In Vivo Relationship Between Insulin Clearance and Action in Healthy Subjects and IDDM Patients

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The relationship between plasma clearance rate of insulin (PCR) and insulin-stimulated glucose disposal was investigated in 15 healthy subjects and 30 insulin-dependent diabetes mellitus (IDDM) patients with the sequential euglycemic (5 mM) clamp technique (insulin infusion rates of 0.5, 1, 2, and 5 mU · kg^{-1} · min^{-1} in 2-h steps). In IDDM patients, insulin-stimulated glucose disposal was decreased at low insulinemia (steps 1–3), whereas at maximal insulinemia (step 4), insulin action was normal. In the healthy subjects, strong positive correlations were found for PCR versus steady-state glucose infusion rate (SSGIR): r = 0.71 ($P < 0.005$), 0.72 ($P < 0.005$), 0.72 ($P < 0.005$), and 0.78 ($P < 0.001$) for steps 1–4, respectively. In contrast, in the IDDM patients, no relationship was observed: r = 0.01, −0.03, 0.06, and 0.01 (NS) for steps 1–4, respectively. In univariate analyses of PCR, no differences were found between patient subgroups with values for percentage of tracer binding below or above 5% or insulin-antibody–binding capacities and equilibrium constants below or above the median. In multiple regression models, adjusting for insulin antibodies, preceding glycemic control (HbA_1 or fructosamine), and duration of IDDM, correlations for PCR versus SSGIR remained nonsignificant. In conclusion, insulin action is correlated to insulin clearance in healthy subjects, suggesting a functional relationship from an in vivo perspective. No such relationship was present in patients with IDDM, even after adjusting for insulin antibodies, preceding glycemic control, and duration of IDDM. *Diabetes* 39:333–39, 1990

In vitro studies have indicated that insulin internalization and action are linked (1,2). In vivo, insulin clearance and action were also found to be related. For example, in patients with extreme insulin resistance, decreased insulin clearance was associated with decreased glucose utilization (3,4). Furthermore, a positive correlation between insulin clearance and action was observed in the combined data sets of healthy subjects and patients with obesity, hyperthyroidism, and Cushing's syndrome (5).

This study investigated whether a similar relationship between insulin clearance and action can be found within sizable groups of healthy subjects and patients with insulin-dependent diabetes mellitus (IDDM). We analyzed the insulin-clamp data of 15 healthy subjects and 30 patients with IDDM studied previously (6). The steady-state conditions of the sequential euglycemic (5 mM) clamp allowed for the quantification of insulin action and clearance at three submaximal and at maximal insulin levels.

RESEARCH DESIGN AND METHODS

Fifteen islet cell–antibody–negative healthy subjects participated in the study. None had a family history of diabetes, and all had normal oral (75-g) glucose tolerance test (7). Thirty patients with IDDM of similar age and body weight also participated. Characteristics of all subjects are shown in Table 1. The patients received a combination of short- and intermediate-acting insulin 2–3 times/day; 24 patients used semisynthetic human insulin, 5 used pork insulin, and 1 used beef insulin. One patient had coronary heart disease and hypertension, which were treated with digoxin and propranolol. Another patient had epilepsy, hypertension, diabetic retinopathy, and neuropathy and was treated with phenobarbital, diphenylhydantoin, furosemide, and atenolol. Six patients had background retinopathy. All patients had normal liver and kidney function.

All subjects consumed a weight-maintaining diet containing ≥200 g carbohydrate/day for at least 3 days before study. Informed consent was obtained before participation. The study protocol was approved by the Ethical Committee of the University Hospital Leiden.

Insulin action in vivo. The sequential euglycemic clamp technique was used to quantify insulin-stimulated glucose

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TABLE 1
Characteristics of study participants

	Healthy subjects	IDDM patients
n (M/F)	8/7	16/14
Age (yr)	30.1 ± 2.9	28.6 ± 1.7
Body weight (kg)	70.4 ± 1.7	70.7 ± 1.5
Body mass index (kg/m^2)	23.2 ± 0.4	22.8 ± 0.3
Duration of IDDM (yr)		9.3 ± 1.5
Insulin dose (U/kg)		0.73 ± 0.04
HbA_1 (%)	6.7 ± 0.2†	10.5 ± 0.4
Stimulated C-peptide* (min/nM)	11.8 (8.5–13.0)†	0.1 (0.1–2.1)

Values are means ± SE where indicated. IDDM, insulin-dependent diabetes mellitus.
*Stimulated by injection of 1 mg glucagon i.v. Values are medians of total areas under the curve of C-peptide levels at 0, 4, 6, and 10 min by trapezoidal method with 25th–75th percentile in parentheses.
†$P < 0.001$ vs. IDDM patients.

disposal in vivo (8,9). A full description has been given elsewhere (6,10). Briefly, after a 10- to 12-h fast, insulin (Actrapid human, Novo, Copenhagen) was infused at rates of 0.5, 1, 2, and 5 mU · kg^{-1} · min^{-1} in four consecutive steps of 2 h preceded by a priming bolus of 2.5, 5, 10, and 50 mU/kg. Plasma glucose was measured every 5 min in arterialized venous blood. By infusing 20% glucose at variable rates, the plasma glucose level was maintained at ~5 mM. Each 30 min and at 10-min intervals during steady state (90th–120th min of each step), plasma was collected for insulin determinations. C-peptide was measured at 90 and 120 min of each step. The steady-state glucose infusion rate (SSGIR) was taken as measure of insulin action.

IDDM patients were admitted to the hospital the day before the study at 1800. Their evening dose of insulin was withheld, and instead, short-acting insulin (Actrapid human, Novo) was administered intavenously by a syringe perfusor to maintain normoglycemia (4–10 mM) during the night until the start of the clamp study. A gradual restoring of blood glucose levels was achieved: glucose levels were 10.1 ± 1.0, 8.3 ± 0.8, 6.4 ± 0.6, 5.3 ± 0.5, 4.5 ± 0.3, and 5.4 ± 0.3 mM at 1800, 2100, 2400, 0300, 0600, and 0730, respectively. Control subjects were admitted on the day of the study after an overnight fast (0730).

Plasma clearance rate of insulin (PCR). PCR (ml · kg^{-1} · min^{-1}) was calculated with the formula (11,12)

$$PCR = \frac{IIR_i}{[INS_i] - ([CPEP_i/CPEP_b] \times [INS_b])}$$

where IIR_i is the insulin infusion rate at step 1 (mU · kg^{-1} · min^{-1}), INS_i is steady-state plasma free insulin at step 1 (mU/L), $CPEP_i$ is steady-state plasma C-peptide at step 1 (nM), $CPEP_b$ is basal plasma C-peptide (nM), and INS_b is basal plasma free insulin (mU/L).

Assays. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman, Palo Alto, CA) after immediate centrifugation (10 s). Glycosylated hemoglobin (HbA_1) was measured by Corning electrophoresis (Palo Alto, CA). Fructosamine was determined in an autoanalyzer with a colorimetric reaction at 500 nm. Samples for hormone determinations were kept on ice and centrifuged within 0.5 h of collection; plasma was collected and stored at −20°C until assay. Plasma insulin levels were determined by radioimmunoassay with dextran-coated charcoal to separate bound from free insulin. When anti-insulin antibodies were detected, plasma was treated with polyethylene glycol (PEG) before insulin was assayed (13). In earlier experiments, excellent agreement was found for insulin levels determined in PEG-treated samples of control subjects versus untreated samples (regression line, $y = 0.98x + 0.81$; $r = 0.99$; $n = 30$; $P < 0.001$). We found no differences in insulin levels in the plasma of IDDM patients when PEG was added immediately after sampling or just before assay (regression line, $y = 0.97x + 0.62$; $r = 0.99$; $n = 20$; $P < 0.001$). Intra- and interassay variations of the insulin assay were 6.8 and 8.1%, respectively, with an assay sensitivity of 2 mU/L. C-peptide was measured by radioimmunoassay (Novo); intra- and interassay variations were 7.1 and 8.8%, respectively, with an assay sensitivity of 0.005 nM. Anti-insulin antibodies were measured by Christiansen's (14) method. When percentage of tracer binding was >5% ($n = 22$), antibodies were characterized further with a modification of Langmuir's absorption isotherm (15). Thereby, the equilibrium constant (K) and binding capacity (Cap) were determined by Scatchard analysis modified according to Reeves (16) for polyclonal antiserums.

Statistical methods. Comparisons between data from nondiabetic and IDDM subjects were performed with the unpaired Student's t test. Simple and multiple least-squares regression analyses were performed with standard methods (17). Data are presented as means ± SE or as medians with 25th–75th percentiles as indicated.

RESULTS

Baseline characteristics. Median percentage of tracer binding for insulin antibodies (IA) was 15% (25th–75th percentile, 6–21%). In 22 patients, IA was >5%, allowing for a further characterization: K was 2.24 ± 0.29 × 10^8 ml/mmol, and Cap was 1.18 ± 0.16 × 10^{-8} mmol/ml. The correlation between K and IA was $r = 0.47$ ($P < 0.05$) and between Cap and IA was 0.61 ($P < 0.002$); correlation for K versus Cap was $r = -0.01$ (NS). In multiple regression analysis relating K and Cap to IA, the partial correlation coefficient for K versus IA was $r = 0.60$ ($P < 0.01$) and for Cap versus IA was $r = 0.68$ ($P < 0.001$). The square of the correlation coefficient for both variables (percentage of variance in IA attributable to K and Cap) was $R^2 = 0.58$ ($P < 0.001$).

Clamp studies. Basal plasma glucose was 4.8 ± 0.3 mM in nondiabetic and 5.4 ± 0.3 mM in IDDM subjects (NS); respective values for basal plasma free insulin were 10 ± 1 and 21 ± 2 mU/L ($P < 0.001$). In both nondiabetic and IDDM subjects, steady-state glucose levels averaged 4.9–5.1 mM, with coefficients of variation ranging from 3 to 7%.

Steady-state plasma free-insulin levels were lower in IDDM than nondiabetic subjects (Table 2). No significant correlations were found between IA, K, or Cap and the achieved insulin levels ($r < 0.40$). Coefficients of variation for SSGIR varied between 2 and 8%. SSGIRs were lower in IDDM patients than control subjects during steps 1–3 (at submaximal insulinemia) but not step 4 (at maximal insulinemia) (Table 2).

PCR. During all four infusion steps, calculated PCR was

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TABLE 2
Steady-state plasma free-insulin levels, glucose infusion rates (SSGIRs), and plasma clearance rates of insulin (PCRs) during euglycemic (5 mM) clamp studies

	Healthy subjects (n = 15)	IDDM patients (n = 30)
Plasma free insulin (mU/L)		
Step 1	43 ± 2*	38 ± 2
Step 2	85 ± 5*	70 ± 4
Step 3	168 ± 10*	148 ± 7
Step 4	666 ± 45†	535 ± 32
SSGIR (μmol · kg^{-1} · min^{-1})		
Step 1	24 ± 2‡	13 ± 1
Step 2	48 ± 2‡	34 ± 1
Step 3	61 ± 3‡	50 ± 2
Step 4	64 ± 3	60 ± 3
PCR (ml · kg^{-1} · min^{-1})		
Step 1	12.0 ± 0.7*	14.2 ± 0.6
Step 2	12.0 ± 0.7†	16.1 ± 0.8
Step 3	12.5 ± 0.7*	14.8 ± 0.7
Step 4	7.7 ± 0.5†	10.9 ± 0.7

Values are means ± SE. Insulin was infused in 4 2-h steps at rates of 0.5, 1, 2, and 5 mU · kg^{-1} · min^{-1}; steady-state was defined as the 90th–120th min of each step. IDDM, insulin-dependent diabetes mellitus.
*P < 0.05, †P < 0.01, ‡P < 0.001, vs. IDDM patients.

higher in IDDM than nondiabetic subjects (Table 2). Scatter diagrams of PCR versus SSGIR are shown in Fig. 1*A* for healthy subjects and in Fig. 1*B* for IDDM patients. In healthy subjects, regression analysis showed strong positive correlations (r = 0.71, P < 0.005; r = 0.72, P < 0.005; r = 0.72, P < 0.005; and r = 0.78, P < 0.001 for steps 1–4, respectively; Fig. 1*A*). In contrast, in the IDDM patients, no relationship was observed (r = 0.01, −0.03, 0.06, and 0.01 [NS]; Fig. 1*B*).

Data of the 8 patients with IA ≤5% compared with the remaining 22 patients with IA >5% showed no significant differences in PCR; PCRs in patients with IA <5% were 13.6 ± 1.4, 15.0 ± 1.7, 13.8 ± 1.3, and 10.0 ± 1.1 ml · kg^{-1} · min^{-1} for steps 1–4, respectively, vs. 14.3 ± 0.7, 16.5 ± 1.0, 15.1 ± 0.8, and 11.1 ± 0.8 ml · kg^{-1} · min^{-1} in the remaining 22 patients (all NS). With simple regression analysis, R^2 values for PCR versus SSGIR were slightly higher but still nonsignificant in the 8 patients with IA <5% versus the 22 patients with IA >5% (R^2 for steps 1–4: 9.6, 0, 9.6, and 8.4% vs. 0.8, 0, 0.5, and 0.3%, respectively). The maximum difference in R^2 was 9.1% for step 3 (NS). When the 22 patients with IA >5% were divided into two groups according to Cap, no significant differences were found for PCR. For the group with Cap below the median of 1.12 × 10^{-8} mmol/ml (n = 11), PCRs for steps 1–4 were 14.0 ± 1.2, 14.1 ± 1.1, 13.3 ± 0.9, and 10.1 ± 1.0 ml · kg^{-1} · min^{-1}, respectively, vs. 14.7 ± 1.1, 15.6 ± 1.4, 15.2 ± 1.3, and 12.3 ± 1.2 ml · kg^{-1} · min^{-1}, respectively, for the group with Cap above the median (n = 11, all NS). A similar division of K values yielded PCRs of 14.5 ± 0.8, 16.5 ± 1.2, 15.3 ± 1.2, and 12.0 ± 1.5 ml · kg^{-1} · min^{-1} for the group with K above the median of 1.75 × 10^8 ml/mmol (n = 11) vs. 14.3 ± 1.2, 16.6 ± 1.5, 15.1 ± 1.1, and 10.5 ± 0.7 ml · kg^{-1} · min^{-1} in steps 1–4, respectively, for the group with K below the median (NS). Simple regression analysis of PCR versus SSGIR indicated nonsignificant relationships in all subgroups for steps 1–4 (r < 0.40).

To examine the role of other potentially confounding variables, linear multiple regression models were constructed with PCR, HbA_1 or fructosamine, insulin antibodies (IA, K, Cap, or K and Cap), and duration of IDDM as independent variables. SSGIR was used as the outcome variable. The model with Cap as controlling variable for insulin antibodies and HbA_1 for preceding glycemic control is shown in Table 3. Inclusion of extra variables into the regression model only slightly improved the relationship between PCR and SSGIR for step 2 (from 0.09% = [0.03^2 × 100%] to 0.38%) and step 4 (from 0.01% = [0.01^2 × 100%] to 0.47%). However, the relationship between PCR and SSGIR remained nonsignificant. Similar results were found when fructosamine was used instead of HbA_1 and when IA, K, or K and Cap were used as controlling variables for insulin antibodies (data not shown).

In the first regression model (Table 3), HbA_1 evolved as an important negative determinant for insulin action in the IDDM patients. A second restricted model was constructed with PCR and HbA_1 as independent variables both in nondiabetic and IDDM subjects (Table 4). Compared with the simple regression analysis (Fig. 1*A*), PCR remained strongly related to SSGIR in the healthy subjects, whereas HbA_1 was unrelated to SSGIR. In the IDDM patients, the correlations between PCR and SSGIR slightly improved compared with the model in Table 3 (maximum gain in step 3 of 5.3% [5.3 − 0.0 × 100%], NS), whereas the correlations between HbA_1 and SSGIR all increased.

DISCUSSION

We studied the relationship between PCR and insulin action in vivo. In healthy subjects, high positive correlations were found for PCR versus insulin-stimulated glucose disposal (r = 0.71–0.78, steps 1–4, 0.001 < P < 0.005). In contrast, in the IDDM patients, no relationship was observed (r = 0.01, −0.03, 0.06, and 0.01 [NS], respectively). In univariate analyses of PCR, no differences were found between patient subgroups with values for percentage of tracer binding below or above 5% or IA Cap and equilibrium constants below or above the median. In multivariate analyses with adjustments for insulin antibodies, preceding long- and short-term glycemic control, and duration of IDDM, the correlation for PCR versus SSGIR remained nonsignificant.

The calculated PCR was higher in patients with IDDM. Possibly the distribution volume for insulin was greater (18,19). Because in the clamp studies insulin was administered per kilogram of body weight, which was similar to control subjects, a greater distribution volume with an unchanged PCR would yield lower steady-state insulin levels and thus higher calculated PCRs. In other studies of IDDM, insulin clearance was found to be increased (20,21), decreased (22), or unchanged (23). Differences in the methods used to assess insulin clearance or heterogeneity among the diabetic patients studied may be the cause of these discrepant findings. In none of these studies was the relationship between insulin clearance and action investigated.

Insulin antibodies could also account for the lower plasma free-insulin levels. If so, subjects with the most antibodies would be expected to have the lowest free-insulin levels. We

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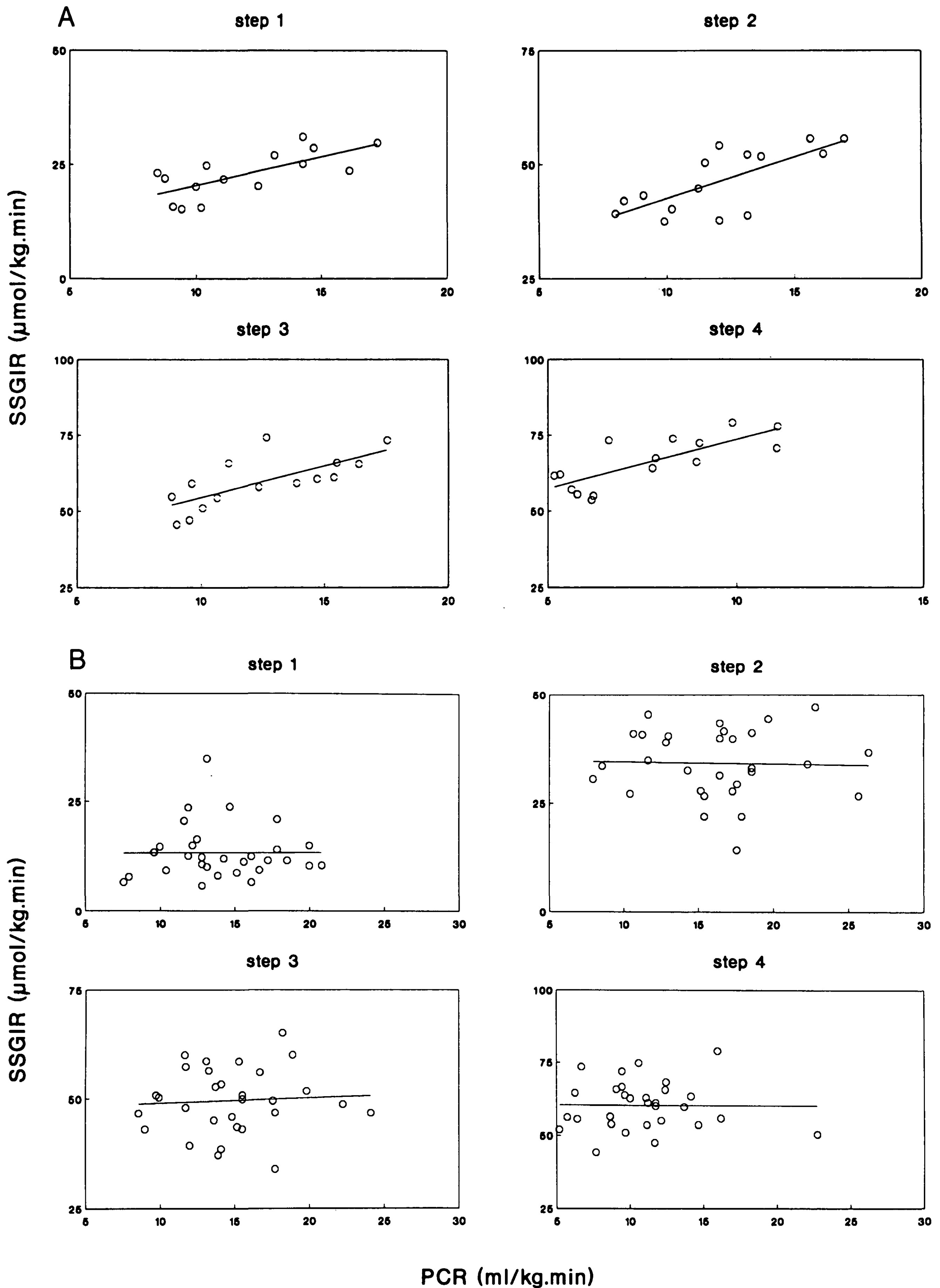

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TABLE 3
Multiple linear regression analysis in 22 insulin-dependent diabetes mellitus (IDDM) patients with plasma clearance rate of insulin (PCR), glycosylated hemoglobin (HbA_1), insulin antibodies (binding capacity [Cap]), and duration of IDDM as independent variables and steady-state insulin-stimulated glucose disposal (SSGIR) as outcome variable

SSGIR	PCR ($ml \cdot kg^{-1} \cdot min^{-1}$)	HbA_1 (%)	Insulin antibodies (Cap, 10^{-8} mmol/ml)	Duration of IDDM (yr)
Step 1				
Regression coefficient ± SE	0.16 ± 0.47	−0.75 ± 0.65	0.10 ± 0.21	−0.14 ± 0.22
R^2	0	7.4	1.4	2.5
Step 2				
Regression coefficient ± SE	−0.14 ± 0.55	−1.79 ± 0.75	0.25 ± 0.29	0.20 ± 0.25
R^2	0.4	25.0*	4.2	3.6
Step 3				
Regression coefficient ± SE	−0.01 ± 0.54	−1.66 ± 0.62	0.33 ± 0.23	0.07 ± 0.20
R^2	0	30.0†	10.7	0.8
Step 4				
Regression coefficient ± SE	−0.14 ± 0.51	−1.50 ± 0.73	0.54 ± 0.33	0.11 ± 0.23
R^2	0.5	20.0*	12.2	1.3

SSGIR was assessed by sequential euglycemic (5 mM) clamps with insulin infusion rates of 0.5, 1, 2, and 5 $mU \cdot kg^{-1} \cdot min^{-1}$; steady state was considered the 90th–120th min of a step and was taken as a measure of insulin action. Insulin antibodies were determined by measuring their binding capacity by a modification of Langmuir's absorption isotherm (see RESEARCH DESIGN AND METHODS). Partial regression coefficients are given with standard errors. (Partial squared correlation coefficients are percentages of variation in SSGIR attributable to a variable after the influence of all other variables has been removed). Regression constants ± SE for steps 1–4: 20.9 ± 8.5, 50.6 ± 8.6, 61.3 ± 7.1, and 70.0 ± 7.6, respectively.
*$P < 0.05$, †$P < 0.02$.

characterized antibodies by measuring percentage of tracer binding according to Christiansen's (14) method and, if IA was >5%, by modified Scatchard analysis (Langmuir's absorption isotherm), which allows for the derivation of Cap and *K*. However, no significant correlation was found between IA, Cap, or *K* and the achieved free-insulin level. Furthermore, Waldhäusl et al. (20) reported no significant differences in achieved free-insulin levels, either submaximal

TABLE 4
Multiple regression analysis with plasma clearance rate of insulin (PCR) and glycosylated hemoglobin (HbA_1) as independent variables and steady-state insulin-stimulated glucose disposal (SSGIR) as outcome variable

	Healthy subjects (n = 15)		IDDM patients (n = 30)	
SSGIR	PCR ($ml \cdot kg^{-1} \cdot min^{-1}$)	HbA_1 (%)	PCR ($ml \cdot kg^{-1} \cdot min^{-1}$)	HbA_1 (%)
Step 1				
Regression coefficient ± SE	1.26 ± 0.37	0.34 ± 1.46	0.14 ± 0.31	−1.18 ± 0.47
R^2	49.0*	0.4	0.8	18.8†
Step 2				
Regression coefficient ± SE	1.94 ± 0.53	1.35 ± 2.02	0.20 ± 0.29	−1.96 ± 0.56
R^2	52.9*	3.6	1.8	31.1*
Step 3				
Regression coefficient ± SE	2.08 ± 0.60	0.07 ± 2.44	0.40 ± 0.33	−1.89 ± 0.52
R^2	49.8*	0.1	5.3	32.6*
Step 4				
Regression coefficient ± SE	3.26 ± 0.79	0.41 ± 2.23	0.48 ± 0.40	−2.02 ± 0.63
R^2	58.5*	0.3	4.9	27.5*

SSGIR was assessed by sequential euglycemic (5 mM) clamps with insulin infusion rates of 0.5, 1, 2, and 5 $mU \cdot kg^{-1} \cdot min^{-1}$; steady state was considered the 90th–120th min of a step and was taken as a measure of insulin action. Partial regression coefficients are given with standard errors. Partial squared correlation coefficients are percentages of variation in SSGIR attributable to a variable after the influence of all other variables has been removed. Regression constants ± SE for steps 1–4: healthy subjects, 5.5 ± 11.9, 13.9 ± 17.0, 33.3 ± 19.7, and 38.2 ± 17.8, respectively; insulin-dependent diabetes mellitus (IDDM) patients, 23.7 ± 6.1, 51.5 ± 6.6, 63.5 ± 6.5, and 76.4 ± 6.6, respectively.
*$P < 0.02$, †$P < 0.01$.

FIG. 1. Scatter diagrams of plasma clearance rate of insulin (PCR) versus steady-state glucose infusion rate (SSGIR) determined in sequential euglycemic (5 mM) clamps in 15 healthy subjects (*A*) and 30 insulin-dependent diabetic patients (*B*). Steps 1–4 signify steps during which insulin was administered intravenously at rates of 0.5, 1, 2, and 5 $mU \cdot kg^{-1} \cdot min^{-1}$, respectively. PCR was calculated by dividing insulin infusion rate by achieved plasma insulin level corrected for endogenous insulin secretion (see RESEARCH DESIGN AND METHODS). Equations for regression lines are $y = 1.24x + 8.05$, $y = 1.81x + 24.43$, $y = 2.07x + 33.82$, and $y = 3.22x + 41.27$ in *A* and $y = 0.01x + 13.22$, $y = -0.05x + 35.00$, $y = 0.11x + 47.93$, and $y = -0.01x + 60.44$ in *B* for steps 1–4, respectively. Correlation coefficients are $r = 0.71$ ($P < 0.005$), $r = 0.72$ ($P < 0.005$), $r = 0.72$ ($P < 0.005$), and $r = 0.78$ ($P < 0.001$) in *A* and r = 0.01, 0.03, 0.06, and 0.01 (all NS) in *B* in steps 1–4, respectively.

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or maximal, between IDDM patients with low and moderate amounts of antibodies in clamp studies. In our study, patients had low amounts of insulin antibodies: 8 of 30 had IA <5% (below which further quantitation by Langmuir's isotherm is not possible), and only 5 of 30 had IA >25%. Taken together, this makes a major influence by insulin antibodies unlikely.

The main findings of our study are a strong relationship between PCR and insulin action in healthy subjects and the total lack of this relationship in IDDM patients. To our knowledge, this study is the first to demonstrate such a relationship in vivo at different (submaximal and maximal) insulin levels within a sizable group of healthy subjects. In another study, the combined data sets of healthy subjects and patients with obesity, hyperthyroidism, and Cushing's syndrome displayed a positive correlation between insulin clearance and action (5). In case studies of acanthosis nigricans (3,4) and lipoatrophy (3), extreme insulin resistance was associated with markedly decreased insulin clearance. We previously reported a patient with hyperthyroidism and newly diagnosed IDDM who had initially marked increases in both insulin clearance and action; with the amelioration of the hyperthyroid state, both processes became normal (24). In studies of obesity (25,26) and aging (27), decreased insulin action coexisted with decreased insulin clearance. No such relationship was found in our group of IDDM patients. For unknown reasons, in our IDDM patients, although insulin action was decreased at low insulinemia, there was no relationship between insulin action and clearance, whereas at maximal insulinemia, when insulin action was similar to healthy subjects, we still found no relationship between insulin action and clearance.

Factors that could have obscured the relationship were explored in several ways. First, univariate analyses were performed on PCR values with patients divided into subgroups according to percentage of IA, Cap, or K. No differences in PCR were found between patients with IA below versus above 5% (below which further characterization in terms of Cap and K is impossible) or between patients with Cap or K below versus above the median. Moreover, in simple regression analyses of these subgroups, no significant relationships between PCR and SSGIR were found.

Second, multiple linear regression models were constructed with insulin antibodies percentage of IA, K, Cap, or K and Cap, glycemic control (HbA_1 and fructosamine; in healthy subjects, insulin clearance was found to be decreased during hyperglycemia; 28,29), and duration of IDDM as controlling variables, and insulin-stimulated glucose disposal was used as the outcome variable. For PCR, IA, K, Cap, and duration of IDDM, nonsignificant correlations were found with SSGIRs, whereas HbA_1 was significantly inversely correlated to insulin action in IDDM, as reported previously (6,30). When these factors were included in the multiple regression model, only minor improvements in the relationship between PCR and SSGIR were found (maximum gain in step 4, partial R^2 from 0.01 to 0.47%).

Third, hepatic glucose output (HGO) could have obscured the relationship. HGO was not determined in this study. Fasting HGO in patients kept normoglycemic overnight is comparable to healthy control subjects (31,32). At insulin levels ~45 mU/L (step 1 in this study), HGO is suppressed by 70% in both diabetic and nondiabetic subjects (9,30). Fasting HGO averages 10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (31–33). If 70% suppression is assumed during step 1, whole-body glucose disposal would be underestimated by 3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Even if in the diabetic patients insufficient suppression is assumed (e.g., 20 instead of 70%), the maximum difference in underestimation of whole-body glucose disposal would be 5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (8 − 3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). This factor, although theoretically small, could introduce noise when relating PCR to SSGIR in step 1. However, at higher insulin levels during steps 2–4, HGO is completely inhibited, and SSGIRs can be equated with glucose disposal rates. Then HGO cannot be expected to obscure the relationship between PCR and SSGIR. These facts taken together make a major influence of insulin antibodies, prior glycemic control, duration of IDDM, and HGO on the relationship between PCR and insulin action unlikely.

We found a strong positive correlation between insulin clearance and action in the healthy subjects, suggesting a functional relationship. This finding supports from an in vivo perspective the in vitro evidence linking insulin action to insulin internalization. Although not fully understood, internalized insulin-receptor complexes were found to be involved in physiological actions of insulin. For instance, in rat adipocytes, monensin (1) or chloroquine substrate analogues (2) inhibited insulin internalization in parallel with inhibition of insulin-stimulated glucose transport. It is not known how such processes might be related. It was hypothesized that insulin internalization and insulin-stimulated glucose transport shared a step in vesicular trafficking, common to both the processing of insulin-receptor complexes to the interior of the cell and the recruitment of glucose-transporter units to the plasma membrane (34). This process may have become distorted in the IDDM patients due to hyperglycemia.

In conclusion, insulin action is correlated to insulin clearance in healthy subjects, suggesting a functional relationship from an in vivo perspective. No such relationship was present in patients with IDDM, even after adjusting for insulin antibodies, preceding glycemic control, and duration of IDDM.

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