Original Article

Insulin Resistance and Microalbuminuria

A Cross-Sectional, Case-Control Study of 158 Patients With Type 2 Diabetes and Different Degrees of Urinary Albumin Excretion

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Microalbuminuria is a risk factor for renal and cardiovascular disease. A role for insulin resistance in the pathogenesis of microalbuminuria has been suggested but is still unproven. In this case-control, cross-sectional study, we compared glucose disposal rate (GDR), measured by hyperinsulinemic-euglycemic clamp, in 50 pairs of matched type 2 diabetic patients with micro- or normoalbuminuria (main study) and in 29 matched pairs of diabetic patients with macro- or microalbuminuria (substudy). In the main study, GDR was ~25% lower in micro- than in normoalbuminuric patients (5.20 ± 1.91 vs. 6.86 ± 2.88 mg · kg^{-1} · min^{-1}, P < 0.05) and was independently associated with microalbuminuria (P = 0.002), with each 1 mg · kg^{-1} · min^{-1} decrease predicting ~40% increased prevalence (odds ratio 1.37 [95% CI 1.14–1.70]). Microalbuminuria was threefold more frequent in patients with GDR ≤7.50 ± 2.56 mg · kg^{-1} · min^{-1} than in those with higher GDR (60% vs. 20%, P < 0.005). In the substudy, GDR in macro- and microalbuminuric patients was comparable (5.52 ± 2.56 vs. 5.16 ± 1.61 mg · kg^{-1} · min^{-1}) and independent of macroalbuminuria. GDR was significantly correlated with urinary albumin excretion rate in the main study (P = 0.004) but not in the substudy (P = 0.60). In type 2 diabetes, more severe insulin resistance is independently associated with microalbuminuria. Longitudinal studies are needed to clarify the role of insulin resistance in the pathogenesis of microalbuminuria and related complications. *Diabetes* 55: 1456–1462, 2006

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Insulin resistance plays a major role in the pathogenesis of type 2 diabetes (1). Virtually all patients with type 2 diabetes are insulin resistant, and the insulin-resistant status normally precedes the onset of diabetes by 1 to 2 decades (2,3). Consistently, amelioration of insulin sensitivity may delay or even prevent the onset of type 2 diabetes (4). Although largely sustained by acquired factors such as decreased physical activity and obesity (5), familial clustering of insulin resistance suggests that genetic factors may also contribute to reduced insulin sensitivity (6). Insulin resistance may arise from defects in fatty acid oxidation (7), and secondary β-cell lipotoxicity may contribute to defects in insulin resistance and hyperglycemia (8). Thirty to 40% of patients with type 2 diabetes develop microalbuminuria (9), which in 5–10% of case subjects may already be present at the diagnosis of the disease (10,11). Every year, 2–5% of those with normal urinary albumin excretion develop microalbuminuria (11,12), 2–3% of those with microalbuminuria progress to macroalbuminuria (11,13), and 2–3% of those with macroalbuminuria progress to renal insufficiency that may ultimately require dialysis or transplantation (11,14). Moreover, 40–50% of patients with type 2 diabetes who have microalbuminuria eventually die of cardiovascular disease (15); this is three times as high a rate of death from cardiac causes as among patients who have diabetes but have no evidence of renal disease (15). Thus, microalbuminuria is a major risk factor for renal and cardiovascular events, and the early identification and treatment of patients at increased risk for microalbuminuria may be instrumental to limit the excess renal and cardiovascular disease associated with type 2 diabetes. This is of paramount importance because the risk of developing microalbuminuria can be effectively reduced, as demonstrated by data from the BENEDICT trial (16).

Evidence suggests that insulin resistance precedes and probably contributes to the development of microalbuminuria in type 1 diabetic patients (17) and in nondiabetic subjects (18). Data in type 2 diabetic patients are less clear, because an association between insulin resistance and microalbuminuria has been suggested by some studies (19–24) but has not been confirmed by others (25–28). These conflicting findings were likely explained by the small number of patients included in the above studies

and, in some circumstances, by the use of indirect markers of insulin resistance (29), which reduced the power of the analyses and increased the risk of false-positive and false-negative results. However, the hypothesis of an association between insulin resistance and microalbuminuria has been revived in a recent study showing that in a large cohort of type 2 diabetic patients, the homeostasis model assessment index, a surrogate of insulin sensitivity, was significantly associated with the albumin-to-creatinine ratio measured in spot urine collections (30).

To formally test this possibility, we compared the total-body glucose disposal rate (GDR) in 50 matched pairs of type 2 diabetic patients with micro- or normoalbuminuria. In these patients, we also evaluated the relationship between insulin sensitivity and the prevalence of microalbuminuria (main study). To explore whether the degree of albuminuria may in turn affect the severity of insulin resistance, we also compared the GDR in 29 pairs of type 2 diabetic patients with macro- or microalbuminuria (substudy). In both studies, the GDR was quantified by means of a euglycemic-hyperinsulinemic clamp technique (31), the gold standard measurement of insulin sensitivity. The results of these studies formed the basis of the present report.

RESEARCH DESIGN AND METHODS

We studied all consecutive type 2 diabetic patients referred to the Department of Renal Medicine of the Clinical Research Centre for Rare Diseases "Aldo e Cele Daccò" of Ranica (Bergamo, Italy) and to the Units of Metabolic Diseases of the University of Padova and of Nephrology and Dialysis of the Ospedali Riuniti of Bergamo, Italy, who satisfied the selection criteria and provided written informed consent according to the Declaration of Helsinki guidelines. Inclusion criteria were diagnosis of type 2 diabetes according to American Diabetes Association criteria (32) and age of 18–75 years. Exclusion criteria were any evidence of nondiabetic renal disease, obstructive uropathy, severe renal insufficiency (serum creatinine >177 μmol/l [2.0 mg/dl]), severe heart failure (New York Heart Association class III or more), liver disease, cancer, autoimmune disease, and any other condition that in the investigator's judgment could affect study participation or confound data interpretation. All patients were on a hypocaloric diet and had moderate amount of physical activity (33). No patient was involved in competitive sports. No change in diet, lifestyle, and pharmacological treatment had been introduced during the 3 months before patients' selection. The Ethical Committee of the Clinical Research Centre approved the study protocol.

Study design

Main study. Patients with type 2 diabetes and microalbuminuria (see METHODS) were identified as case subjects. For each case subject, one type 2 diabetic patient with normoalbuminuria was identified as a control subject. Case and control subjects were matched for sex, age (±5 years vs. case subjects), BMI (±2 kg/m^2 vs. case subjects), and presence or absence of arterial hypertension (defined as systolic and/or diastolic blood pressure ≥140/90 mmHg and/or concomitant antihypertensive therapy) (34) and satisfied the same inclusion/exclusion criteria. Subjects were matched by using a dedicated software program in SAS platform for random selection of control subjects, where each case subject was matched with the first control subject as identified on the basis of the criteria described above. Twenty nondiabetic subjects without family history of diabetes and hypertension, matched with case subjects for sex, age, and BMI, served as healthy control subjects.

Substudy. Consecutive patients with type 2 diabetes and macroalbuminuria (27) were identified as case subjects. For each case subject with macroalbuminuria, one diabetic patient with microalbuminuria, matched for sex, age, BMI, and blood pressure who satisfied the same inclusion/exclusion criteria, was identified as a reference case subject.

Methods

In addition to a complete physical and laboratory evaluation, all patients had their insulin sensitivity, albumin excretion rate (AER), and glomerular filtration rate (GFR) evaluated as specified below. All relevant demographic, clinical, and laboratory data were reported in a dedicated case record form.

Insulin sensitivity. Patients were admitted to the metabolic ward at 7:30 A.M. To avoid the confounding effect of concomitant insulin therapy (if any), the last doses of long-acting and rapid insulin were given the morning and the evening before the clamp study, respectively. Weight and height were measured without shoes. After 15 min of rest, sitting blood pressure was measured three times to the nearest 2 mmHg in the dominant arm with a random-zero sphygmomanometer, and the average of the three readings was recorded. The clamp was started at 8:00 A.M. after an overnight fasting. One polyethylene tube was inserted into an antecubital vein (for infusion of insulin and glucose), and another one was inserted retrogradely into a wrist vein surrounded by heated box at 55°C (for sampling of arterialized venous blood). Fasting blood samples were taken for measurements of plasma insulin, creatinine, glycosylated hemoglobin (HbA_{1c} [A1C]), and lipids.

The peripheral insulin sensitivity was assessed during a hyperinsulinemic-euglycemic clamp (31). Insulin was infused at a constant rate of 2 mU · kg^{-1} · min^{-1}. Blood glucose concentration was allowed to decrease during the insulin infusion to 90 ± 5 mg/dl, at which level it was maintained for 2 h by a variable glucose infusion through an IVAC pump (IVAC 560, San Diego, CA). Whole-blood glucose concentration was assayed by glucose oxidized method every 5 min. During the last 30 min of the clamp, three blood samples were taken every 10th min for insulin measurements to confirm a steady-state plasma insulin concentration.

Mean insulin levels achieved during 90–120 min of the clamp were ~200 mU/l in all groups. Because at the achieved plasma insulin concentration, the hepatic glucose production is totally suppressed, the amount of glucose required to maintain steady-state euglycemia was assumed to equal the total-body glucose disposal. Thus, total-body GDR (mg · kg^{-1} · min^{-1}) was calculated as the mean of the glucose infusion rate during the last 30 min of the clamp.

GFR. GFR was measured by the plasma clearance of unlabeled iohexol after a single, intravenous injection of 5 ml iohexol solution (647 mg/ml Omnipaque 300; Nycomed Amersham Sorin, Milano, Italy), as previously described (35).

TABLE 1
Main clinical and laboratory characteristics of patients with microalbuminuria (case subjects) and normoalbuminuria (control subjects) included in the main study

Variable	Microalbuminuria (case subjects)	Normoalbuminuria (control subjects)
Patients (*n*)	50	50
Sex (male/female)	42/8	42/8
Age (years)	59.1 ± 8.7	58.4 ± 8.6
Duration of diabetes (months)	98.9 ± 84.2	95.5 ± 78.1
Smoking (no/yes/ex-smokers)	24/18/8	24/21/5
BMI (kg/m^2)	28.7 ± 3.5	28.5 ± 3.6
sBP (mmHg)	141 ± 18	140 ± 18
dBP (mmHg)	83 ± 9	83 ± 11
MAP (mmHg)	103 ± 11	102 ± 12
A1C (%)	7.09 ± 2.13*	6.05 ± 1.92
GDR (mg · kg^{-1} · min^{-1})	5.20 ± 1.91*	6.86 ± 2.88
Serum creatinine (μmol/l)	77 ± 20	77 ± 18
GFR (ml/min per 1.73 m^2)	107.9 ± 24.63	115.80 ± 24.28
AER (μg/min)	61.41 ± 40.52*	7.58 ± 4.85
Total cholesterol (mmol/l)	5.23 ± 0.83	5.05 ± 0.75
HDL cholesterol (mmol/l)	1.11 ± 0.21	1.11 ± 0.31
LDL cholesterol (mmol/l)	4.12 ± 0.83	3.91 ± 0.73
Triglycerides (mmol/l)	1.64 ± 0.80	1.41 ± 0.62
Diet/sulfonylureas/metformin/insulin†	3/37/24/10	14/34/16/6
ACEi/diuretics/α-blockers/CCB†	4/19/7/13	0/7/8/4
Statins/fibrates	4/0	7/0

Data are frequency or means ± SD. ACEi, ACE inhibitor; CCB, calcium channel blocker; dBP, diastolic blood pressure; MAP, mean arterial pressure; sBP, systolic blood pressure. *$P < 0.05$ vs. patients with normoalbuminuria. †Fisher's exact test: diet, $P = 0.006$; ACEi, $P = 0.044$; diuretics, $P = 0.006$; CCB, $P = 0.01$.

TABLE 2
Main characteristics of type 2 diabetic patients with macroalbuminuria (case subjects) and microalbuminuria (control subjects) with serum creatinine <1.5 mg/dl included in the substudy

Variable	Macroalbuminuria (case subjects)	Microalbuminuria (control subjects)
Patients (n)	29	29
Sex (male/female)	27/2	27/2
Age (years)	60.0 ± 8.7	59.0 ± 8.7
Duration of diabetes (months)	178.9 ± 113.5*	90.8 ± 80.0
Smoking (no/yes/ex-smokers)	14/11/4	15/10/4
BMI (kg/m^2)	29.3 ± 4.1	29.9 ± 4.9
sBP (mmHg)	149 ± 18	145 ± 15
dBP (mmHg)	85 ± 10	84 ± 9
MAP (mmHg)	106 ± 12	104 ± 9
A1C (%)	7.73 ± 1.73*	6.32 ± 1.31
GDR (mg · kg^{-1} · min^{-1})	5.16 ± 1.61	5.52 ± 2.56
Serum creatinine (μmol/l)	98 ± 19*	82 ± 20
GFR (ml/min per 0.1.73 m^2)	89.95 ± 25.90	100.90 ± 26.24
AER (μg/min)	1,027.69 ± 737.79*	71.21 ± 48.87
Total cholesterol (mmol/l)	5.34 ± 0.75	5.15 ± 0.75
HDL cholesterol (mmol/l)	1.14 ± 0.36	1.04 ± 0.26
LDL cholesterol (mmol/l)	4.22 ± 0.88	4.12 ± 0.75
Triglycerides (mmol/l)	2.63 ± 1.67*	1.72 ± 1.11
Diet/sulfonylureas/metformin/insulin	0/14/14/13	2/15/17/9
ACEi/diuretics/α-blockers/CCB†	18/23/5/7	10/11/3/8
Statins/fibrates	8/6	2/2

Data are frequency or means ± SD. ACEi, ACE inhibitor; CCB, calcium channel blocker; dBP, diastolic blood pressure; MAP, mean arterial pressure; sBP, systolic blood pressure. *$P < 0.05$ vs. patients with microalbuminuria. †Fisher's exact test: diuretics, $P = 0.0029$.

Albumin excretion rate. AER was measured in three consecutive overnight urine collections by rate nephelometry (Array 360 System; Beckman, Milano, Italy). The sensitivity of the assay was 2 mg/l. Normo-, micro-, and macroalbuminuria were defined as AER <20, 20–200, and >200 μg/min, respectively, in at least two of three consecutive overnight urine collections confirmed in at least two visits 2 months apart.

Laboratory tests. Serum creatinine, potassium, and lipid concentration and other routine laboratory parameters were measured by automatic analyzer Beckman Synchron CX5. Glycosylated hemoglobin was measured by high-performance liquid chromatography (normal laboratory range 3.53–5.21%; Beckman System Gold Chromatograph).

Sample size estimation

Main study. The sample size was calculated on the basis of an expected difference in the primary outcome variable (e.g., GDR) between microalbuminuric (case subjects) and normoalbuminuric (control subjects) diabetic patients. Assuming an average GDR of 5.50 mg · kg^{-1} · min^{-1} among case subjects and a difference of 1.50 mg · kg^{-1} · min^{-1} between case subjects and corresponding control subjects, it was estimated that to give the main study an 80% power to detect as statistically significant ($P < 0.05$) the expected difference, 45 patients per group had to be included. On the basis of a preliminary estimate of the prevalence of type 2 diabetic patients satisfying the inclusion/exclusion criteria of the main study regularly attending the outpatient clinics of the involved centers, it was predicted that the 100 patients (50 case and 50 control subjects) needed for the analyses should be identified throughout a screening period of ~6 months (estimated dropout of 10%).

Substudy. The sample size was not established a priori on the basis of an expected difference because this was a secondary, explicative study aimed to explore/exclude mechanisms possibly explaining differences (if any) in blood GDR between normo- and microalbuminuric patients included in the main study. Thus, we planned to include in the substudy all patients with macroalbuminuria and matched patients with microalbuminuria who were identified up to 6 months after completion of the main study.

Statistical analyses. The characteristics of different groups were assessed by methods of descriptive statistics, Shapiro-Walk test of normality, and method of percentiles and compared by Student's unpaired t test, Wilcoxon's rank-sum test, Somer's d test for directional measures, or χ^2 test, as appropriate. Correlation analysis was done by Pearson's r coefficient. The association between different variables listed in Tables 1 and 2 with micro- or macroalbuminuria was evaluated by univariate analyses. Logistic regression analysis was applied by entry and backward stepwise methods with adjustment for covariate effects (logit link function with likelihood ratio or conditional tests, as appropriate) to those variables that were significantly associated with micro- or macroalbuminuria at univariate analyses. The statistical significance of the tests was assumed at $P < 0.05$. Before the analyses the skewed distribution of diabetes duration, AER, A1C, and triglycerides was normalized by log-transformation. Receiver operating characteristic curves were used to determine the true positive rate (sensitivity) as the proportion of case subjects (e.g., with microalbuminuria) who were classified at lower values of the predictor (e.g., GDR) and the true negative rate (specificity) as the proportion of control subjects who were classified at higher values, at the usually accepted probability cutoff level of 0.50. As a rule, a larger area under the curve indicates better performance of the predictor, thus providing a scrutiny approach for an acceptable internal validation of the fitted logistic regression models. All evaluations were done with SAS software (version 8.0). Data are means (SD or SE) or number and percent frequency, unless otherwise stated.

RESULTS

The main clinical and laboratory features of type 2 diabetic patients included in the main study and in the substudy are given in Tables 1 and 2, respectively. Only four patients (three males) denied their consent to study participation and were not included. Their characteristics were similar to those of included patients. Regardless of the degree of albuminuria and renal function, all study patients were insulin resistant, with their average whole-body GDR (6.03 ± 2.57 mg · kg^{-1} · min^{-1}) ~50% lower ($P < 0.0001$) than that of 20 nondiabetic healthy subjects without family history of diabetes and hypertension (11.0 ± 1.50 mg · kg^{-1} · min^{-1}) evaluated under the same experimental conditions by an euglycemic-hyperinsulinemic clamp. As expected on the basis of the matching criteria, in both studies the two patient groups were very well comparable for sex distribution, age, BMI, and prevalence of arterial hypertension.

Comparative analyses

Main study. Micro- and normoalbuminuric patients were similar not only for the matching variables, but also for diabetes duration, smoking habit, blood pressure control, renal function, and lipid profile (Table 1). Patients with microalbuminuria, however, had higher A1C levels and were more frequently on antihypertensive therapy.

GDR was ~25% lower ($P = 0.005$, power 92.5%) in case subjects than in control subjects (Table 1; Fig. 1*A*). At multivariate stepwise regression analysis (considering the variables listed in Table 1 that were significantly associated with microalbuminuria at univariate analysis), GDR, A1C, and GFR were significantly and independently associated with microalbuminuria (Table 3). Of note, each decrease in GDR of 1.0 mg · kg^{-1} · min^{-1} was associated with ~40% increased risk of microalbuminuria (adjusted odds ratio [OR] 1.37 [95% CI 1.14–1.70]). The multivariate model (Table 3) revealed the independent role of GDR within a nonlinear logistic conditional relationship to

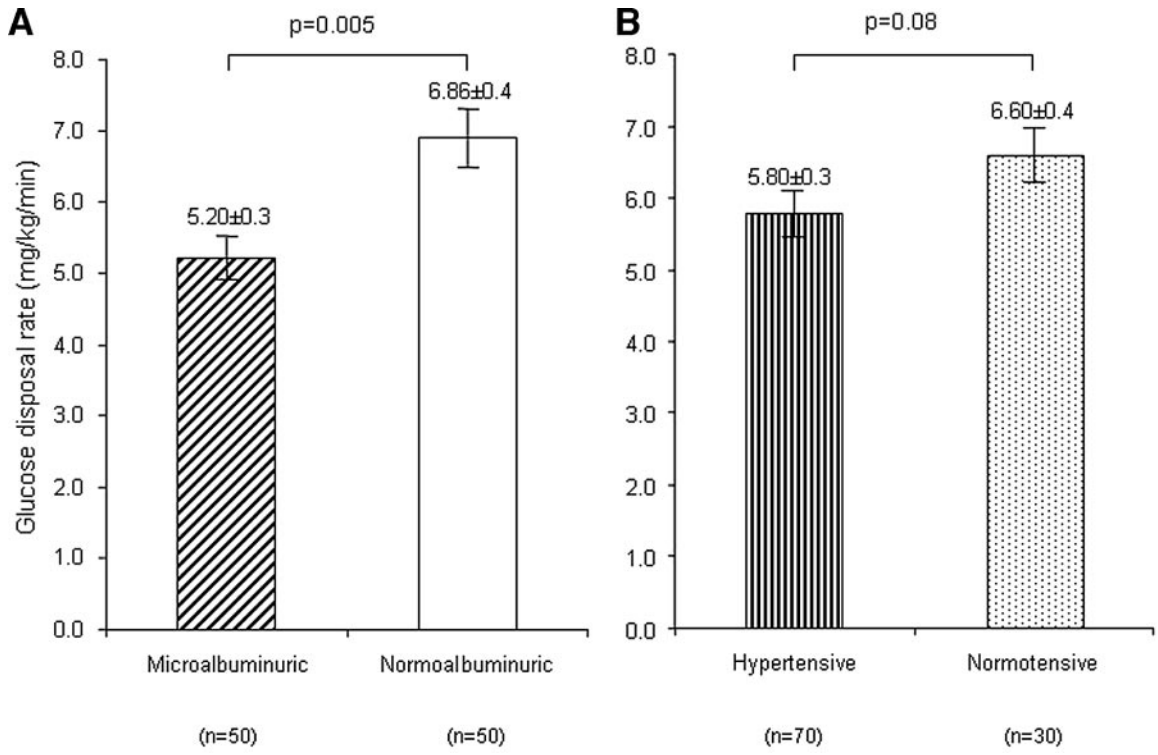

FIG. 1. GDR in 100 patients with type 2 diabetes considered according to the presence of micro- or normoalbuminuria regardless of arterial blood pressure (*A*) or considered according to the presence of arterial hypertension or normal blood pressure regardless of the urinary albumin excretion (*B*).

albuminuria with 76.1% accuracy, and sensitivity and specificity >72%, at 0.50 probability cutoff value. Receiving operator characteristics curve analysis confirmed the predictive power of GDR (area under the curve 0.66, 95% CI 0.55–0.77, $P = 0.005$, best theoretical cutoff value 5.81 mg · kg^{-1} · min^{-1}). The univariate consideration of GDR indicated a 70% specificity at the range of cutoff values from 4.60 to 5.11 mg · kg^{-1} · min^{-1} (range 68–74%). The GDR cutoff value of 7.50 mg · kg^{-1} · min^{-1} discriminated between the absence or presence of microalbuminuria (89% specificity, 39% sensitivity). Among patients with a GDR ≤7.50 mg · kg^{-1} · min^{-1}, the prevalence of microalbuminuria was threefold higher (60 vs. 20%, $P < 0.005$) than in those with a GDR ≥7.51 mg · kg^{-1} · min^{-1} (Fig. 2). All patients with GDR ≤2.48 mg · kg^{-1} · min^{-1} were microalbuminuric, whereas all patients with GDR ≥8.85 mg · kg^{-1} · min^{-1} were normoalbuminuric. There was no independent association between GDR and concomitant treatment with insulin or oral antidiabetic agents, blood pressure–lowering medications, or ACE inhibitors.

Hypertensive patients ($n = 70$) compared with normotensive patients ($n = 30$) tended to be more insulin resistant, but the difference in GDR between the two groups failed to achieve the assumed level of statistical significance ($P = 0.08$) (Fig. 1*B*). With the above patients, the analysis had an 80% power to detect at two-tailed $P < 0.05$ a GDR difference of 1.40 mg · kg^{-1} · min^{-1} or more. Mean GDR was also significantly lower in case subjects than in control subjects in a subgroup analysis considering the 70 hypertensive patients (microalbuminuria, 5.09 ± 1.98 mg · kg^{-1} · min^{-1}; and normoalbuminuria, 6.64 ± 3.10 mg · kg^{-1} · min^{-1}) and the 30 normotensive patients (microalbuminuria, 5.73 ± 1.40 mg · kg^{-1} · min^{-1}; and normoalbuminuria, 7.34 ± 2.30 mg · kg^{-1} · min^{-1}) as shown on Fig. 3. At multivariate stepwise regression analysis, GDR retained a significant and independent association with abnormal AER (e.g., hypertensive, $b = 0.18$, $P = 0.017$; normotensive, $b = 2.24$, $P = 0.038$).

Substudy. Macro- and microalbuminuric patients were similar not only for the matching variables, but also for smoking habit, blood pressure control, GFR, and total and LDL cholesterol. Macroalbuminuric patients had significantly (twofold) longer diabetes duration, higher A1C, serum creatinine, and triglyceride levels than microalbuminuric patients. A higher proportion of macroalbuminuric patients was on diuretic therapy (Table 2). The GDR, however, was comparable in the two groups. With the above patients, the analysis had an 80% power to detect at two-tailed $P < 0.05$, a difference in GDR of 1.58 mg · kg^{-1} · min^{-1} or more. Thus, the substudy was powered to detect as statistically significant a difference even inferior to that observed in the main study (Fig. 1, *left panel*). At multivariate backward stepwise regression analysis (considering the variables listed in Table 2 that were significantly associated with macroalbuminuria at univariate analysis with the only exception of AER), the diabetes duration,

TABLE 3
Logistic backward conditional regression analysis for prediction of microalbuminuria in diabetic patients included in the main study

Independent variable	Risk of microalbuminuria	*P*
GDR	1.370 (1.140–1.697)	0.0018
Ln A1C	0.105 (0.029–0.316)	0.0002
GFR	1.021 (1.004–1.039)	0.0166

Data are adjusted OR (95% CI). Model accuracy 76.1%; $P < 0.0001$.

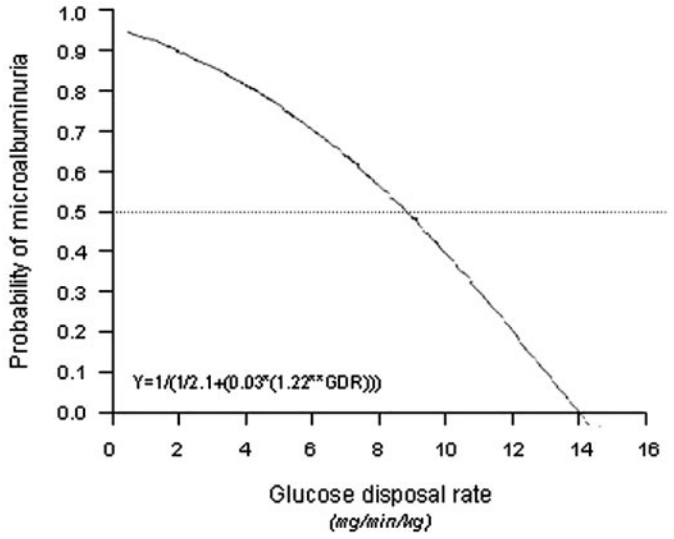

FIG. 2. Nonlinear inverse relationship between GDR and probability of microalbuminuria in the 100 patients with type 2 diabetes included in the main study (P_{model} = 0.001); *x*-axis, GDR; *y*-axis, probability of microalbuminuria (where 0 = normoalbuminuria; 1 = microalbuminuria).

serum creatinine, HDL, and triglycerides but not GDR were significantly associated with the presence of macroalbuminuria (Table 4). There was no independent association between GDR and concomitant treatment with insulin or oral antidiabetic agents, blood pressure–lowering medications, or ACE inhibitors.

DISCUSSION

In this cross-sectional study of a large cohort of patients with type 2 diabetes and different degrees of albuminuria, we provided the evidence that those with microalbuminuria were more insulin resistant than those with a normal urinary albumin excretion. The magnitude of insulin resistance was independently associated with microalbuminuria, and for each decrease in GDR of 1 mg · kg^{-1} · min^{-1}, the prevalence of microalbuminuria increased by ~40%.

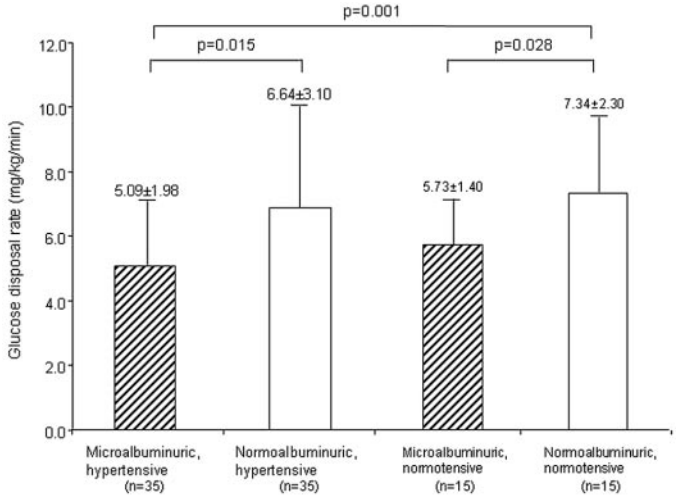

FIG. 3. GDR in 35 normo- and 35 microalbuminuric patients with type 2 diabetes and arterial hypertension and in 15 normo- and 15 microalbuminuric patients with type 2 diabetes and normal blood pressure. GDR is significantly higher in normo- than in microalbuminuric patients but is comparable between patients with normal or high blood pressure, regardless of normo or microalbuminuria.

Of note, among patients with a GDR of 7.50 mg · kg^{-1} · min^{-1} or less, the prevalence of microalbuminuria was threefold higher than among patients with a higher GDR. Of interest, there was a clear association between more severe insulin resistance and microalbuminuria also in the subgroup of patients with normal blood pressure considered separately from those with arterial hypertension. Moreover, normotensive patients with microalbuminuria were more insulin resistant than hypertensive patients with normoalbuminuria. Altogether, these findings lend support to the possibility that insulin resistance is directly associated with microalbuminuria, regardless of its relationships with arterial hypertension. On the other hand, in the substudy, insulin sensitivity was similar in patients with macro- or microalbuminuria and was not correlated with the degree of urinary albumin excretion.

These findings were obtained in well-characterized patients through gold-standard procedures not only for the evaluation of insulin sensitivity, but also for the measurement of GFR and albuminuria. All consecutive, eligible patients were included in the study. Thus, the study patients reliably represent those who normally attend a Diabetology Unit who, in turn, represent the average population of subjects with type 2 diabetes referred to the Health Care System in Italy. Moreover, any change in their concomitant treatments was intentionally avoided to keep from interfering with their routine care and, therefore, to avoid introducing systematic bias in data interpretation. Thus, our present findings can be reasonably generalized to the average population of patients with type 2 diabetes. The case-control design allowed also achieving a good matching for potential confounders such as age, sex, BMI, and blood pressure. Other possible confounders such as diabetes duration, GFR, and lipid profile were also very well comparable in the two groups of patients with normo- or microalbuminuria. These findings, combined with the evidence of an independent association between insulin resistance and microalbuminuria at multivariate analyses, can reasonably exclude the possibility of a systematic confounding effect of the above factors on the study findings. Also, the more frequent use of blood pressure–lowering medications in micro- and macroalbuminuric patients did not appreciably affect the results, as demonstrated by the finding that the antihypertensive treatment was not independently associated with insulin sensitivity. Conceivably, this was explained by the opposite effects of drugs that may increase (ACE inhibitors) or decrease (diuretics) insulin sensitivity.

The difference in A1C likely did not explain the difference in GDR between patients with micro- or normoalbuminuria. No difference in GDR was found between patients with micro- or macroalbuminuria, despite an even more consistent difference in A1C. Consistent with results of multivariate analyses, the above findings confirm that reduced insulin sensitivity is independently associated with microalbuminuria in people with type 2 diabetes, as already documented in nondiabetic populations (18). The finding that known diabetes duration was similar in patients with micro- or normoalbuminuria and was not associated with GDR can be taken to rule out any appreciable role also for this potential confounding factor. This is consistent with previous evidence that diabetes duration is not a major determinant of insulin sensitivity (36) and with our present data that in patients with micro- and macroalbuminuria insulin sensitivity was comparable, de-

TABLE 4
Logistic backward regression analysis for prediction of microalbuminuria in albuminuric diabetic patients with serum creatinine <1.5 mg/dl included in the substudy

Independent variables	Risk of microalbuminuria	*P*
Ln duration	0.20 (0.07–0.59)	0.004
Creatinine	0.004 (0.001–0.28)	0.011
Ln HDL	0.003 (0.001–0.21)	0.008
Ln TG	0.012 (0.001–0.16)	0.001

Data are OR (95% CI). Model accuracy 94.1%; $P < 0.0001$. TG, triglyceride.

spite the longer diabetes duration reported in those with macroalbuminuria.

Different mechanisms may link insulin resistance to abnormal albuminuria. In response to defective responsiveness of peripheral tissues and vasculature, plasma insulin may rise to supranormal concentrations that may sustain glomerular hyperfiltration (37), endothelial dysfunction (38), and increased vascular permeability (39), effects that eventually result in increased albumin ultrafiltration and leakage into the urine. Furthermore, impaired insulin sensitivity is associated with altered renal cellular metabolism and electrolyte composition, mesangial hyperplasia, and renal hypertrophy and increased endothelial cell proliferation and lipid and hyaluronate deposition in the renal matrix and inner medulla, effects that may directly contribute to progressive kidney damage, even independently of hyperglycemia (40). Finally, central actions of insulin stimulating the sympathetic nervous system activity and renal effects enhancing renal sodium reabsorption may contribute to the etiology of arterial hypertension, a frequent concomitant of the insulin-resistant state (31) that may further contribute to renal damage by increasing glomerular capillary pressure and protein traffic (40).

In conclusion, the evidence presented here supports the presence of a strong, independent relationship between the severity of insulin resistance and microalbuminuria in patients with type 2 diabetes. Longitudinal studies are needed to better clarify the role of insulin resistance in the pathogenesis of microalbuminuria and related micro- and macrovascular complications of type 2 diabetes.

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