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Relationships Between Insulin Resistance Measured with the Minimal Model and Microalbuminuria in Type 2 (Non-insulin-dependent) Diabetics

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ABSTRACT

Using the minimal model technique we investigated whether insulin resistance is associated with microalbuminuria (a marker of increased renal and vascular risk in diabetes) and which parameter of glucose assimilation is more related to microalbuminuria: insulin sensitivity (SI) or glucose effectiveness (Sg). A frequent sampling intravenous glucose tolerance test (FSIVGTT) was performed in a group of 10 microalbuminuric type 2 (non-insulin-dependent) diabetic patients (NIDDM) with an overnight urinary albumin of 41 ± 6.5 μg/min (group A) and a group of 12 normoalbuminuric NIDDM patients (group B) matched with A for age, HbA1c, sex and body mass index. The NIDDMs were compared to a matched control group of non-diabetic subjects. Sg and SI were lower in the diabetic patients ($P<0.01$) than in the controls. Sg was similar in the two diabetic groups and its reduction was explained by a low basal insulin effectiveness (BIE). The insulin resistance index given by the homeostatic model assessment (IR HOMA) was lower in the groups of NIDDM patients compared to controls ($P<0.01$), resulting in lower values of insulin-mediated glucose uptake (IMGU; $P<0.01$) and total glucose uptake (TGU; $P<0.02$) than in the controls. SI was higher in group B [$1.97 \pm 0.54\ min^{-1}/(\mu U/ml) \times 10^{-4}$] than in group A [$0.817 \pm 0.33\ min^{-1}/(\mu U/ml) \times 10^{-4}$]; $P<0.05$], consistent with a higher IMGU (2.55 ± 1.5 vs 0.28 ± 0.09 mg/min^{-1}/kg^{-1}; $P<0.01$) and a lower IR HOMA in this group (3.43 ± 0.5 vs 4.66 ± 0.53 mol/μU/l^{-2}; $P<0.01$). There was a correlation between microalbuminuria and IMGU ($r=0.440$; $P<0.05$). These data confirm that microalbuminuria is associated with a more pronounced insulin resistance in NIDDM subjects.

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INTRODUCTION

Microalbuminuria is a widely recognized predictor of renal and cardiovascular disease in both type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes [1–4]. In the case of type 2 diabetes, it appears to be associated with a cluster of metabolic alterations [5]. Some of these alterations are common

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with those of the insulin resistance syndrome, or 'Syndrome X' [6]. Thus, microalbuminuria may be an additional feature of insulin-resistant states. Consistent with this concept, lowered insulin sensitivity has been reported to be associated with microalbuminuria in both NIDDM patients [7,8] and type 1 (insulin-dependent) diabetes (IDDM) [9]. Insulin resistance might be an additional pathogenetic factor for diabetic renal disease. Glucose clamp studies in NIDDM patients [7,8] suggest that insulin resistance is predominantly associated with hypertension and microalbuminuria, while NIDDM patients without those abnormalities have normal insulin sensitivity. Moreover, Nosadini [7] showed in a longitudinal study that extrahepatic defects in insulin sensitivity seem to precede the development of hypertension and microalbuminuria.

The importance of these studies comes from the support they give to the concept of a link between insulin resistance and the factors of vascular risk in diabetics, and the possibility that some patients with normal values of insulin sensitivity could be less prone to vascular complications [7–9]. However, studies investigating this question have employed the glucose clamp technique which has the advantage of allowing the separate measurement of hepatic and extrahepatic glucose metabolism, but does not easily differentiate insulin-mediated glucose uptake from non-insulin-mediated glucose uptake.

The minimal model technique, which is now well validated [10], gives results equivalent to those of the glucose clamp [11] with the advantage of measuring with a simple procedure not only insulin sensitivity (SI), which is mostly a measurement of insulin-mediated glucose uptake but also glucose effectiveness (Sg), that is the ability of glucose itself to promote its own assimilation, independent of any change of insulinaemia. This latter parameter Sg cannot be easily measured by the clamp, unless multiple steps of insulinaemia are performed. It is largely explained by glucose effectiveness at zero insulin (GEZI) which represents glucose uptake independent of any insulin action [12]. To our knowledge, the relationships between microalbuminuria in NIDDM patients and these parameters SI and Sg has not been investigated. If the minimal model technique, which is simpler than the clamp and can be used routinely, was able to detect among NIDDM patients those who are prone to later develop microalbuminuria and vascular disease, it could have a potential clinical application. Thus, our purpose in this study was to determine (a) whether the minimal model technique detects a relationship between impaired glucose assimilation and microalbuminuria; (b) which minimal model parameter (SI or Sg or both) seems to be more closely involved in this process.

MATERIALS AND METHODS

Diabetic patients

Two groups of NIDDM patients were studied. All patients underwent routine clinical examination, and information on family and personal history was obtained by direct questioning. Diagnosis of NIDDM had been made when fasting plasma glucose (FPG) was >7.8 mmol on two different occasions [13]. Blood pressure (BP) was measured three times in both arms in the sitting position after a 15-min rest. In agreement with the Statement of the Working Group on Hypertension in Diabetes [14], hypertension was considered to be BP >145/90 mmHg. These measurements were made by the same person using a calibrated standard mercury manometer (Spengler, Paris, France). Mean BP was calculated as the diastolic BP plus one-third of pulse pressure. At least three overnight urine collections were requested from each patient to evaluate the albumin excretion rate. The total volume of urine was recorded and the albumin excretion rate (AER) was computed and expressed as micrograms per minute. This was considered normal if all urine collections were <15 μg/min. Microalbuminuria was defined as an albumin excretion rate >15 μg/min in two or three urine collections. On the basis of AER, these patients were divided into two groups. Group A consisted of 10 patients with NIDDM (five women and five men, age 54.7 ± 2.3 years; body mass index 26.9 ± 0.8 kg/m^2) who were microalbuminuric (overnight AER >15 μg/min; mean ± SEM: 41 ± 6.5, range: 15–82.5 μg/min). Group B subjects (six women and six men, age 58.2 ± 3.1 years; body mass index 28.2 ± 2.6 kg/m^2) were normoalbuminuric (<10 μg/min) NIDDM patients matched to patients of group A for age, HbA1c, sex and body mass index. None of the NIDDM patients had ever undergone antihypertensive treatment. They were treated with glibenclamide (15 mg/day) and metformin (2550 mg/day) together with dietary advice, except for one patient in each subgroup in whom oral antidiabetic therapy had been replaced by two daily insulin injections. All these treatments were stopped 12 h before the intravenous glucose tolerance test. The clinical features of the NIDDM patients are listed in Table 1.

A control group of normotensive patients with normal glucose tolerance was also studied. It consisted of 12 subjects (six men, six women) with a mean age of 53.17 ± 2.4 years (range: 44–73 years) and a mean body mass index of 26.3 ± 0.79 kg/m^2 (range: 20.9–30 kg/m^2). This group was matched with the two groups of NIDDM patients described above for age, sex and body mass index. These subjects also underwent an intravenous glucose tolerance test.

Table 1 Clinical data in the two groups of NIDDM patients studied (mean ± SEM). The two groups were matched for age, sex and body mass index. Glycosylated hemoglobin HbA1c was not significantly different in the two groups. Abbreviations: BMI: body mass index; AER: albumin excretion rate; HbA1c: glycated hemoglobin A1c; BP: blood pressure (syst: systolic; diast: diastolic).

Group	Sex M/F	Age (yr)	BMI (kg/m^2)	AER (μg/min)	HbA1c (%)	Duration of diabetes (yr)	Mean BP (mmHg)	BP_{syst} (mmHg)	BP_{diast} (mmHg)
A	5/5	54.7 ±2.3	26.9 ±0.8	41 ±6.5	10.3 ±0.5	6.3 ±1.3	107 ±5	142 ±6	90 ±6
B	6/6	58.2 ±3.06	28.2 ±2.6	6.7 ±1.2	9.0 ±0.6	8.5 ±2.3	99 ±1	144 ±2	76 ±2[a]

[a] $P<0.05$ vs group A.

Laboratory measurements

Urinary albumin excretion rate was measured by radioimmunoassay with the kit 'Albumin RIA 100' from Pharmacia AB, S-75182 Uppsala, Sweden. This technique uses a double antibody radioimmunoassay. Its within-assay coefficient of variation ranges between 2.9 and 4.1% and its detection limit is <0.4 mg/l. HbA1c was measured with high pressure liquid chromatography. Plasma insulin was measured by a radioimmunoassay (kit SB-INSIK-5 from Sorin Biomedica 13040 Saluggia, Italy) and plasma glucose with a Beckman glucose analyser. The within-assay coefficient of variation (CV) for insulin was determined by repeated measurements of the same sample and was between 8.6% (low values) and 9.7% (high values). The between-assay CV for insulin was between 12.5% (low values) and 14.4% (high values). The sensitivity (lowest detectable value) was 2 μU/ml.

Intravenous glucose tolerance test (IVGTT) protocol

After a 12-h fast, at 09.00 h, a cannula was placed in the cephalic vein at the level of the cubital fossa for blood sampling. A glucose injection (0.5 g/kg, solution at 30%) was administered in the contralateral cephalic vein, slowly over precisely 3 min. Blood samples were drawn twice before the glucose bolus and at 1, 3, 4, 8, 10, 15, 19, 20, 22, 30, 41, 70, 90 and 180 min following glucose injection. Insulin (0.02 units/kg body weight, i.e. 1 or 2 units) was injected intravenously immediately after the 19 min sample. The 1 and 3 min samples were used for the determination of insulin early secretory phase [15]. The other samples were necessary for minimal model calculations [16].

Glucose assimilation coefficient 'Kg'

In addition to the minimal model analysis, a separate approach using a classical monoexponential model of glucose disappearance was used. The least square slope of the log of the absolute glucose concentration between 4 and 19 min after the glucose bolus was used as an index of glucose tolerance Kg_{4-19}. The more classical Kg_{10-30} which measures the decrease in blood glucose between 10 and 30 min [17] was not employed in this study since its results may be influenced by the insulin injection at the 19th min, while Kg_{4-19} cannot be influenced by this injection.

Measurement of insulin sensitivity and glucose effectiveness

Minimal model analysis of the IVGTT was performed according to Bergman's method [10,18–19] with the software 'TISPAG' from the Department of Physiology of the University of Montpellier I, France [20–22] which uses a non-linear least square estimation. This programme gave the values of insulin sensitivity (SI) and glucose effectiveness (Sg). SI and Sg are calculated from the following equations:

$$dG(t)/dt = -(p1 + X(t))\ G(t) + p1\ Gb \quad (1)$$

$$G(0) = Go \quad (2)$$

$$dX(t)/dt = -p2\ X(t) + p3\ (I(t) - Ib) \quad (3)$$

$$X(0) = 0 \quad (4)$$

where G(t) and I(t) are plasma glucose and insulin concentrations, X(t) is the insulin in a compartment remote from plasma ('insulin action'), and p1–p3 are model parameters. Go is the glucose concentration that one would obtain immediately after injection, if there were instantaneous mixing in the extracellular fluid compartment. Gb and Ib are basal values of glucose and insulin. Parameter p1 represents Sg, i.e. the fractional disappearance rate of glucose, independent of any insulin response. p3 and p2 determine the kinetics of

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

Calculation of the two components of glucose effectiveness

Sg was divided into its two components [12]: the contribution of hyperglycaemia per se to tissue glucose utilization and the effect of basal insulin on glucose uptake. The basal insulin component of Sg is termed the basal insulin effect (BIE) and can be calculated as the product of basal insulin Ib and SI:

$$BIE = Ib \times SI \quad (5)$$

Thus the contribution of non-insulin-dependent glucose uptake (glucose effectiveness at zero insulin, GEZI) to glucose uptake is the difference between total Sg and the BIE:

$$GEZI = Sg - (Ib \times SI) \quad (6)$$

Calculation of insulin and non-insulin-mediated glucose uptake rates

Using the method of Welch *et al.* [23] glucose uptake rates normalized to a plasma glucose level of 11 mmol/l were also calculated from the minimal model data. Insulin-mediated glucose uptake (IMGU) at any level of insulin can be calculated from SI as follows:

$$IMGU = SI \times G_{11} \times I_{11} \times V_D \quad (7)$$

where G_{11} and I_{11} are glucose and insulin values interpolated from individual determinations using the 11 mmol/l value for each patient, usually no more than 1–2 min from the 11 mmol/l value. V_D is the glucose distribution space and is assumed to be 0.16 l/kg. Similarly, non-insulin-mediated glucose uptake (NIMGU) can be calculated as follows:

$$NIMGU = GEZI \times G_{11} \times V_D \quad (8)$$

The sum of NIMGU and IMGU represented total glucose uptake (TGU). All these values (IMGU, NIMGU and TGU) were corrected for body weight and thus expressed as $mg/min^{-1}/kg^{-1}$ body weight.

Validation studies of this minimal model procedure

The validity of our procedure using a reduced number of sampling times has been tested on 13 IVGTTs with values of SI ranging between 0.55 and 16.94 $min^{-1}/(\mu U/ml) \times 10^{-4}$. We compared the results given by the software with a classical protocol including 24 samples (1, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, 180 min, see ref. [10] and with the reduced number of samples proposed by Steil and co-workers [16] and used here. Values of Sg ($r=0.983$; slope: 0.964 intercept: 0.13) and SI ($r=0.974$; slope: 0.863 intercept: 1.20) were highly correlated. The mean relative deviation (defined as the percentage of difference between parameters calculated from the full sample protocol and parameters calculated from the reduced sample protocol) was -1.67 ± 2.46 for Sg and $-10 \pm 5\%$ for SI. This apparently high deviation for SI is explained by changes of less than 0.5 $min^{-1}/(\mu U/ml) \times 10^{-4}$ in NIDDM patients with values of SI lower than 1 $min^{-1}/(\mu U/ml) \times 10^{-4}$. The mean absolute difference between SI calculated with the full protocol and SI calculated with the reduced sampling protocol was 0.91 ± 0.26 $min^{-1}/(\mu U/ml) \times 10^{-4}$. Intra-subject reproducibility of minimal model parameters and Kg has been investigated in eight subjects (including four NIDDM patients) after they underwent two IVGTT under the same conditions at an interval varying between 3 days and 1 year. Coefficients of variation (CV) for paired samples [24] were calculated as mean SD divided by the mean of all the results. Mean SD is the square root of the sum of the SD for each pair divided by the number of paired observations minus one. The SD for each pair is the difference between tests divided by the $\sqrt{2}$. The mean difference between the two tests is $+0.55 \pm 0.38$ $min^{-1}/(\mu U/ml) \times 10^{-4}$ for SI (i.e. a CV of 30.2%), $+0.36 \pm 0.08$ $min^{-1} \times 10^{-2}$ for Sg (i.e. a CV of 18.3%), $+0.34 \pm 0.07$ $min^{-1} \times 10^{-2}$ for GEZI (i.e. a CV of 19.4%) and $+0.29 \pm 0.08$ $min^{-1} \times 10^{-2}$ for Kg (i.e. a CV of 21.4%). Concordance between the two sets of IVGTT parameters was also studied by their correlations: for SI ($r=0.999$; slope: 1.18 intercept: -0.08); for Sg ($r=0.879$; slope: 0.824 intercept: 0.49); for GEZI ($r=0.912$; slope: 0.704 intercept: 0.61); for Kg ($r=0.888$; slope: 1 intercept: -0.22).

Assessment of beta cell function

First phase insulin secretion [15] was calculated by the sum of insulin concentration at the 1st and the 3rd min after the end of glucose injection (I_{1+3}). In NIDDM where first phase is minimal or abolished, I_{1+3} is not a reliable index and may be influenced by high values of Ib. Thus,

incremental first phase insulin response (ΔI_{1+3}) was also measured as $I_{1+3} - (2 \times Ib)$. Since exogenous insulin was added after time 19, the second phase insulin secretion could not be measured.

Homeostasis model assessment (HOMA)

An attempt to evaluate insulin sensitivity was made with the homeostasis model assessment (HOMA), a simple calculation which has been validated in comparison with the euglycaemic clamp [25]. Insulin resistance index is defined as:

$$IR = \text{insulin}/(22.5\, e^{-\ln \text{glucose}}). \quad (9)$$

The lowest of the two baseline values of glucose and insulin before IVGTT was employed for this calculation.

Statistical analysis

Values are expressed as mean ± SEM. Comparisons of insulin and glucose curves were performed with repeated measures ANOVA. Comparisons for metabolic parameters were performed with two-tailed tests for unpaired data. Unpaired *t*-tests were used for comparisons between the groups when the data was normally distributed and the Mann–Whitney *U*-test was used for comparisons between groups when the data was not normally distributed. Correlation by the least squares method was used to determine the association between microalbuminuria and other parameters. Log transformation of the non-normally distributed data was used in regression analysis. Significance was accepted at the $P<0.05$ level.

RESULTS

Table 1 shows that groups A and B were matched for age, sex, HbA1c, and mean blood pressure, with no statistical differences between the groups for these variables. Diastolic BP was lower in group B than in group A ($P<0.05$), but other measurements of BP were not different between the groups. Groups A and B had the same duration of diabetes. The two highest values of BP were 160/100 mmHg (i.e. a mean BP of 120) and corresponded to values of AER of 24.7 and 45.6 μg/min.

Figures 1 and 2 show the blood glucose and insulin levels during IVGTT in the two groups of NIDDM patients. Insulin levels were not statistically different overall, but there were some differences in blood glucose levels which were higher in group A at time −15, 90 and 180 min ($P<0.05$).

IVGTT parameters and the results of the minimal model analysis in the two groups of NIDDM patients and in controls are shown in Tables 2 and 3. Kg, I_{1+3} and ΔI_{1+3} were lower in diabetics than in the control subjects ($P<0.01$) but not significantly different between groups A and B. SI was markedly lower than control values in all NIDDM patients ($P<0.01$). Sg was reduced in 22 of the 32 NIDDM patients. There was a significant difference ($P<0.01$) between controls and the two subgroups combined. However, when groups of NIDDM patients were considered separately, there was no difference between groups A and B. I_{1+3} and BIE were lower in NIDDM patients than in the controls ($P<0.01$). All subgroups of NIDDM patients compared to controls had a lower BIE ($P<0.01$). However, GEZI was not reduced in NIDDM patients compared to controls. Values of Sg, BIE and GEZI were similar in groups A and B. Values of IMGU and TGU were calculated for the value of insulinaemia (I_{11} in equation 7) when the glucose level was 11 mmol/l, which was 53.7 ± 12.25 μU/ml in controls, 24 ± 10.7 μU/ml in group A and 32.75 ± 9.2 μU/ml in group B. IMGU and TGU were lower in all diabetic patients than in controls ($P<0.01$ and $P<0.02$) while values of NIMGU were similar. IMGU was lower in group A than in group B (−89%; $P<0.01$). The two groups had the same value of NIMGU and a considerable overlap in values of TGU which resulted in no significant difference for TGU between them. The insulin resistance index given by HOMA was lower in the groups of NIDDM patients compared to controls ($P<0.01$). SI values were lower in group A than in group B ($P<0.05$), as was the insulin resistance index given by HOMA ($P<0.01$).

In the whole sample of 22 subjects the correlation coefficient between SI and AER was $r = -0.19$ NS. The correlation coefficient between the insulin resistance index given by HOMA and AER was $r = 0.09$ NS. The correlation coefficient between SI and BP was $r = -0.19$ NS. Two patients from group A and three patients of group B were not insulin resistant (SI $>2\ \text{min}^{-1}/\mu\text{U/ml} \times 10^{-4}$). These patients had, however, the same treatment and similar glycaemic control to the other patients. There was a non-linear correlation between AER and IMGU ($r = 0.440$, $P<0.05$) which was found after log transformation of non-normally distributed data (Fig. 3). NIMGU and TGU were not significantly correlated with AER (respectively $r = -0.271$, $r = -0.20$), neither were they correlated with blood pressure. There was no significant correlation between AER and HbA1 ($r =$

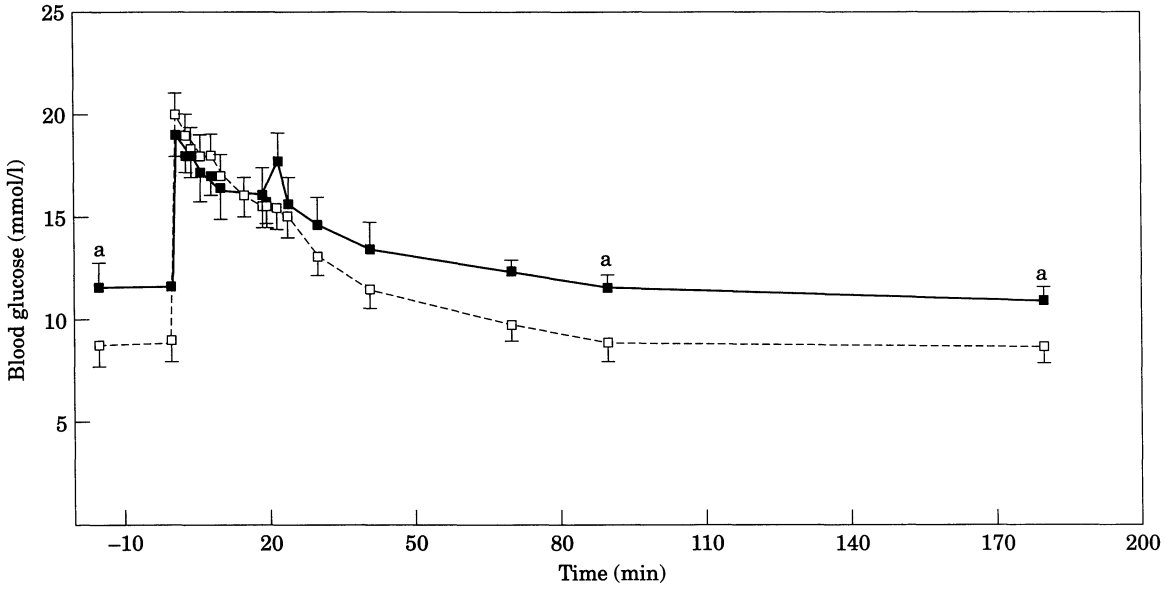

Fig. 1 Blood glucose response to IVGTT in the two groups of NIDDM patients. Group A ($n=10$, black squares and full lines) and group B ($n=12$, white squares and dashed lines). Values given as mean ± SEM. [a] $P<0.05$.

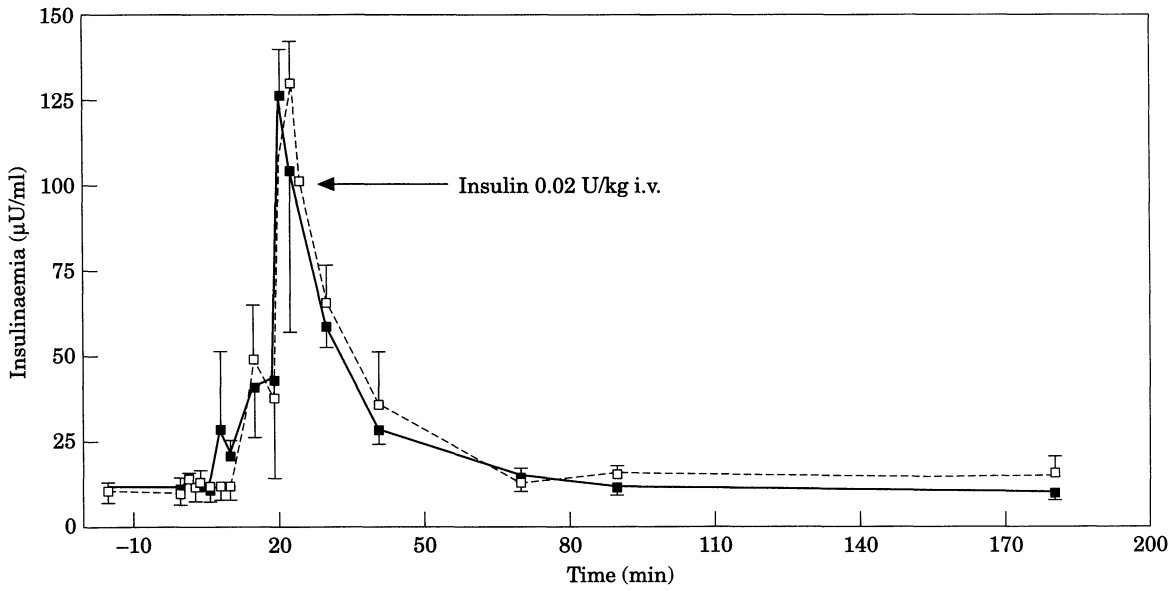

Fig. 2 Insulinaemic response to IVGTT in the two groups of NIDDM patients. Insulin is injected just after time 19 min. Group A ($n=10$, black squares and full lines) and group B ($n=12$, white squares and dashed lines). Values given as mean ± SEM. No significant differences between the two groups.

0.267) and mean blood pressure ($r=0.253$). SI and the insulin resistance index given by HOMA showed a negative correlation ($r=-0.608$, $P<0.01$) in the 22 diabetics and these two measurements of insulin resistance were also correlated in the whole sample of 34 subjects (NIDDM and controls, $r=0.408$, $P<0.05$). The insulin resistance index given by HOMA was negatively correlated to IMGU ($r=-0.459$, $P<0.05$). SI was correlated to IMGU ($r=0.859$, $P<0.01$).

DISCUSSION

The aim of this study was to investigate the association of insulin resistance and microalbuminuria in patients with NIDDM. As far as we know, this study is the first which investigates this association with the minimal model procedure. With this technique, glucose disposal can be described as a result of two mechanisms: insulin action, measured by insulin sensitivity (SI), and glucose

Table 2 IVGTT parameters in the two groups of NIDDM patients and normotensive controls with normal glucose tolerance matched for age, sex and body mass index (mean ± SEM). Ib: baseline insulinaemia; Gb: baseline blood glucose level; G180: blood glucose at the 180th min of IVGTT; Kg_{4-19}: slope of the exponential glucose decrease between 4 and 19 min after glucose infusion; I_{1+3}: sum of insulin values at 1 and 3 min after the end of glucose infusion; ΔI_{1+3}: sum of incremental insulin values at 1 and 3 min after the end of glucose infusion. IR (HOMA) parameter of insulin resistance given by the homeostatic model assessment.

Group	Ib μU/ml	Gb mmol/l	G180 mmol/l	Kg_{4-19} %/min	I_{1+3} μU/ml	ΔI_{1+3} μU/ml	IR (HOMA) mol/μU.l^{-2}
Controls ($n=12$)	7.8 ±0.6	4.6 ±0.1	4.6 ±0.2	2.0 ±0.21	70.4 ±12.9	54.7 ±12	1.7 ±0.26
A ($n=10$)	10.7 ±1.82	10.2 ±0.6[c]	10.9 ±0.7[c]	0.88 ±0.12[c]	22.8 ±3.1[c]	3.7 ±0.9[c]	4.66 ±0.53[c]
B ($n=12$)	8.4 ±1.02	8.0 ±0.8[c]	8.6 ±1[c]	1.10 ±0.22[b]	23.6 ±3.2[c]	7.4 ±3.3[c]	3.43 ±0.51[c,d]
All NIDDMs (A+B) ($n=22$)	9.4 ±1	8.9 ±0.6[c]	9.3 ±0.7[c]	1.0 ±0.1[c]	23.3 ±2.2[c]	5.7 ±1.9[c]	3.99 ±0.39[c]

[a] $P<0.05$ vs group A; [b] $P<0.02$ vs controls; [c] $P<0.01$ vs controls; [d] $P<0.01$ vs group A.

Table 3 Parameters calculated from the minimal model in two groups of NIDDM patients and normotensive controls with normal glucose tolerance matched for age, sex and body mass index (mean ± SEM). Abbreviations: SI: insulin sensitivity; Sg: glucose effectiveness; BIE: basal insulin effectiveness; GEZI: glucose effectiveness at zero insulin; IMGU: insulin-mediated glucose uptake at a blood glucose level of 11 mmol/l; NIMGU: non-insulin-mediated glucose uptake at a blood glucose level of 11 mmol/l; TGU: total (insulin-mediated and non-insulin-mediated) glucose uptake at a blood glucose level of 11 mmol/l.

Group	SI $min^{-1}/(\mu U/ml) \times 10^{-4}$	Sg %/min	BIE %/min	GEZI %/min	IMGU mg/min^{-1}/kg^{-1}	NIMGU mg/min^{-1}/kg^{-1}	TGU mg/min^{-1}/kg^{-1}
Controls ($n=12$)	8.26 ±1.87	2.51 ±0.26	0.59 ±0.12	1.9 ±0.3	8.2 ±2.7	6.2 ±0.8	14.4 ±2.8
A ($n=10$)	0.817 ±0.33[c]	1.86 ±0.17	0.07 ±0.02[c]	1.69 ±0.2[c]	0.28 ±0.09	5.7 ±0.8	5.9 ±0.8
B ($n=12$)	1.97 ±0.54[a,c]	1.86 ±0.27	0.12 ±0.02[c]	1.73 ±0.27	2.55 ±1.51[c,d]	6.0 ±1.0	8.6 ±2.2
A+B ($n=22$)	1.44 ±0.35[c]	1.86 ±0.17[c]	0.10 ±0.01[c]	1.71 ±0.18	1.47 ±0.83[c]	5.8 ±0.6	7.3 ±1.3[b]

[a] $P<0.05$ vs group A; [b] $P<0.02$ vs controls; [c] $P<0.01$ vs controls; [d] $P<0.01$ vs group A; [e] $P<0.05$ vs controls.

effectiveness (Sg). The latter is less frequently investigated, but has been demonstrated to be an important factor in glucose tolerance [26,27]. Mean values of SI and Sg found in this study in normal and NIDDM subjects are similar to those of other reports using the minimal model [10,18,23]. Consistent with these reports, we found markedly reduced values of SI and Sg in patients with NIDDM. When Sg was divided into its two components BIE and GEZI, BIE appeared to explain almost all the reduction of Sg, while GEZI was not significantly lower than in controls.

The minimal model procedure is now recognized as a reproducible technique [28] which gives results equivalent to those of the glucose clamp when the integrated insulin concentration above baseline is sufficiently high [11]. Modified protocols [23,29–31] have been validated for measuring SI and Sg in diabetic patients. The insulin protocol employed here has been described by Bergman *et al.* [29]. Since there was a lack of studies indicating the reproducibility of this insulin protocol, we made our own evaluation, as indicated in 'Methods'. The CV of 30.2% for SI in this range of values is higher than the CVs reported in other studies [27,28], although they are similar to CVs reported for the glucose clamp technique [32]. These other studies have not measured SI in the same range of values. A change of 0.5 $min^{-1}/(\mu U/ml) \times 10^{-4}$ for values of SI lower than one may represent a high percentage of the absolute value

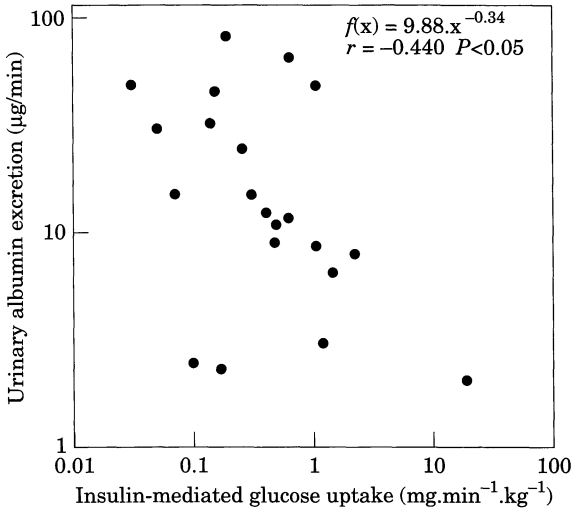

Fig. 3 Correlation between urinary albumin excretion rate and the evaluation of insulin-mediated glucose uptake (IMGU) from minimal model measurements in the 22 NIDDM subjects of the study. Due to the lack of normal distribution the correlation was calculated between log-transformed data and then expressed as a power relationship.

of SI, although it is a minor variation within the range of values of this parameter (from 0.01 to 20).

Recently, a multicentre study compared the results of insulin sensitivity measurements obtained with the glucose clamp and the minimal model [33]. This study showed that the minimal model gave lower values of SI than the clamp in NIDDM patients, when a modified protocol including insulin injection was used (as used in the present study). The reasons for this discrepancy remain unclear. Among the explanations which have been proposed, Saad *et al.* [33] suggests that the rapid rise and fall of the insulin level in the FSIVGTT may be a different stimulus from the sustained hyperinsulinaemia of the glucose clamp in markedly insulin resistant subjects. Since insulin-stimulated glucose utilization increases with time [34], insulin sensitivity measured with the clamp can be higher than insulin sensitivity measured with the IVGTT for reasons related to the type of insulin stimulus. It has been also suggested by Cobelli *et al.* [35], with labelling studies, that the monocompartmental assumption of glucose kinetics in the minimal model is a too simplistic description of glucose disappearance, which leads to an underestimation of the effect of insulin and glucose on glucose uptake. Thus, in the low range of values of SI, results given by the minimal model are likely to be lower than results given by the clamp. Although this important methodological issue requires more investigation, we think that there is now a large body of literature which supports the concept that the minimal model is a simple, safe and satisfactory method of evaluation of SI and Sg over the whole range of glucose tolerance [10,18]. The clinical relevance of this method is further supported by its predictive value for the occurrence of NIDDM in follow up studies [36]. In addition we used a less sophisticated evaluation of insulin sensitivity: the homeostasis model assessment (HOMA) which gives an index of insulin resistance correlated to clamp measurements [25]. It is interesting to note that we found a good correlation of this index with SI, and that results from both methods are in agreement.

The calculation of NIMGU, IMGU and TGU from the minimal model data by the procedure reported by Welch *et al.* [23] enables the comparison of the results of the present study with those of a complex clamp experiment [37–39]. When we compare our values of IMGU, NIMGU and TGU to those reported by Baron *et al.* [37,38] in control subjects, our results are very similar (see Table 4). Since these parameters are calculated

Table 4 Rates of glucose disposal measured in control subjects and NIDDM subjects with the glucose clamp in the studies of Baron *et al.* [37–39] or with the minimal model in the study of Welch *et al.* [23] or in this study. IMGU: insulin-mediated glucose uptake at a blood glucose level of 11 mmol/l; NIMGU: non-insulin-mediated glucose uptake at a blood glucose level of 11 mmol/l; TGU: total (insulin-mediated and non-insulin-mediated) glucose uptake at a blood glucose level of 11 mmol/l.

Authors	IMGU mg/min^{-1}/kg^{-1}	NIMGU mg/min^{-1}/kg^{-1}	TGU mg/min^{-1}/kg^{-1}
Controls			
Baron *et al.*	7.5	5.5	13.0
Welch *et al.*	6.2	6.4	12.7
This study	8.2	6.2	14.4
NIDDMs			
Baron *et al.*	0.7	3.6	4.3
Welch *et al.*	1.3	3.9	5.8
This study	1.5	5.8	7.3

as a predictor of a lower vascular and renal risk, as suggested by Nosadini [7], remains an attractive hypothesis which should be investigated in follow-up studies.

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for similar values of insulinaemia and glycaemia, they suggest a good agreement between clamp and minimal model measurements in controls when the results are expressed with similar indices. In the NIDDM subjects (Table 4), IMGU was very similar to the value reported by Welch *et al.* [23], but higher than the value found by Baron *et al.* [39]. Thus, the average insulin sensitivity of our sample is not lower than that found with the clamp, as could be expected from the paper of Saad *et al.* [33]. The major discrepancy we have found is for NIMGU. Our values of NIMGU in NIDDM are the same as those of our non-diabetic controls. This is in agreement with the findings of Baron *et al.* [39] who reported a high rate of NIMGU in NIDDM patients, suggesting that there was no defect in this pathway in NIDDM patients. However, values of NIMGU in the present study are higher than those reported by Welch *et al.* [23], and by Baron *et al.* [39] when they are expressed in the same units, i.e. corrected for body weight. Baron *et al.* [39] hypothesize that abnormal free fatty acid levels may result in measurements giving an underestimation of the true value of NIMGU [39]. The heterogeneity of NIDDM may explain this discrepancy.

What new information does our minimal model study provide concerning the relationships between microalbuminuria and insulin sensitivity? First, no relationship between AER and Sg (as well as its compounds BIE and GEZI) could be found. Non-insulin-mediated glucose uptake (which is considered as the major determinant of GEZI and Sg) does not seem to be linked with microalbuminuria. This was an important point to clarify, since insulin-independent glucose uptake is a major determinant of the total glucose uptake. In some situations this may explain marked differences in glucose tolerance [26,27], which can be interpreted as changes in insulin sensitivity in clamp studies [10, 26]. Moreover, Baron *et al.* suggested that elevated values of NIMGU resulting from elevated fasting glycaemia, in association with normal values of Sg, could play a role in the late complications of diabetes mellitus [39]. It has been recently suggested that the single pool model of glucose disposal which is an assumption of Bergman's minimal model [10,11,18,19] may lead to an overestimation of glucose disappearance [40]. Whilst the minimal model values of Sg correctly describe the initial 20 min of the glucose decrease they could overevaluate glucose uptake over longer periods.

By contrast, we observed a relationship between insulin sensitivity (measured by SI, IMGU or the parameter IR of HOMA) and AER. NIDDM patients with microalbuminuria were more insulin resistant (i.e. had a 58% lower SI) than normoalbuminuric normotensive patients. This result is confirmed by a higher value of IR (HOMA) in microalbuminuric subjects, and further illustrated by the non-linear correlation between AER and the minimal model derived evaluation of IMGU. Although blood pressure is a major determinant of albumin excretion, and tends to be higher in microalbuminuric diabetics [1–5], mean blood pressure was not significantly different in group A and group B, and the classical correlation between blood pressure and microalbuminuria was not found. This suggests that differences in blood pressure are not the explanation for the differences in insulin sensitivity. The association of microalbuminuria with a higher degree of insulin resistance is consistent with previously reported results obtained with the glucose clamp procedure [7,8].

Results obtained with the glucose clamp by Nosadini *et al.* [7] and Groop *et al.* [8] show that NIDDM patients can be subdivided into two subgroups: one with insulin resistance, microalbuminuria, hypertension, and another with almost normal insulin sensitivity and none of these disorders. In contrast in the present study, SI in the normoalbuminuric NIDDMs, although higher than in diabetics with microalbuminuria, was still markedly lower than in non-diabetic matched controls. This is probably related to the methodological differences outlined above.

In studies which have used the minimal model to investigate NIDDM patients, reduced SI and Sg have been found to be a typical feature of the disease [10, 18,23]. In our unit we have previously observed, in a study of 47 NIDDM patients, that SI was lower than the control range in 87% of subjects, while Sg was lower in 75% of them [41]. Thus, if the assumption that vascular risk in NIDDM patients is confined to those with insulin resistance [8] is true, this risk is present in a majority of these patients. Whether diabetic patients with less impaired SI are at a lower risk for renal or vascular complications [7–9] is an attractive hypothesis, but remains undemonstrated. Two of our microalbuminuric patients exhibited a value of SI $>2\ min^{-1}/(\mu U/ml) \times 10^{-4}$, i.e. within the range of control values for non-diabetic subjects.

It should be also stressed that NIDDM is a heterogeneous disease and that a link between AER and insulin resistance may exist in some patients but not others. NIDDM patients in whom insulin resistance is a factor involved in the deterioration of renal function may be only a subgroup in this category of patients. Other hemodynamic, metabolic or genetic factors are probably responsible for glomerular alterations in NIDDM patients in whom values of SI remain within a physiologic range [5].

In conclusion, this study confirms that NIDDM patients, regardless of their AER and BP, have a low (and in most cases a very low) value of SI. However, normoalbuminuric NIDDM patients are a little less insulin resistant than microalbuminuric ones. The potential relevance of a less reduced SI in NIDDM patients

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