravated metabolic profile; this was not so for patients treated with an angiotensin II receptor blocker (ARB), if needed combined with a calcium antagonist. Chen et al [14] reported that, in a

population of patients where some were taking ACE inhibitors (predicted to increase insulin sensitivity) whereas others were taking drugs such as diuretics, β-blockers, and calcium channel blockers (predicted to decrease insulin sensitivity), no significant change was found in mean insulin sensitivity determined by glucose clamp in the same subjects on and off medication, although individual responses did vary. Similarly to the study by Chen et al [14], each of the H patients involved in the present study was taking different drugs from various classes of antihypertensive agents: ACE inhibitor (2 patients), ACE inhibitor added to diuretic (1), ACE inhibitor added to α-blocker (1), ARB (2), ARB in combination with low-dose diuretic (1) added to α-blocker (1 other), β-blocker (1), and β-blocker added to calcium-channel antagonist (1). Based on studies cited above, such a variety of medications was likely to have no significant impact in our comparative analysis of mean metabolic parameters from the H and N groups. Therefore, risks of suspension of therapy were avoided; and our H patients were studied while taking their usual medications.

Each subject underwent an OGTT for measurement of plasma glucose, insulin, and C-peptide concentrations. Starting time was 8:30 AM after overnight fast. A 75-g glucose load was administered, and 22 blood samples were taken within 300 minutes. 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 [21]. Blood was promptly centrifuged, and glucose was immediately measured by the glucose oxidase method with an automated glucose analyser. 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%. C-peptide was measured by commercially available radioimmunoassays (Biochem Immunosystems Italia, Milan, Italy). Sensitivity and intra- and interassay precision of the C-peptide were 0.025 pmol/mL, 5.8% ± 2.4%, and 5.7% ± 0.8%, respectively.

2.2. Quantification of insulin sensitivity

2.2.1. IE method

To measure insulin sensitivity from oral glucose tests, the classic minimal model of glucose kinetics was coupled by Caumo et al [33] 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 insulin sensitivity index (S_I) [33]:

$$S_I = \frac{f \cdot \frac{D}{W} \cdot \frac{\mathrm{AUC}[\Delta G(t)/G(t)]}{\mathrm{AUC}[\Delta G(t)]} - \mathrm{GE} \cdot \mathrm{AUC}[\Delta G(t)/G(t)]}{\mathrm{AUC}[\Delta I(t)]} \qquad (1)$$

In this equation, AUC denotes the area under the curves of the quantities in brackets, in the time frame from t = 0 to the end of test (t = 5 hours); $\Delta G(t)$ and $\Delta I(t)$ are glycemia and insulinemia above basal, respectively; GE is glucose effectiveness ($\mathrm{min}^{-1}\cdot\mathrm{dL}\cdot\mathrm{kg}^{-1}$), given by the product of fractional glucose effectiveness, S_G (min^{-1}), and glucose distribution volume, V (dL/kg); D/W is the dose of ingested glucose per unit body weight (mg/kg); and f is the fraction of ingested glucose that actually appears in the systemic circulation (ie, survives gastrointestinal absorption and 1-pass hepatic uptake). If plasma glucose and insulin concentrations are expressed as mg/dL and pmol/L, respectively, measure units of S_I are $\mathrm{min}^{-1}\cdot\mathrm{dL}\cdot\mathrm{kg}^{-1}/(\mathrm{pmol/L})$.

Population values of f and GE are to be filled into Eq. (1) to calculate S_I [33]. In accordance with Dalla Man et al [39], computations in our N and H groups were performed after assuming f = 0.87 and GE = 3.7×10^{-2} $\mathrm{min}^{-1}\cdot\mathrm{dL}\cdot\mathrm{kg}^{-1}$ (this was obtained from the product of S_G = 0.028 min^{-1} and V = 1.34 dL/kg). Suitability of these assumptions was discussed in our previous work [34].

2.2.2. Quantitative insulin sensitivity check index

The QUICKI was calculated as previously defined [14,35]:

$$\mathrm{QUICKI} = \frac{1}{(\log I_b + \log G_b)}, \qquad (2)$$

where I_b and G_b are fasting plasma insulin (μU/mL) and glucose (mg/dL) concentrations, respectively. Because QUICKI is the reciprocal of the log-transformed product of fasting glucose and insulin, it has been considered a dimensionless index [14].

2.3. Quantification of β-cell responsivity

Recently, it has been demonstrated that, by extending to the OGTT the C-peptide minimal model developed for intravenous glucose infusion, it is possible to assess β-cell responsivity indexes from plasma glucose and C-peptide concentrations measured during the test [21,24,31,32]. A scheme of the model is illustrated in Fig. 1; and, for clarity, model equations, redrawn from Breda et al [21] and Toffolo et al [31], are summarized in the Appendix A.

The model of Fig. 1 [32] features a dynamic and a static component of insulin secretion in response to glucose challenge. The former is likely to represent secretion of promptly releasable insulin and is proportional to the rate of increase of glucose concentration through a parameter, Φ_d (10^{-9}), that defines the dynamic responsivity index. The latter relates to provision of new insulin to the releasable pool and is characterized by a static index, Φ_s (10^{-9} min^{-1}), and a delay, T (min), presumably reflecting, at least in part, the time it takes for new granules to reach the releasable pool. An estimate of glycemia threshold, h, above which insulin secretion occurs, is also provided. After parameter estimation is accomplished, an overall β-cell responsivity index, Φ_{oral} (10^{-9} min^{-1}), can be determined from a

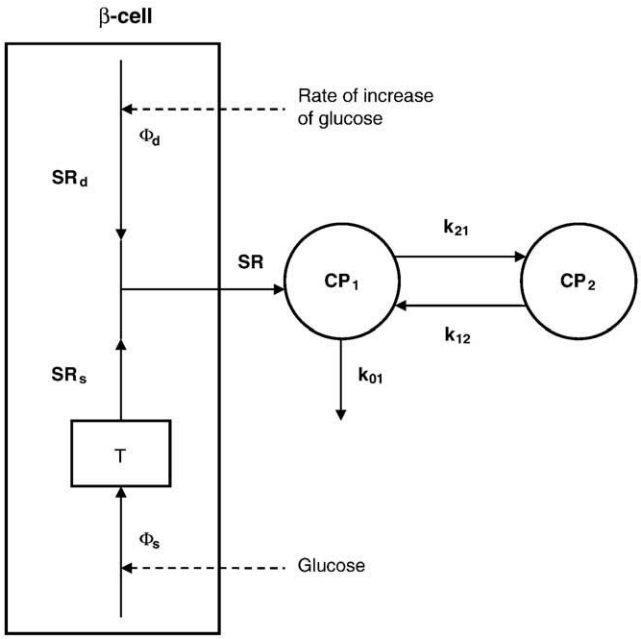

Fig. 1. C-peptide minimal model adapted to OGTT. Φ_d and Φ_s are indexes of dynamic and static β-cell responsivity to glucose challenge, respectively; T is delay in provision of new insulin; k_{01}, k_{21}, and k_{12} are characteristic parameters of C-peptide kinetics; and SR_d and SR_s are dynamic and static components of β-cell secretion rate, SR.

combination of Φ_d and Φ_s (see Appendix A and Breda et al [21]). In addition to Φ_d, Φ_s, and Φ_{oral}, a basal responsivity index, Φ_b (10^{-9} min^{-1}), which is equal to the ratio between basal insulin secretion rate per unit distribution volume, ISR_b ($pmol \cdot L^{-1} \cdot min^{-1}$), and basal glycemia, G_b (mmol/L), is of interest because it gives a basal, nonstimulated index complementing the stimulated ones [24].

2.4. Parameter estimation

Characteristic parameters of C-peptide kinetics (see Appendix A) were adjusted to subject's anthropometric data as proposed by Van Cauter et al [27]. C-peptide (and insulin) secretion parameters T, h, Φ_d, and Φ_s were estimated in each subject, together with a measure of their precision (CV%), from plasma C-peptide and glucose data by nonlinear least squares [40] using SAAM II software [41]. Weights were optimally chosen, that is, equal to the inverse of the variance of C-peptide measurement errors. When T was elevated and estimated with poor precision, the Bayesian approach implemented in SAAM II was used, with normal a priori distribution (mean = 10, SD = 5, min). Errors in C-peptide measurements were assumed uncorrelated, Gaussian and zero mean, with a variance linked to C-peptide (CP) measurements according to the model: $\sigma^2(CP) = 2000 + 0.001 \cdot CP^2$ [42], where C-peptide concentrations are expressed in pmol/L. Thus, the coefficients of variations of C-peptide measurements, on average, ranged from approximately 10% at basal level down to 4% at highest levels, over all participants. Glucose concentration, interpolated with spline between data, and its time derivative were assumed as error-free model inputs. Basal values were identified with pretest samples. Area under curves was calculated using the trapezoidal rule. The indexes Φ_{oral} and Φ_b, and the time course of the insulin secretion rate, ISR (pmol/min), were subsequently computed for individual cases (Appendix A). The integral of ISR(t) over the 5 hours of our OGTT yielded the total amount of insulin secreted (TIS, pmol) by the β-cells.

2.5. Insulin clearance

Circulating insulin concentrations are dependent upon β-cell insulin release through insulin distribution and clearance [9,22,43]. Alterations of insulin clearance in hypertension have been reported [4-7,9,11]. The ability of our hypertensive patients to remove insulin from the circulation was tested in the present study by computing the C-peptide vs insulin molar ratio [5], that is, the ratio between the areas under the curve of C-peptide (CPAUC) and insulin (IAUC).

Renal functionality was assessed by estimating the creatinine clearance, CRC (mL/min), with the Cockcroft formula (http://www.intmed.mcw.edu/clincalc/creatinine.html):

$$CRC = \frac{(140 - age) \times BW}{72 \times SCC}, \quad (3)$$

where BW is body weight (kg) and SCC is serum creatinine concentration (mg/dL). In female patients, the result was multiplied by 0.85.

2.6. Disposition index

In the context of IVGTT, Bergman et al [19] postulated that, if β-cells are normal, the insulin sensitivity vs β-cell function relationship could most efficiently be expressed as a rectangular hyperbola. In other words, the product of insulin sensitivity and β-cell function would be equal to a constant, which was named *disposition index* (DI). If β-cells of an individual respond to a decrease in insulin sensitivity by adequately increasing insulin secretion, the DI is unchanged and normal glucose tolerance is retained. In contrast, if there is not an adequate compensatory increase in β-cell function to the decreased insulin sensitivity, the individual is expected to develop glucose intolerance [19,20,43]. More recently, the question arose as to whether the relationship between an insulin secretion index and an insulin sensitivity index actually conforms to a hyperbola. Indeed, given that different empirical and model-derived indexes have been used for insulin secretion and sensitivity, the hyperbolic relation cannot assume general value [22-24]. Based on C-peptide minimal model interpretation of IVGTT and OGTT data, disposition indexes have been defined as the product of each β-cell sensitivity (static, dynamic, global, and basal) index and an insulin sensitivity index [21,24]. The total plasma insulin response to OGTT (ie, IAUC) has also been used to define one more empirical disposition index, among several others [22]. In the present

study, a general expression was considered in the form of power function:

$$\text{Insulin secretion index} = DI \times (\text{insulin sensitivity index})^{-\alpha} \quad (4)$$

with both DI and α assumed as free parameters to be estimated by fitting to data. The particular case of a hyperbolic function, obtained under the assumption of α equals 1, was also considered [22-24].

2.7. Statistical analysis

All data and results are given as means ± SE or means and 95% confidence interval (CI) when useful. The Lilliefors test [44], which is 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 the N and H groups. A value of P less than .05 was considered as statistically significant. Wilcoxon (Mann-Whitney) rank sum test was used to compare samples that were not normally distributed (significance was set at 5% level).

3. Results

3.1. Clinical data

Our H and N groups featured no significant differences in fasting and 2-hour glycemia. Fasting and 2-hour plasma insulin and C-peptide concentrations, triglycerides, and diastolic blood pressure were significantly higher in the H group than in the N group. No significant differences were observed in all other variables reported in Table 1.

Mean plasma glucose, $G(t)$; insulin, $I(t)$; and C-peptide, CP(t), responses to a 75-g oral glucose challenge are illustrated in Fig. 2A, B, and C, respectively. Compared with the N group, our H group featured a significant increase ($P < .05$, unpaired Student t test) in plasma glucose concentration in the time frame between 10 and 30 minutes, whereas a significant increase in plasma C-peptide ($P < .05$, unpaired Student t test) and insulin ($P < .05$, Wilcoxon rank sum test) concentrations occurred in the time frame between 0 and 135 minutes. At the 90th minute after glucose challenge, plasma insulin concentration showed, in the H group, a huge peak, followed by a steep decrease (Fig. 2B).

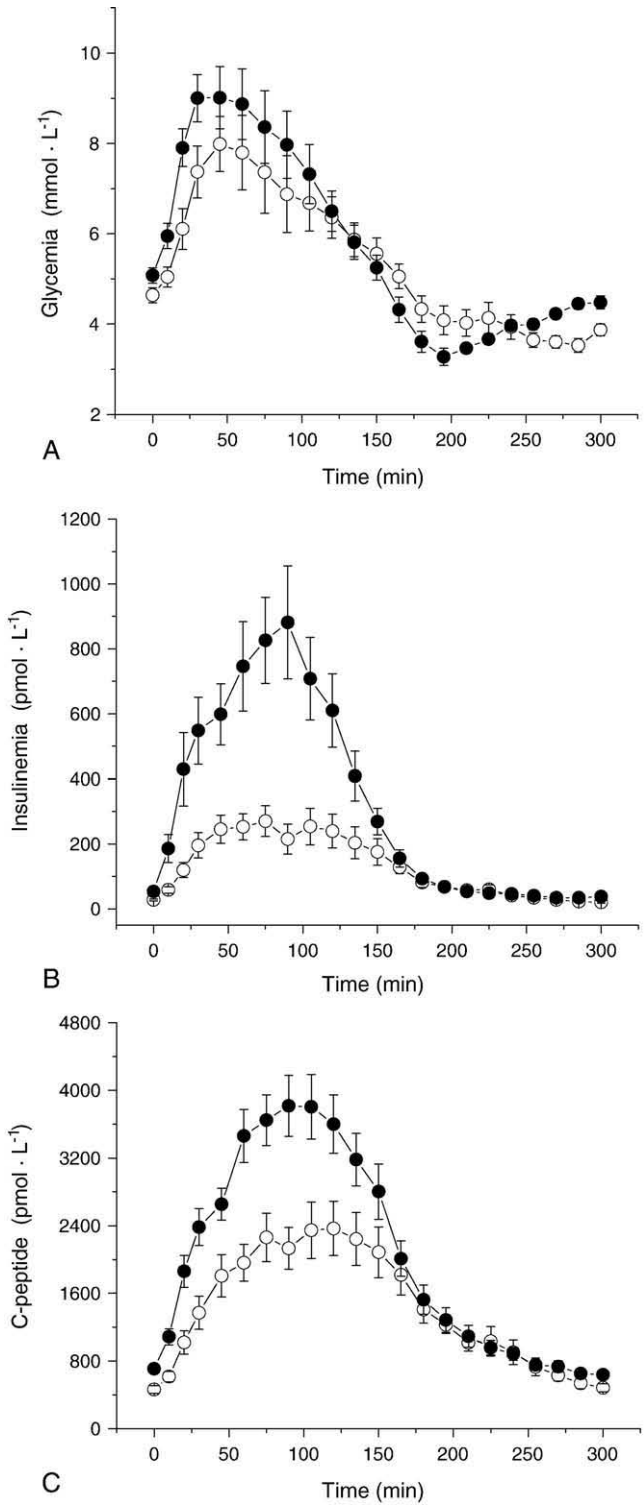

Fig. 2. Time course of plasma glucose (A), insulin (B), and C-peptide (C) concentrations during OGTT in our groups of N subjects (open circles) and H patients (closed circles). Values are expressed as mean ± SE over 10 cases.

3.2. Insulin action

Mean S_I (95% CI) values obtained from the IE method were 2.75 (1.41-4.10) × 10^{-4} $min^{-1}·dL·kg^{-1}$/(pmol/L) for the N group and 0.87 (0.43-1.32) × 10^{-4} $min^{-1}·dL·kg^{-1}$/(pmol/L) for the H group. Mean QUICKI (95% CI) values were 39.1 (37.4-40.9) × 10^{-2} for the N group and 35.1 (33.1-37.0) × 10^{-2} for the H group. Thus, in the absence of MS, the hypertensive state was characterized by a significant 68% reduction ($P < .01$, unpaired Student

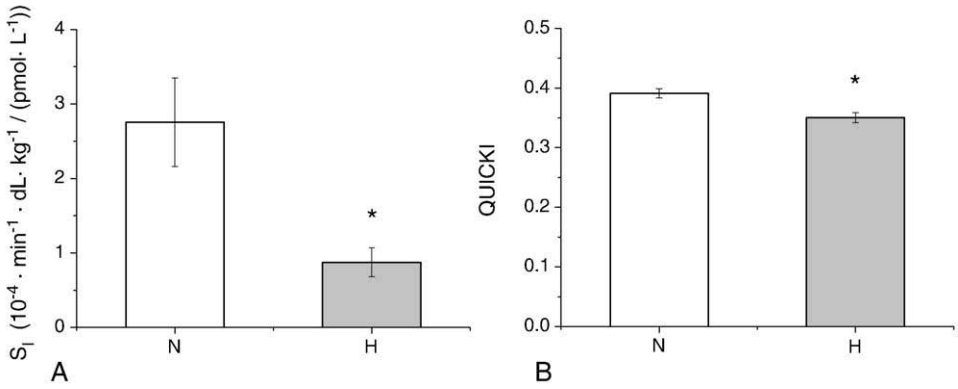

Fig. 3. A, Mean ± SE estimates of S_I index obtained from application of IE method (Eq. [1]) to OGTT data from N subjects (open bar) and H patients (shaded bar). B, Mean ± SE estimates of QUICKI obtained from fasting glycemia and insulinemia (Eq. [2]) of N subjects (open bar) and H patients (shaded bar). *$P < .01$ in comparing H and N groups (unpaired Student t test).

t test) of S_I and a significant (although much smaller) 10% reduction ($P < .01$, unpaired Student t test) of QUICKI (Fig. 3A and B).

3.3. Insulin secretion

C-peptide data fit provided by the model was good as judged from the mean weighted residuals, which showed no systematic deviation from zero, behavior consistent with the hypothesis that the measurement error was a random variable normally distributed around zero. Mean CV% (±SE) over all N and H individuals gathered together, associated with the parameter estimates provided by the C-peptide oral minimal model, were as follows: 16.3 ± 1.7 for Φ_d, 2.9 ± 0.3 for Φ_s, 17.7 ± 2.1 for T, and 1.0 ± 0.1 for h.

No significant differences ($P > .05$, unpaired Student t test) were observed in mean Φ_d, Φ_s, and Φ_{oral} between our H and N groups (Fig. 4A, B, and C, respectively), whereas a significant 38% increase of mean Φ_b ($P < .02$, unpaired Student t test) characterized the H group (Fig. 4D). No differences were observed in mean delay constant, T (10.4 ± 2.0 minutes in the N group and 10.4 ± 4.1 minutes in the H group, $P > .05$, Wilcoxon rank sum test); mean glucose threshold, h (3.97 ± 0.11 mmol/L in the N group and 4.23 ±

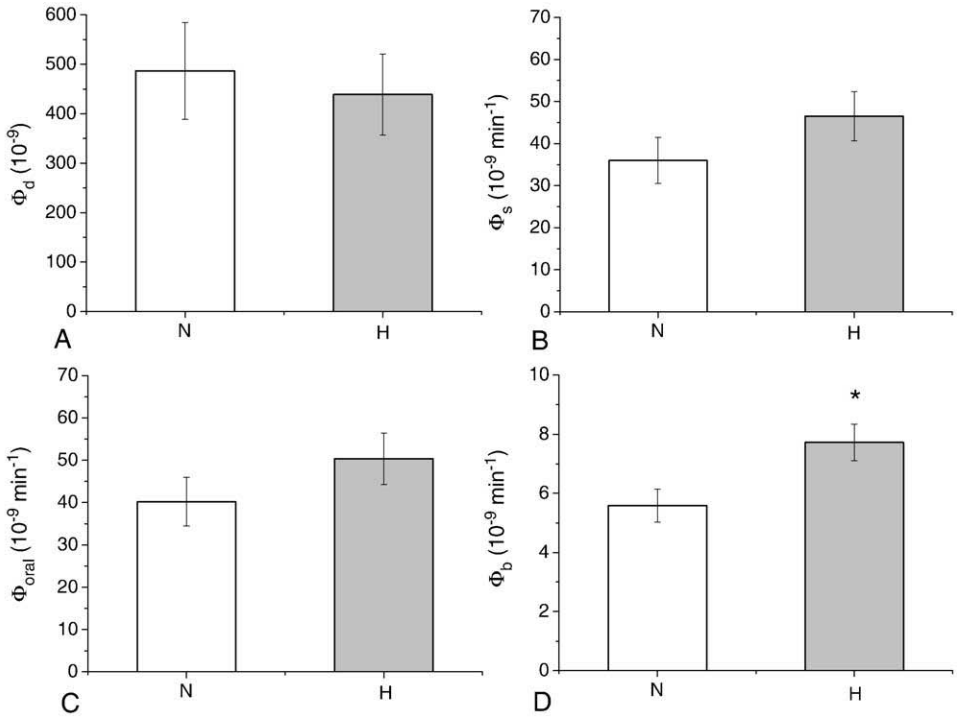

Fig. 4. β-Cell responsivity quantified by dynamic (Φ_d, A), static (Φ_s, B), global (Φ_{oral}, C), and basal (Φ_b, D) sensitivity indexes in N subjects (open bar) and H patients (shaded bar). *$P < .02$ in comparing H and N groups (unpaired Student t test).

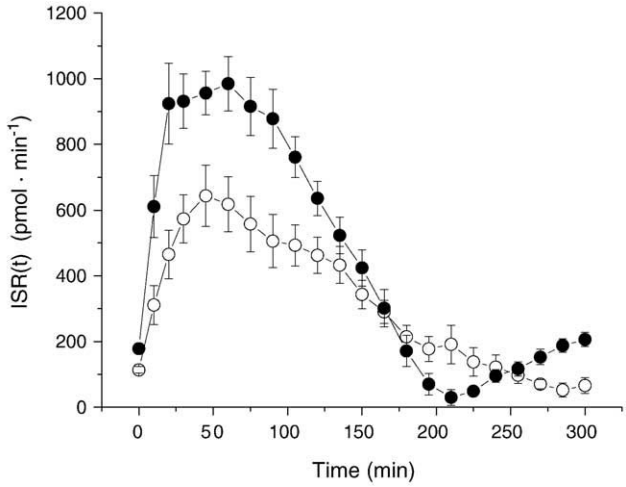

Fig. 5. Time course of insulin secretion rate (ISR, Eq. [A13]) during OGTT in our groups of N subjects (open circles) and H patients (closed circles). Values are expressed as mean ± SE over 10 cases.

0.12 mmol/L in the H group, $P > .05$, unpaired Student t test); and mean distribution volume, V_1 (4.2 ± 0.1 L in the N group and 4.1 ± 0.1 L in the H group, $P > .05$, unpaired Student t test).

Mean basal insulin secretion rate, SR_b, showed a significant increase (unpaired Student t test, $P < .002$) in the H group compared with the N group (38.8 ± 2.6 vs 25.6 ± 2.5 pmol·min^{-1}·L^{-1}). Mean profiles of the insulin secretion rate, ISR(t), predicted by the C-peptide minimal model during the entire OGTT are illustrated in Fig. 5. A significant increase ($P < .05$, unpaired Student t test) of ISR (t) occurred in the H group in the time frame between 0 and 120 minutes. Integration of ISR(t) over 5 hours showed a significant ($P < .02$, unpaired Student t test) 42% increase in model-predicted TIS in the H group (Fig. 6A), which is consistent with a 42% increase ($P < .02$, unpaired Student t test) in mean CPAUC (Fig. 6B). Compared with these markers of β-cell insulin secretion, a much higher increase (143%, $P < .005$, unpaired Student t test) of the IAUC was observed in the H group (Fig. 6C). As a consequence, the CPAUC/IAUC ratio was, in this group, 34% lower ($P < .01$, unpaired Student t test). These results demonstrate that, on average, throughout the 5-hour duration of our OGTT, there was a much higher availability of peripheral insulin in hypertension than could be expected from increased TIS and CPAUC.

3.4. Relationship between insulin action and secretion

Scatter plot of our estimates of Φ_d, Φ_s, and Φ_{oral} vs S_I (Fig. 7A, B, and C) showed no sign of the expected inverse relationship between β-cell sensitivity to glucose challenge and insulin sensitivity. Thus, neither a generalized power function (Eq. [4]) of the form $\Phi_i = DI \times S_I^{-\alpha}$ (with $i = d$, s, and oral) nor a hyperbolic function ($\alpha = 1$) applied to these data. An increasing trend is visible in Φ_b with decreasing QUICKI (Fig. 7D). However, scatter of data and limited range of variation of both Φ_b and QUICKI did not allow a suitable fit by nonlinear regression.

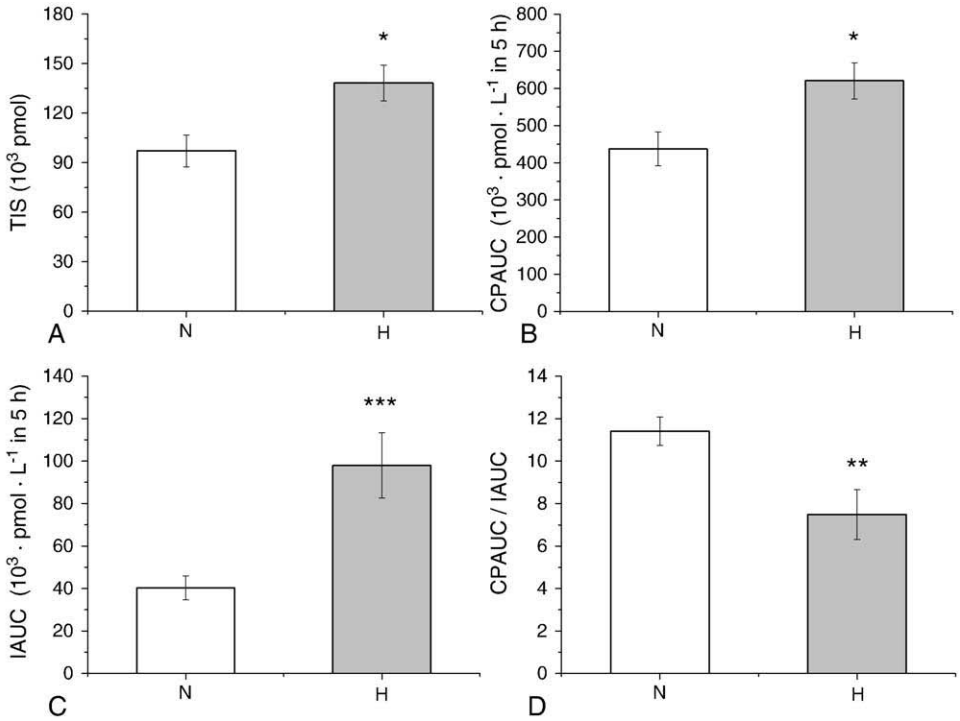

Fig. 6. The OGTT-based mean ± SE values of C-peptide model-predicted TIS (A), CPAUC (B), IAUC (C), and CPAUC/IAUC (D) in N subjects (open bar) and H patients (shaded bar). *$P < .02$, **$P < .01$, and ***$P < .005$ in comparing H and N groups (unpaired Student t tests).

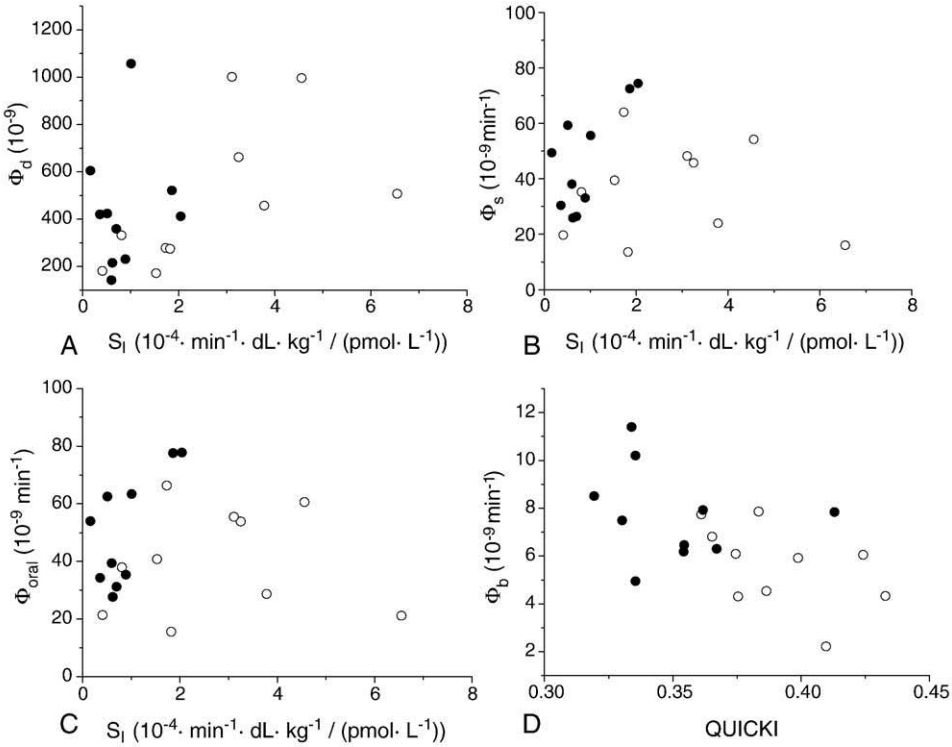

Fig. 7. Scatter plots of dynamic (Φ_d, A), static (Φ_s, B), and global (Φ_{oral}, C) responsivity indexes vs S_I after OGTT in N subjects (open circles) and H patients (closed circles). D, Scatter plot of basal (Φ_b) sensitivity index vs QUICKI in N subjects (open circles) and H patients (closed circles).

In contrast to β-cell responsivity vs S_I estimates of Fig. 7, a net nonlinear inverse relationship is visible in the scatter plot of IAUC vs S_I displayed in Fig. 8. Data of the N and H groups were best fitted by a nonlinear regression line of the form IAUC=DI × $S_I^{-\alpha}$ (solid line). Estimated DI (CV%) and α (CV%) were 6.6 (8.5) min^{-1}·dL·kg^{-1} in 5 hours and 0.59 (12), with r = 0.88. This power function yielded a significantly better fit than the traditional hyperbolic disposition index function, IAUC = DI × S_I^{-1} (dash dot line), with DI = 4.1 (10) min^{-1}·dL·kg^{-1} in 5 hours and r = 0.71.

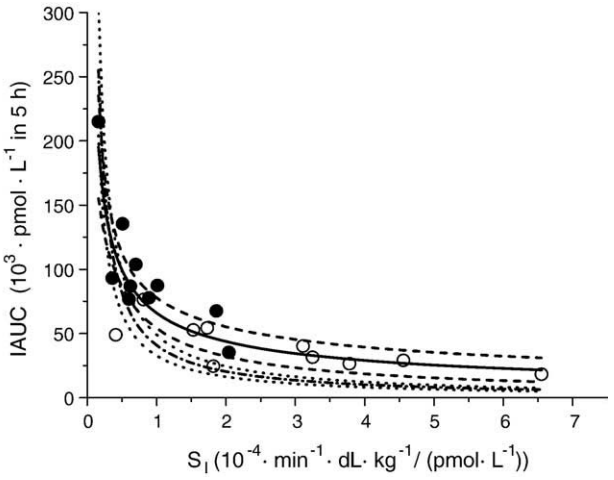

Fig. 8. The OGTT-based scatter plot of IAUC data vs S_I in N subjects (open circles) and H patients (closed circles). Continuous line is the best fitting curve, with 95% CI (dashed lines), described by a power function (DI = IAUC × S_I^{α}). Dash-dot line is best fitting disposition index curve, with 95% CI (dotted lines), described by a hyperbolic function (DI = IAUC × S_I). See "Results" for details on DI and α parameter estimates.

4. Discussion

Although several studies have reported evidence that patients with essential hypertension are insulin resistant and hyperinsulinemic compared with normotensive individuals, there is not yet unanimous consensus as to whether or not there is a physiologic relationship among insulin resistance, compensatory hyperinsulinemia, and high blood pressure [15]. Discrepancies in reports from different research groups may depend on differences in methods used to quantify characteristic parameters of insulin sensitivity and β-cell responsiveness, as well as on differences in selection criteria of hypertensive and control samples. Furthermore, statistical technique of factor analysis, often used to evaluate the role of hypertension among a cluster of abnormalities that associate with insulin resistance and/or hyperinsulinemia, yields conclusions that are likely to be affected by etiologic and clinical heterogeneity of patients with essential hypertension [15].

The present study exercised special care in recruiting the participants to avoid confounder effects of age, sex, obesity, and MS in evaluating the relationships between insulin

action and secretion in essential hypertension. This limited the number of participants, but improved the quality of our analysis of insulin action and secretion in relation to hypertension on its own right.

All participants underwent a 5-hour, 22–blood sample OGTT. Data were interpreted by the oral C-peptide minimal model [21,24,31,32] and the "IE" derived from adaptation of the minimal model of glucose kinetics to oral glucose tests [33]. A previous study by us [34] showed that the IE, compared with other empirical and model-based formulas, is able to discriminate the insulin-resistant state in hypertension and has the advantage of providing an S_I index characterized by measure units consistent with the definition of insulin sensitivity as the ability of insulin to enhance glucose effectiveness. Estimates of S_I in response to glucose challenge were contrasted with QUICKI estimates to infer information as to the relative contribution of the liver and peripheral tissue (ie, skeletal muscle and adipose tissue) to metabolic insulin sensitivity [14], as discussed below.

A significant reduction of S_I (Fig. 3A) and QUICKI (Fig. 3B) in our H group compared with the N group confirmed an extant insulin-resistant state in hypertension associated with significantly higher fasting and 2-hour plasma insulin concentration, without confounding effects of age, sex, obesity, and MS factors (Table 1). This finding meets the conclusions of the European Group for the Study of Insulin Resistance [8]. In this report, after examining the relationship between a specific measure of insulin-mediated glucose disposal, fasting insulin concentration, and blood pressure in 333 normotensive individuals from 20 different clinical research centers, blood pressure was shown to be related to both insulin resistance and insulin concentration independently of age, sex, and degree of obesity.

In quantitative terms, the significant 68% reduction of S_I obtained here from IE-based interpretation of OGTT data (Fig. 3A) is in agreement with the significant (40%-70%) reductions found in previous studies based on minimal model interpretation of IVGTT [6,13,16] and euglycemic-hyperinsulinemic clamp [1,9]. Moreover, the significant reduction of QUICKI in hypertension found here (Fig. 3B) agrees with a previously reported conclusion by Chen et al [14] that QUICKI is a useful tool for evaluating and following the insulin resistance of hypertensive patients. A relevant aspect that the present OGTT-based study shares with our previous IVGTT-based study [16] is that a significant deterioration of insulin action occurs in hypertension, in the absence of MS defined by the ATP III criteria [18].

The QUICKI and S_I indexes portray somewhat different information on insulin resistance components [14]. Because QUICKI (Eq. [2]) is based on fasting glucose and insulin values, this index primarily reflects hepatic insulin sensitivity (ie, the ability of insulin to suppress hepatic glucose production). The S_I index, computed by the IE (Eq. [1]) derived from minimal model of glucose kinetics adapted to oral glucose test, lumps together the contribution of the liver and peripheral tissue to metabolic insulin sensitivity [33]. The much higher reduction (68% vs 10%) of S_I compared with QUICKI suggests a prominent role of peripheral insulin over hepatic sensitivity impairment [14].

Insulin sensitivity and plasma insulin concentration are mutually related, and it is expected that insulin resistance is compensated by an increased insulin secretion [2,8,15,19,20,22-24,43]. Quantitative relationship between these 2 factors has been investigated here by estimating indexes of glucose-stimulated (dynamic, Φ_d; static, Φ_s; and global, Φ_{oral}) and nonstimulated (basal, Φ_b) β-cell responsiveness from C-peptide oral minimal model, to be contrasted with insulin sensitivity indexes. No significant variations of mean Φ_d, Φ_s, and Φ_{oral} (Fig. 4A, B, and C) were observed between our H and N groups in response to glucose challenge. Thus, insulin sensitivity deterioration in our H group was not mirrored by a reciprocal change in β-cell responsivity, such that there was no evidence of hyperbolic relationship between β-cell sensitivity and S_I (Fig. 7A, B, and C). This result is consistent with those found in previously reported studies [6,13] based on the application of C-peptide models to IVGTT data. Higher values of β-cell sensitivity indexes during OGTT (present study) than during our previous IVGTT-based study [13] are consistent with reported findings that incretin effect, more involved in insulin response using oral as opposed to intravenous glucose, enhances insulin secretion in response to given glucose concentration and its rate of change [29,30].

With no significant changes in β-cell responsivity, a significant increase of instantaneous β-cell insulin secretion rate, ISR(t), in the H group, as predicted by the model (Fig. 5), during the first 2 hours of the test appears to be a consequence of S_I worsening and related increase of instantaneous glycemia level (Fig. 2A). Consistently, the TIS index obtained from integration of ISR(t) over the 5 hours of test duration showed, in the H group, a significant increase comparable with that of CPAUC (Fig. 6A and B).

In the present study, β-cell responsivity was complemented by a basal, nonstimulated sensitivity index, Φ_b, which, differently from glucose-load stimulated indexes, Φ_d, Φ_s, and Φ_{oral}, showed a significant increase in our H group (Fig. 4D). As a consequence, under basal condition, in the presence of no significant difference in fasting glycemia, a significant compensatory β-cell secretory response (SR_b) occurred ("Results"). This finding is consistent with results from an IVGTT-based study previously reported by others [6].

By envisioning the β-cell compensating for insulin resistance, Bergman et al [19] postulated that, if β-cells are normal, the relationship between β-cell and insulin sensitivity indexes could most efficiently be expressed as a rectangular hyperbola; that is, DI is a constant in Eq. (4), with $\alpha = 1$. DI was named *disposition index*. Recent criticism of this approach relates to theoretical and experimental assessment of a power function (ie, Eq. [4] with

variable α) as a more appropriate descriptor of glucose tolerance, which is, therefore, characterized by 2 parameters (DI and α) rather than 1 (DI) [22-24]. Scatter plots of β-cell sensitivity to glucose vs insulin sensitivity found here (Fig. 7 A, B, C, and D) could not be fitted with a hyperbola or by the power function of Eq. (4), such that no DI and α parameters could be reliably determined. From this observation, one can infer that β-cell compensatory response to insulin resistance would be insufficient unless a further regulatory mechanism, in addition to increased insulin secretion, contributed to actuate, in the H group, the 143% ($P < .005$) increase of circulating insulin concentration (Fig. 6C), which is the most important determinant of glucose disposal. In accordance with previously reported findings, this additional compensatory response can be ascribed to reduced insulin clearance [4,5,7,9,11,43]. Information on possible alteration of insulin clearance was inferred here by comparing C-peptide vs insulin molar ratio (CPAUC/IAUC) measured in our H and N groups [5]. C-peptide is cosecreted with insulin in equimolar concentration, is not extracted by any appreciable extent by the liver, and has a constant metabolic clearance rate over a wide range of physiologic serum concentration. In contrast, circulating insulin concentrations are dependent upon β-cell insulin release through insulin distribution and clearance [22-24,43]. Based on these considerations, the significant 34% ($P < .01$) reduction of CPAUC/IAUC found here (Fig. 6D) is likely to be due to a decreased ability of hypertensive patients to remove insulin from the circulation. Insulin elimination occurs mainly through metabolic degradation, which involves the liver and kidney and is thought to be largely receptor mediated [9, 43]. No alteration in renal degradation of insulin is inferred from no difference in serum creatinine and creatinine clearance across our H and N groups (Table 1). A significant increase of triglycerides in our hypertensive patients (Table 1) may suggest an increase of free fatty acid such that both these factors might decrease insulin sensitivity and clearance [43]. The role of liver is controversial. Reduced hepatic insulin degradation in hypertension was inferred by Giugliano et al [5] from decreased C-peptide to insulin ratio. A similar conclusion can also be inferred from results of the present study. This is, however, in contradiction with the elevated hepatic insulin extraction in patients with essential hypertension reported by Kautzky-Willer et al [6]. Etiologic and clinical differences in patients with hypertension may explain contradictory findings. Differently from our hypertensive patients, those involved in the study by Kautzky-Willer et al [6] featured, on average, significantly higher fasting plasma glucose concentration (although no one had overt diabetes), whereas BMI ranged from 19 to 32 kg/m^2, thus involving obesity. One more relevant difference with respect to our study is that total insulin delivery (ie, the amount of insulin in the periphery), although elevated in hypertensive patients considered by Kautzky-Willer et al [6], was not statistically significant compared with control subjects. Eventually, as discussed above, incretin effect, more involved in insulin response using oral (present study) than intravenous [6] glucose, makes a further difference [29,30].

Although the data presented here do not allow us to specifically assign a mechanism for insulin clearance impairment in our hypertensive patients, in accordance with previous reports [4,5,7,9,11,43], we can speculate that decreased insulin clearance is a further regulatory mechanism, in addition to increased insulin secretion, to compensate for insulin resistance. Up-regulation of circulating insulin results in an inverse relation between IAUC vs S_I data (Fig. 8) that, gathering H and N subjects together, was described by the power function IAUC = DI $\times$ $S_I^{-\alpha}$ (with DI and α equal to 6.6 min^{-1}·dL·kg^{-1} in 5 hours and 0.59, respectively) better than by a hyperbola ("Results"). This power function encompasses the idea that the ability of up-regulation of circulating insulin in response to a decrement in insulin sensitivity preserves glucose tolerance in nonobese and normoglycemic hypertensive patients not even affected by MS. Because we performed a nonlinear regression on original IAUC vs S_I data, by considering S_I as an independent variable rather than using a regression with error in 2 variables, the DI and α estimates might be not fully correct [24]. Our assumption of no error on S_I, however, was consequent to the fact that the IE-based computation of S_I (as well as all methods that provide an insulin sensitivity value by means of a formula and not by a parameter estimation from data fit) does not provide an estimation error on the computed value. The uncertainty in the *DI* and α estimates is likely to be limited in our study by the fact that, hypertension being the only discriminator between our H and N groups, all data are pretty close to the best fitting power function curve (Fig. 8).

4.1. Conclusions

Significant reductions of both QUICKI and S_I indexes in our H group compared with the N group confirm an association between insulin resistance and hypertension, in the absence of MS. Interpretation of OGTT data by C-peptide oral minimal model indicates that this insulin-resistant state does not affect dynamic β-cell responsivity (Φ_d, Φ_s, and Φ_{oral}) to glucose challenge. Rather than being mirrored by a reciprocal change in β-cell responsivity (as would commonly be expected), in our clinical setting, insulin resistance was compensated by an adequate up-regulation of peripheral insulin, as demonstrated by a 143% ($P < .005$) increase of the IAUC in the H group and by power-function fit of IAUC vs S_I data from all participants (Fig. 8). A 34% ($P < .01$) reduction of C-peptide vs insulin molar ratio (CPAUC/IAUC) in hypertensive patients yielded further evidence that up-regulation of peripheral insulin in the H group was more effective than could be expected from the limited compensatory response by the β-cells. Our results support the hypothesis that decreased insulin clearance in our hypertensive patients, not affected by MS, is a further regulatory mechanism, in addition to increased insulin secretion, to compensate for decreased insulin sensitivity.

Acknowledgment

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Appendix A

With reference to the 2-compartment model depicted in Fig. 1, equations of C-peptide kinetics are as follows [21,26,31,32]:

$$\dot{CP}_1(t) = -(k_{01}+k_{21})\cdot CP_1(t) + k_{12}\cdot CP_2(t) + SR(t); \quad CP_1(0)=0 \tag{A1}$$

$$\dot{CP}_2(t) = k_{21}\cdot CP_1(t) - k_{12}\cdot CP_2(t); CP_2(0)=0\,, \tag{A2}$$

where the over-dot indicates time derivative; CP_1 and CP_2 (pmol/L) are C-peptide concentrations above basal in the accessible and peripheral compartment, respectively; k_{01}, k_{21}, and k_{12} are characteristic parameters (with measure units of min^{-1}) of C-peptide kinetics; and SR ($pmol\cdot L^{-1}\cdot min^{-1}$) is the above-basal β-cell secretion rate, normalized by the volume of distribution of CP_1 compartment.

Pancreatic secretion rate, SR, is expressed by the sum of a component, SR_s, controlled by glucose concentration (static control), and a component, SR_d, controlled by the rate of glucose increase in response to glucose challenge (dynamic control):

$$SR(t) = SR_s(t) + SR_d(t) \tag{A3}$$

The SR_s component is assumed to be equal to the provision of new insulin to the β-cells (Y, $pmol\cdot L^{-1}\cdot min^{-1}$)

$$SR_s(t) = Y(t) \tag{A4}$$

The following relation between plasma glucose concentration, $G(t)$, and $Y(t)$ is assumed:

$$\dot{Y}(t) = -\frac{1}{T}\cdot\{Y(t) - \Phi_s\cdot[G(t)-h]\}; Y(0)=0 \tag{A5}$$

According to Eqs. (A4 and A5), SR_s tends, with time constant T, toward a steady-state value linearly related, through the Φ_s (min^{-1}) parameter, to the difference between instantaneous glucose concentration $G(t)$ and a threshold h (mmol/L). The parameter Φ_s is given the meaning of static β-cell sensitivity, which is a marker of the stimulatory effect of glucose load on β-cell secretion in steady state.

The SR_d component of Eq. (A3) is assumed to represent the secretion of insulin stored in the β-cell in a promptly releasable form (labile insulin). Labile insulin is not homogeneous with respect to the glucose stimulus: for a given glucose step, only a fraction of labile insulin is mobilized, so that more insulin can be rapidly released in response to a subsequent more elevated glucose step. In accordance with the M1 version of C-peptide model described by Toffolo et al [31] and subsequently used by Dalla Man et al [32], the amount of released insulin (dQ) in response to a glucose increase from G to $G + dG$ was assumed proportional to dG:

$$dQ = \Phi_d\cdot dG, \tag{A6}$$

such that the flow of insulin secretion, SR_d, is proportional, through a dimensionless parameter, Φ_d, to the derivative of glucose:

$$SR_d(t) = \begin{cases} \dfrac{dQ}{dt} = \Phi_d\cdot\dfrac{dG}{dt} & \text{if } \dfrac{dG}{dt}>0 \text{ and } G(t)>G_b \\ 0 & \text{otherwise} \end{cases} \tag{A7}$$

The Φ_d parameter describes the dynamic control of glucose on insulin secretion, that is, the effect of the rate of change of glucose on secretion of stored insulin, when glucose concentration is increasing (dG/dt positive). If we define a parameter X_0 (pmol/L) as the amount of insulin (per unit of C-peptide distribution volume) released in response to the maximum glucose concentration, G_{max}, achieved during the experiment:

$$X_0 = \int_{G_b}^{G_{max}} dQ = \int_{G_b}^{G_{max}} \Phi_d\cdot dG = \Phi_d\cdot(G_{max}-G_b) \tag{A8}$$

The Φ_d parameter equals X_0 normalized to glucose increment ($G_{max} - G_b$):

$$\Phi_d = \frac{X_0}{G_{max}-G_b} \tag{A9}$$

and is referred to as an *index of dynamic sensitivity*.

The C-peptide model described by Eqs. (A1-A9) is characterized by the parameters k_{01}, k_{12}, and k_{21} of C-peptide kinetics and by 4 more parameters of C-peptide (and insulin) secretion: T, h, Φ_d, and Φ_s. Distribution volume, V_1, and characteristic parameters of C-peptide kinetics were adjusted to subject's anthropometric data, according Van Cauter et al [27], as described below. Free model parameters T, h, Φ_d, and Φ_s were estimated from fitting to C-peptide data as described in "Materials and methods."

The model also allows quantification of a global index, Φ_{oral} (min^{-1}), of β-cell responsivity to glucose, which is defined as the average increase above basal of β-cell secretion (Eq. [A3]) divided by the average glucose stimulus above the h threshold [21]:

$$\Phi_{oral} = \frac{\int_0^\infty SR(t)\cdot dt}{\int_0^\infty [G(t)-h]\cdot dt} \tag{A10}$$

According to Breda et al [21], this parameter can be computed by the following equation:

$$\Phi_{oral} = \Phi_s + \frac{\Phi_d\cdot(G_{max}-G_b)}{\int_0^\infty [G(t)-h]\cdot dt} \tag{A11}$$

A basal sensitivity index, Φ_b (min^{-1}), measures the ratio of basal insulin secretion rate to basal glucose concentration [21,31]:

$$\Phi_b = \frac{SR_b}{G_b} = \frac{k_{01} \cdot CP_{1b}}{G_b} \quad (A12)$$

Insulin secretion profile, ISR (pmol/min) is described by the following equations:

$$ISR(t) = \{SR_b + SR(t)\} \cdot V_1 = \begin{cases} \left\{k_{01} \cdot CP_{1b} + Y(t) + \Phi_d \cdot \frac{dG}{dt}\right\} \cdot V_1 & \text{if } \frac{dG}{dt} > 0 \text{ and } G(t) > G_b \\ \{k_{01} \cdot CP_{1b} + Y(t)\} \cdot V_1 & \text{otherwise} \end{cases} \quad (A13)$$

In these equations, V_1 (L) is C-peptide distribution volume of the accessible CP_1 compartment of Fig. 1.

From ISR(t), it is possible to compute the 5-hour TIS (pmol) by integrating over the 5 hours of test duration (TD):

$$TIS = \int_0^{TD} ISR(t) \cdot dt \quad (A14)$$

Parameters of Van Cauter et al

In accordance with Van Cauter et al [27], k_{01}, k_{12}, and k_{21} parameters of C-peptide kinetics and distribution volume, V_1, were computed as follows:

$$k_{12} = FRA \cdot B_1 + (1 - FRA) \cdot A_1 \quad (A15)$$

$$k_{01} = \frac{A_1 \cdot B_1}{k_{12}} \quad (A16)$$

$$k_{21} = A_1 + B_1 - k_{12} - k_{01}, \quad (A17)$$

where the parameters FRA, A_1, and B_1 are defined as follows:

$$A_1 = \begin{cases} 0.152 & \text{if BMI} > 29 \\ 0.140 & \text{if BMI} \le 29 \end{cases} \quad (A18)$$

$$FRA = \begin{cases} 0.78 & \text{if BMI} > 29 \\ 0.76 & \text{if BMI} \le 29 \end{cases} \quad (A19)$$

$$B_1 = \frac{0.69315}{0.14 \cdot age + 29.16} \quad (A20)$$

The distribution volume of pool 1 (Fig. 1) was computed as [27]:

$$V_1 = \begin{cases} 1.92 \cdot BSA + 0.64 & \text{for men} \\ 1.11 \cdot BSA + 2.04 & \text{for women} \end{cases} \quad (A21)$$

with BSA (body surface area, m^2) being expressed as a function of body weight, BW (kg), and height, H (cm):

$$BSA = BW^{0.425} \cdot H^{0.725} \cdot 0.007184 \quad (A22)$$

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