Hyperglycemia Facilitates Urinary Excretion of C-Peptide by Increasing Glomerular Filtration Rate in Non–Insulin-Dependent Diabetes Mellitus

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We have evaluated the feasibility of monitoring the 24-hour urinary excretion rate of C-peptide (U-CPR) as a measure of integrated β-cell function in patients with non–insulin-dependent diabetes mellitus (NIDDM). In 37 normoalbuminuric patients, U-CPR of 117.9 ± 9.1 μg/d (mean ± SEM) during the poorly controlled glycemic phase (fasting plasma glucose [FPG], 171 ± 7 mg/dL; hemoglobin A_{1C} [HbA_{1c}], 8.8% ± 0.4%) was significantly higher than the value of 83.3 ± 13.7 μg/d ($P < .001$) during the well-controlled phase (FPG, 135 ± 6 mg/dL; HbA_{1c}, 7.0% ± 0.2%), although the plasma insulin response to meals was lower during the former phase (53.3 ± 6.3 μU/mL) versus the latter phase (65.7 ± 6.6, $P < .005$). Endogenous creatinine clearance (Ccr) was significantly elevated during the poorly controlled phase (105.4 ± 7.3 *v* 88.7 ± 4.7 mL/min, $P < .005$). In 26 microalbuminuric patients, the plasma insulin response was greater during good glycemic control, but U-CPR did not differ between the two phases. Ccr was comparable at two phases in this group (92.7 ± 7.4 *v* 91.1 ± 5.9 mL/min, NS). U-CPR correlated positively with Ccr in both groups ($r = .593$, $P < .001$ in normoalbuminuria; $r = .585$, $P < .001$ in microalbuminuria). In addition, when biosynthetic human C-peptide was infused intravenously at an identical rate in two healthy subjects, resulting steady-state plasma levels of CPR were lower, and fractional U-CPR was higher during the moderately hyperglycemic phase versus the euglycemic phase. The calculated metabolic clearance rate (MCR) of C-peptide was found to be increased in the presence of hyperglycemia. These results suggest that hyperglycemia enhances U-CPR through an increase of glomerular filtration rate (GFR), and therefore U-CPR as an index of residual β-cell function in diabetes should be used cautiously.

C-PEPTIDE AND INSULIN are cosecreted from islet β cells in an equimolar ratio.[1,2] Unlike insulin, C-peptide does not seem to be extracted by the liver in dogs[3] or humans,[4] and is removed largely by the kidneys.[5-7] C-peptide is excreted in urine in even higher concentrations than in plasma. Moreover, the metabolic clearance rate (MCR) of C-peptide has been shown to remain relatively constant over a wide physiologic range of plasma concentrations[3,8] and also to be nearly equivalent in both normal and diabetic subjects.[5] Therefore, the urinary excretion rate of C-peptide (U-CPR) has been generally accepted as a reflection of integrated β-cell secretion in both normal and diabetic states.[9-11] To date, measurement of U-CPR is widely used as a diagnostic aid for insulin-dependent diabetes mellitus (IDDM), because little or no C-peptide excretion is invariably found in this type of diabetes. In contrast, we have observed that U-CPRs show a discordance with the plasma response of C-peptide or insulin in the case of non–insulin-dependent diabetes mellitus (NIDDM), ie, inappropriately large amounts of C-peptide are excreted in the urine in patients who show a low plasma insulin response to ingestion of mixed meals. Obviously, hyperglycemia is of great interest as a potential factor for increasing U-CPR. Therefore, in the present study we evaluated the effects of hyperglycemia on the changes in U-CPR and endogenous creatinine clearance rate (Ccr), an approximation of glomerular filtration rate (GFR), and their relationship in NIDDM patients.

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Submitted September 12, 1994; accepted January 18, 1995.

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0026-0495/95/4409-0016$03.00/0

SUBJECTS AND METHODS

Subjects

The subjects consisted of 63 NIDDM patients whose diabetes was managed by diet alone (n = 20) or by oral hypoglycemic agents (n = 43). None of these patients had advanced diabetic complications, including clinical proteinuria or elevated serum creatinine concentrations. They were divided into two groups according to the degree of urinary albumin excretion: 37 patients were normoalbuminuric (<30 mg/d), and 26 were microalbuminuric (30 to 300 mg/d). Clinical profiles of these subjects are listed in Table 1. Twenty healthy subjects served as controls (12 men; ages 30.7 ± 0.9 years; body mass index, 22.8 ± 0.3). In a separate study, two healthy male volunteers (22 and 25 years old) and one female IDDM patient (46 years old) with no C-peptide response to glucagon and negative for antiinsulin antibodies participated in the C-peptide infusion experiment.

Methods

A 24-hour urine sample was collected at home using a measuring bag that contained sodium azide (NaN_3) as a preservative. Total volume was measured by the patients, and an aliquot of the sample was brought to the hospital on the day of completion. Urinary albumin concentrations were determined using a commercial radioimmunoassay (RIA) kit (Albumin Kit; Eiken, Tokyo, Japan).

Urinary C-peptide concentrations were determined by a double-antibody RIA using a C-peptide RIA kit (Shionogi Laboratories, Osaka, Japan). As reported previously,[12] urine samples were diluted 1:10 with assay buffer (0.01 mol/L phosphate-buffered saline with 0.01 mol/L EDTA and 0.5% bovine serum albumin), which allows most samples to be measured within the optimal range of sensitivity of the standard curve.

After overnight fasting, a meal tolerance test was performed using meals of undefined composition with individual allowance for calories on two occasions, ie, one under metabolically poor control and the other under good control, from several months to 1 year apart. In most cases, the test was performed on the next morning after completion of the 24-hour urine collection. There was no change in the type of treatment for diabetes on these two occasions. The status of normoalbuminuria or microalbuminuria in

Table 1. Clinical Profiles of the Subjects (mean ± SEM)

	Normoalbuminuria (n = 37)		Microalbuminuria (n = 26)	
Characteristic	Phase I	Phase II	Phase I	Phase II
Sex (M/F)	21/16		16/10	
Age (yr)	52 ± 2.1		56 ± 2.1	
Duration of diabetes (yr)	9.8 ± 1.2		8.1 ± 1.2	
Tx				
Diet	16		4	
OHA	21		22	
BMI (kg/m²)	22.8 ± 0.5	22.3 ± 0.5	23.1 ± 0.5	23.8 ± 0.5
S-Cr (mg/dL)	0.9 ± 0.04	0.85 ± 0.03	0.89 ± 0.04	0.87 ± 0.03
U-Alb (mg/d)	7.9 ± 0.8	9.7 ± 1.2	75.3 ± 17.9	77.3 ± 14.3

Abbreviations: OHA, oral hypoglycemic agents; S-cr, serum creatinine; U-Alb, urinary albumin excretion rate; BMI, body mass index; Tx, treatment.

each group of patients stayed consistent during this interval (Table 1).

Plasma glucose (ΣPG) and immunoreactive insulin (ΣIRI) responses were defined as the sum of PG and insulin levels at 0, 60, and 120 minutes after ingestion of the meal. Plasma insulin concentrations were measured using a Phadeseph Insulin RIA kit (Pharmacia, Stockholm, Sweden).

Synthetic human C-peptide and somatostatin were purchased from Sigma Chemical (St Louis, MO) and were prepared for injection by filtration through a membrane filter (Millex 0.22 μm; Millipore, Milford, MA) after being dissolved in 0.9% sterilized saline solution. They both proved negative in a pyrogen test. C-peptide was infused intravenously by a microinfusion pump (Mifuser PSW-11A; Nikkiso, Tokyo, Japan), at a rate of 20 or 40 μg/h. At the start of each infusion, a bolus injection consisting of 4% to 6% of the total dose of C-peptide for the subsequent infusion was given. To suppress endogenous C-peptide secretion, somatostatin infusion preceded C-peptide infusion at a rate of 300 μg/h using a peristaltic pump (Nihon Koden, Tokyo, Japan) over the entire experimental period. During the hyperglycemic phase, 10% glucose was infused at a rate that maintained PG at a level of 180 to 220 mg/dL, which kept plasma insulin and C-peptide suppressed throughout the experiment by somatostatin infusion at this rate. Blood samples for PG, insulin, and C-peptide determinations were drawn from an indwelling catheter in the contralateral hand vein at the intervals indicated in Figs 3 and 4. MCR of synthetic human C-peptide was calculated with the use of measurements of the infusion rate of C-peptide and mean steady-state plasma level of CPR during the two phases of glycemia in two healthy controls and one C-peptide–negative IDDM patient.

Informed consent was obtained from each subject who participated in the C-peptide infusion experiment, and the study was approved by the Institutional Committee of the Diabetes Center.

Statistical analysis was performed using Student's paired or

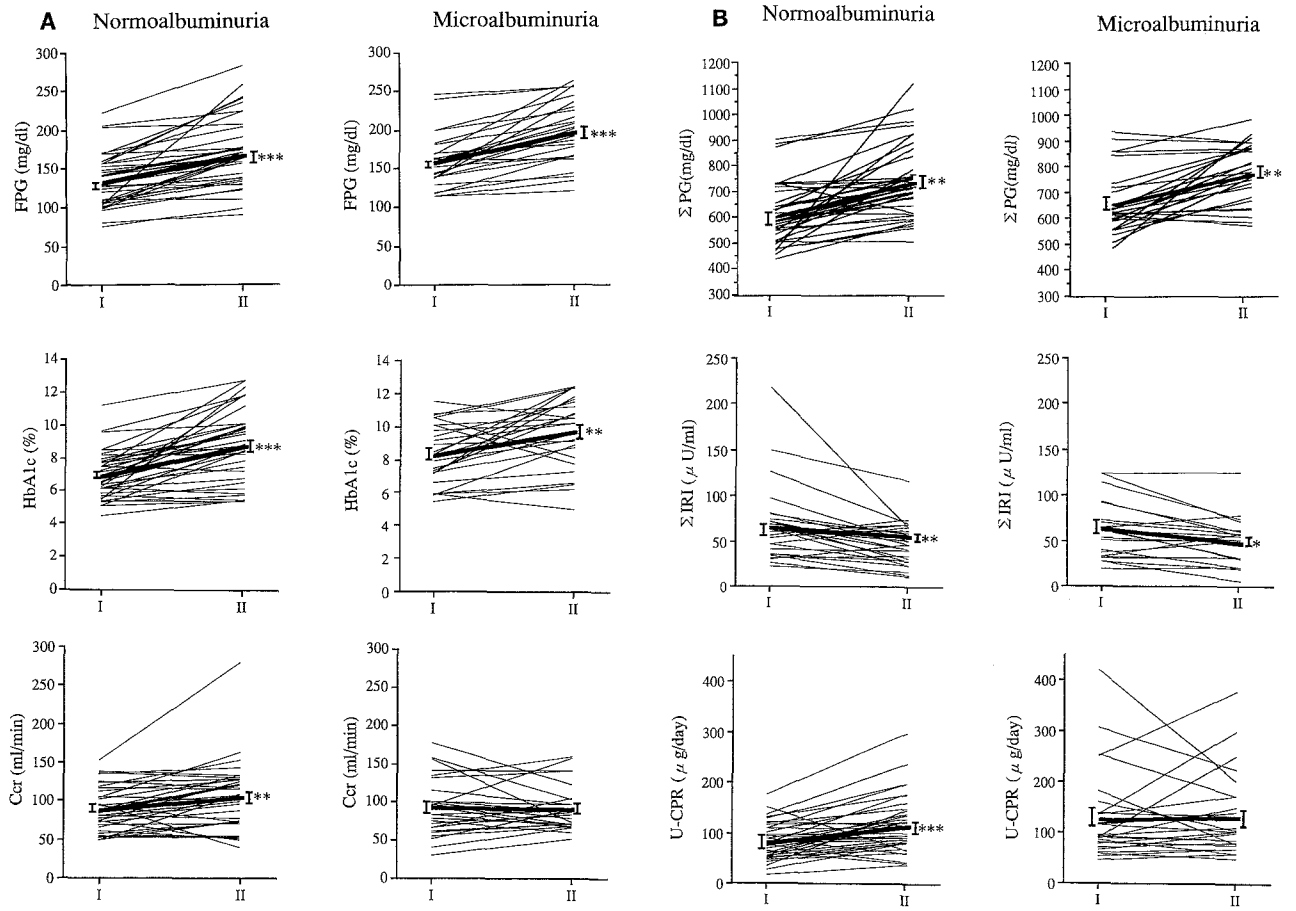

Fig 1. Changes in (A) FPG, HbA_{1c}, and Ccr and in (B) ΣPG and ΣIRI responses and U-CPR between well-controlled (I) and poorly controlled (II) phases of diabetes. Data show changes in the same patient. (▬) Mean ± SEM. *$P < .05$, **$P < .005$, ***$P < .001$.

nonpaired t test and Spearman's correlation coefficient as appropriate. Data are the mean ± SEM.

RESULTS

Mean differences in fasting PG (FPG) and hemoglobin A_{1c} (HbA_{1c}) levels between poorly controlled and well-controlled glycemic phases were similar in the normo- and microalbuminuric groups: approximately 35 to 40 mg/dL for FPG and 1.5% to 2.0% for HbA_{1c} (Fig 1A and B). Plasma insulin response was significantly higher in the good-control phase in the group with normoalbuminuria (65.7 ± 6.6 *v* 53.3 ± 6.3 μU/mL, $P < .005$) and the group with microalbuminuria (65.0 ± 7.8 *v* 48.3 ± 5.0, $P < .01$). In contrast, U-CPR was significantly lower in the good-control phase in the normoalbuminuric group (83.3 ± 13.7 *v* 117.9 ± 9.1 μg/d, $P < .001$), but not in the microalbuminuric group (130.7 ± 17.6 *v* 134.6 ± 16.0). These values for U-CPR in either phase of glycemia were significantly higher than those in normal control subjects (61.5 ± 6.4 μg/d, $P < .01$). The mean Ccr was significantly higher under poor glycemic control in the normoalbuminuric group (105.4 ± 7.3 *v* 88.7 ± 4.6 mL/min, $P < .005$), but was not significantly different between the two glycemic phases in the microalbuminuric group (91.1 ± 5.9 *v* 92.7 ± 7.4). U-CPR correlated with Ccr values in both normoalbuminuria ($r = .593$, $P < .001$) and microalbuminuria ($r = .585$, $P < .001$) (Fig 2A and B).

Figures 3 and 4 show a comparison of steady-state plasma levels of infused human C-peptide between euglycemia (70 to 90 mg/dL) and moderate hyperglycemia (180 to 220) in normal healthy subjects. Plasma insulin levels remained low over the entire experiment, reflecting the effective suppression of endogenous insulin (and also C-peptide) secretion by somatostatin. At the two infusion rates (20 and 40 μg/h), the resulting mean plasma CPR levels in the euglycemic phase were 0.7 ± 0.02 and 3.0 ± 0.1 ng/mL, respectively, and were decreased to 0.3 ± 0.04 and 2.3 ± 0.06, respectively, in the hyperglycemic phase. During the experiment, fractional U-CPR was increased in the hyperglycemic phase (3.6 and 6.1 μg/g creatinine) as compared with the euglycemic phase (3.3 and 4.2, respectively).

MCR of synthetic human C-peptide was 2.7 mL/kg/min during euglycemia and increased to 4.4 during hyperglycemia in one healthy subject. A similar increase from 4.3 mL/kg/min during euglycemia to 10.8 during hyperglycemia was observed in the other healthy subject. In addition, in one IDDM patient, MCR of synthetic human C-peptide was 8.8 mL/kg/min during substantial hyperglycemia (260 to 300 mg/dL; data not shown).

DISCUSSION

The present study demonstrated that hyperglycemia facilitates U-CPR in NIDDM patients whose residual β-cell function is preserved. This conclusion was based on the following findings: First, U-CPR was paradoxically increased during the hyperglycemic phase, while plasma insulin response to mixed meals was substantially reduced in the same patients during this phase. Second, acute elevation of PG to moderate hyperglycemia (~200 mg/dL)

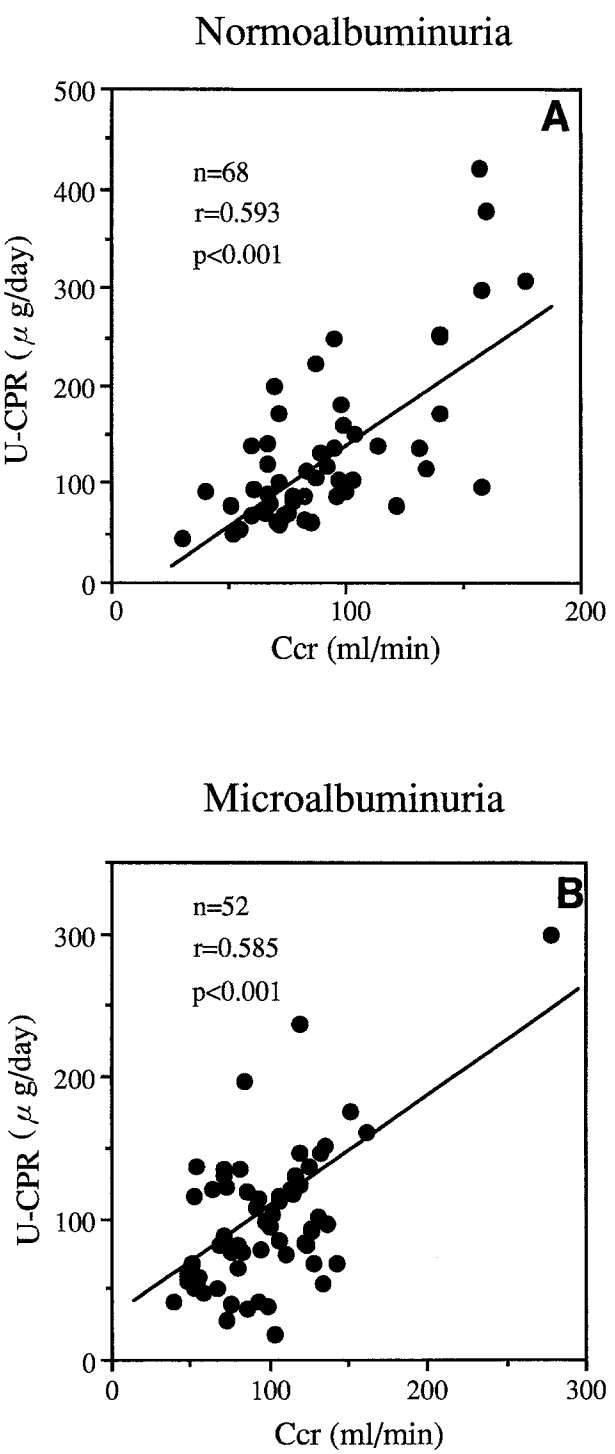

Fig 2. Correlation between U-CPR and Ccr. All paired data from both glycemic controls were included in normoalbuminuric (A) and microalbuminuric (B) groups.

in normal subjects resulted in a lower plasma level of C-peptide and an increase in U-CPR, while the infusion rate of synthetic C-peptide was kept constant. Endogenous insulin and C-peptide concentrations appropriately decreased before the exogenous C-peptide infusion was commenced, and remained low even under hyperglycemia by

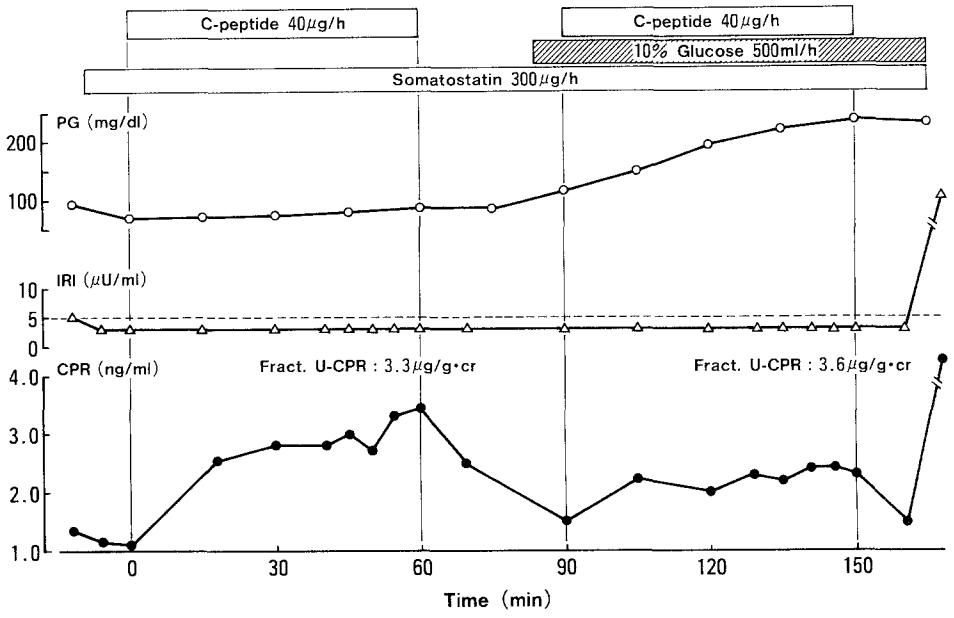

Fig 3. Effects of hyperglycemia on steady-state plasma level of C-peptide and U-CPR during a constant-rate (40 μg/h) infusion of synthetic human C-peptide in a healthy subject (25-year-old man; 175.0 cm; 72.0 kg).

continuous infusion of somatostatin. Although we did not confirm these latter findings in diabetic patients, hyperglycemia is reasonably considered to facilitate U-CPR. Our calculated MCR of C-peptide was in agreement with results reported previously by Meistas et al[9] in normal subjects (3.7 to 5.1 mL/kg/min), and MCR was increased during hyperglycemia in normal subjects and was high in a poorly controlled IDDM patient.

Limited data are available concerning whether differences in glycemic control can affect U-CPR in the same diabetic individuals. Garvey et al[13] reported results relevant to this issue, as follows: (1) 24-hour U-CPR in NIDDM patients did not correlate with integrated serum insulin and C-peptide levels, (2) U-CPR was increased in NIDDM patients, and (3) intensive insulin therapy partially reversed this increase. From these results, they concluded that U-CPR does not predict insulin secretion in NIDDM. Some investigators have also suggested that factors other than peripheral C-peptide levels are important in determining changes in U-CPR in NIDDM.[14-17] However, neither of these investigators specified any mechanisms by which hyperglycemia facilitates U-CPR. In the present study, we demonstrated a significant correlation between U-CPR and Ccr in both normoalbuminuric and microalbuminuric NIDDM patients. Therefore, we speculate that the increased U-CPR is likely to be due to an increase in GFR ("hyperfiltration") secondary to hyperglycemia. Intravenous glucose infusion that causes moderate hyperglycemia in the range of 200 mg/dL has been shown to increase GFR (6% to 10%) in normal subjects.[18-20] In the microalbumin-

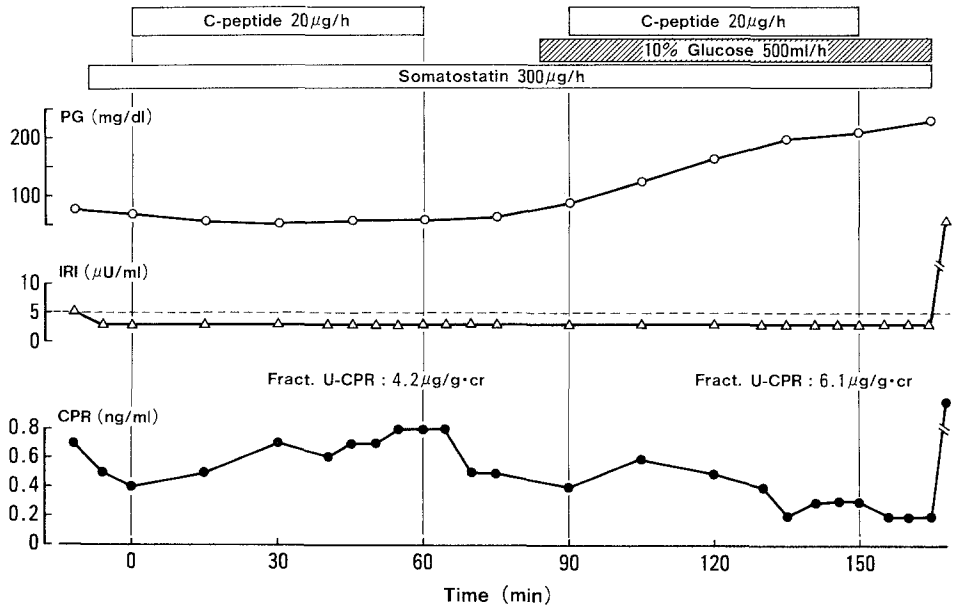

Fig 4. Effects of hyperglycemia on steady-state plasma level of C-peptide and U-CPR during a constant-rate (20 μg/h) infusion of synthetic human C-peptide in a healthy subject (22-year-old man; 165.0 cm; 65.0 kg).

uria group, U-CPR did not differ between the two glycemic phases. This finding may provide additional evidence, because Ccr remained unchanged. Moderate hyperglycemia may not be effective for a further increase of GFR in this early stage of nephropathy.

Faber et al[5] estimated MCR of C-peptide from the plasma disappearance curve after an intravenous injection of synthetic human C-peptide in normal and IDDM subjects. They found no significant difference in MCR between normal and diabetic groups, in contrast to our present results. Although neither the glycemic status nor GFR of their diabetic subjects was given, they stated that the MCR and half-life of plasma C-peptide were inversely correlated in diabetics but not in nondiabetics. Therefore, this latter finding may be explained in part by the effect of hyperglycemia on renal clearance of C-peptide.

Alternatively, hyperglycemia may modulate some enzyme activities responsible for the degradation of C-peptide in renal tubules. Luminal borders of the proximal tubules have been shown to have high peptidase activity.[21] In addition, approximately 80% of C-peptide delivered to the tubules is assumed to be reabsorbed and metabolized therein.[7,13] Consequently, even a mild alteration of tubular functions can greatly influence U-CPR. Although this mechanism seems unlikely, further studies are required to analyze the molecular species of urinary C-peptide.

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