
Food Constituents as a Cause of Variation of C-peptide Excretion in the Urine

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Abstract

Since C-peptide immunoreactivity (CPR) is excreted at a much higher rate than insulin in the urine, the urinary CPR (U-CPR) level could be a good measure of pancreatic B-cell function. In 10 normal subjects and 17 patients with non insulin-dependent diabetes mellitus (NIDDM), the 24-hour U-CPR level was 49.6 ± 4.5 (mean ± SE) µg, and 59.1 ± 7.9 µg, respectively. When measured repeatedly during 4–37 consecutive days, the mean levels of coefficient of variation (c.v.) of 24-hour U-CPRs in each individual in normal and diabetic patients were 23.4 ± 3.2%, and 39.1 ± 1.2%, respectively. Thus, the daily fluctuation of U-CPR was considerably large not only in NIDDM but also in normal healthy subjects. In order to investigate factors responsible for these U-CPR variations, we analyzed the effect of food constituents on U-CPR excretion in this paper. In 8 healthy subjects 5-hour U-CPR excretions were measured after ingesting 5 kinds of isocaloric 300 kcal test meals, i.e. glucose, starch, protein, fat, and mixed meal which consisted of equal kcal of starch, protein and fat. Five hour U-CPR excretion after glucose, starch and protein meal ingestion was 9.5 ± 1.3 µg, 13.7 ± 1.9 µg, and 7.4 ± 0.9 µg, respectively. Fat meal induced no increase in U-CPR excretion. After the mixed meal ingestion, 5-hour U-CPR was 8.2 ± 0.6 µg, which was approximately the mathematical average for the U-CPR after 3 meals. We conclude that the cause of variations in the U-CPR excretion may be ascribed not only to the ingested total calories, but also to the nutritional components of the diet. Therefore, care must be taken in reading a daily U-CPR measurement in assessing pancreatic B cell function.

Connecting peptide (C-peptide), a component of proinsulin, is released into the portal circulation from the B-cell of the pancreas together with insulin in equimolar quantities (Rubenstein et al., 1969). Hepatic metabolism of C-peptide is minimal (Kühl et al., 1978, Stoll et al., 1970), and it is mainly metabolized in the kidney (Katz and Rubenstein, 1973). Though most of its renal clearance is attributed to degradation rather than excretion, C-peptide excreted in the urine (U-CPR) represents about 4–20% of the total secretion, which is a much higher rate than the percentage of insulin excretion (Kaneko et al., 1975, Horwitz et al., 1977, Meistas et al., 1981). Therefore, the amounts of 24-hour U-CPR may be a good index of B-cell function (Meistas et al.,

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The measurement of U-CPR is non-invasive and easily repeatable, rendering frequent blood sampling unnecessary. Furthermore, as urine collection can be done by the patient (or her)-self everyday, the measurement is feasible in the normal conditions of his (or her) daily life without any modification. For the assessment of islet B-cell function in diabetes, especially in insulin requiring diabetes, U-CPR measurement could possibly be used for making a classification of insulin-dependent diabetes mellitus (IDDM) or non-insulin-dependent diabetes mellitus (NIDDM) (National Diabetes Data Group, 1979). However, since successive measurements of daily U-CPR revealed a considerable day to day variation in both diabetic and normal subjects, we have investigated the causes of these variations. A wide daily fluctuation of the U-CPR quantity is assumed to be due to the following causes: 1) a change in total caloric intake as well as in diet composition 2) a variation of daily exercise 3) a variation of C-peptide clearance in the kidney 4) effects of drugs (Hoogwerf and Goetz, 1983).

In this study, we examined the possibility that a wide daily fluctuation of U-CPR excretion could be partly ascribed to daily variation of ingested food constituents. We measured U-CPR in normal subjects after they ingested 5 kinds of isocaloric meal and examined the effect of various nutrients on the amount of U-CPR excretion.

Materials and Methods

Measurement of 24-hours U-CPR

Daily U-CPR was measured in 17 NIDDM patients and 10 normals for 4 to 9 consecutive days. In diabetic patients, 6 were obese, body weight being above 120% of the ideal value calculated with Jones' formula (Nöcker, 1956), and the other 11 were non-obese. The clinical data are shown in Table 1. These patients were all inpatients in our diabetic clinic. All of them showed normal liver and renal functions. Urine samples were collected for 24 hours in plastic containers at room temperature in which 10 ml of NaN₃ had been put beforehand. After measuring urine volume, 4 ml urine was kept frozen at -20°C until assay.

<p>| Table 1. Clinical Data in Non Insulin-Dependent DM |
|-----------------|-----------------|-----------------|-----------------|
|                | Obese           | Non-obese       |</p>
<table>
<thead>
<tr>
<th>No</th>
<th>Age</th>
<th>Sex</th>
<th>%BW*</th>
<th>FPG (mg/dl) mean±SE</th>
<th>Complications**</th>
<th>No</th>
<th>Age</th>
<th>Sex</th>
<th>%BW*</th>
<th>FPG (mg/dl) mean±SE</th>
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<td>54</td>
<td>F</td>
<td>163</td>
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<td>33</td>
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* %BW shows % of the ideal body weight calculated with Jones' formula.
** Complications indicate the presence of retinopathy (R) or neuropathy (N).
Effect of Various Nutrients on U-CPR

Eight healthy volunteers, 7 males and 1 female, aged from 18 to 30 years old were selected for the study. Their diets prior to the study were casual Japanese foods, containing ample carbohydrate. After an overnight fast, they were instructed to consume the next 5 test meals containing 300 kcal within 15 minutes. These test meals were glucose-meal, starch-meal, protein-meal, fat-meal and mixed-meal. Glucose-meal consisted of 75 g of glucose, starch-meal 83 g of mashed potatoes, protein-meal 418 g of boiled egg white and fat-meal 41.6 g of butter. And the mixed-meal consisted of the above 3 kinds of nutrient (starch, protein and fat), 100 kcal of each i.e. 27.7 g of mashed potatoes, 139 g of egg white and 13.9 g of butter. In all 8 subjects, these five test meals were given in random order on separate days. The blood or urine samples were collected every 30–60 minutes or every 60 minutes for 5 hours, respectively.

Analytical Methods

Plasma glucose (PG) was determined by the glucose oxidase method using Beckman’s glucose analyzer. Serum immunoreactive insulin (S-IRI) was measured by the solid phase method using a Shionogi kit which came originally from Pharmacia, Sweden. Serum and urine C-peptide immunoreactivity (S-CPR and U-CPR, respectively) was assayed by the double antibody method using a CPR kit from Shionogi Pharmaceutical Corporation, Osaka Japan. The C-peptide used in the kit was identical with authentic human C-peptide. In the assay, urine samples were routinely diluted 5–20 times with deionized water. Serum and urine creatinine were measured by Jaffe’s method. Statistical significance was assessed by Student’s t-test.

![Graph](image-url)
Results

Twenty-four Hour U-CPR in Healthy Subjects and Patients with NIDDM (Fig. 1)

In 10 healthy subjects, 24-hour U-CPR was measured for 4-9 days. The successive measurements of U-CPR gave a mean of $49.6 \pm 4.5$ (mean $\pm$ SE) $\mu$g/day, with a coefficient of variation (C.V.) of 23.8%. The mean C.V. for 24-hour U-CPR in 10 individuals was $23.4 \pm 3.2\%$. Thus, both the daily fluctuation in an individual and the variation among individuals were quite large. When urine CPR excretion was expressed on the basis of the ratio to the urine creatinine, a similar magnitude of variations was observed.

In 17 patients with NIDDM, the mean level of U-CPR was $63.1 \pm 9.0$ $\mu$g/day in 6 obese patients and $54.3 \pm 10.6$ $\mu$g/day in 11 non-obese patients. No statistical difference was found among these two groups and normal subjects. The C.V. in each patient of 17 NIDDM was 28-47% and the mean value for the C.V.'s was 39.1%. These variations in U-CPR tended to be larger in NIDDM than in healthy subjects.

The Response of PG, S-IRI, S-CPR and U-CPR to Test Meals in Eight Healthy Subjects

1) 75g Glucose Ingestion (Fig. 2)

The mean PG value reached its peak of $129 \pm 11$ mg/dl in 30 minutes from the fasting level of $88 \pm 6$ mg/dl and then decreased to $96 \pm 7$ mg/dl in 60 min. S-IRI and S-CPR reached their peaks of 50 $\mu$U/ml, and 7.9 ng/ml, respectively at 30 min and then decreased to the fasting levels at 3 hours after glucose ingestion. Hourly U-CPR excretion reached its peak at the 2nd hour and decreased to the fasting level at the 4th hour, showing a delayed peak in U-CPR compared to that in S-CPR.

2) Starch Ingestion (Fig. 3)

A peak PG level of $128 \pm 11$ mg/dl was obtained at 30 min then it decreased to $112 \pm 16$ mg/dl at 60 min, and $97 \pm 9$ mg/dl at 90 min after starch meal ingestion. This PG response was similar to that with glucose ingestion though the return of PG to the fasting level was slightly delayed. The maximal S-IRI and S-CPR levels were at-

![Fig. 2. Plasma glucose (panel A), S-CPR (●●), S-IRI (●●●) (panel B), U-CPR (panel C) and urine volume (panel D) during oral glucose ingestion in 8 healthy subjects (mean $\pm$ SE). Asterisks denote significant differences from the basal levels. * P<0.05 ** P<0.01 *** P<0.005]
tained at 30 min and at 60 min, respectively, after starch ingestion and they returned to the fasting level at 3 hour. Although U-CPR on starch ingestion showed a pattern similar to that seen on glucose ingestion, the magnitude of response tended to be greater.

3) Protein Ingestion (Fig. 4)

PG showed no definite change after

Fig. 3. Plasma glucose, S-CPR, IRI, U-CPR and urine volume during starch-meal ingestion in 8 healthy subjects (mean±SE). Other symbols as in Fig. 2.

Fig. 4. Plasma glucose, S-CPR, IRI, U-CPR and urine volume during protein-meal ingestion in 8 healthy subjects (mean±SE). Other symbols as in Fig. 2.
protein meal ingestion. S-IRI before and 2 hr after protein ingestion was 5.5 ± 1.1 and 7.7 ± 1.4 µU/ml, respectively. S-CPR before and 1 hr after protein meal was 1.75 ± 0.15 and 2.06 ± 0.16 ng/ml, respectively. These changes after the ingestion were not statistically significant. However, U-CPR excretion at 3 hr after the protein meal increased to 167% of the basal rate and this increase is statistically significant (P < 0.005).

Although the increase in U-CPR excretion following protein ingestion was smaller than that with glucose or starch ingestion, it persisted for 3 hours.

4) Fat Ingestion (Fig. 5)

After a fat meal ingestion, PG showed no change at all and both S-IRI and S-CPR remained unchanged throughout a 5 hour observation period.

![Fig. 5. Plasma glucose, S-CPR, IRI, U-CPR and urine volume during fat-meal ingestion in 8 healthy subjects (mean ± SE). Other symbols as in Fig. 2.](image)

![Fig. 6. Plasma glucose, S-CPR, IRI, U-CPR and urine volume during mixed-meal ingestion in 8 healthy subjects (mean ± SE). Other symbols as in Fig. 2.](image)
5) Mixed Meal Ingestion (Fig. 6)

PG before and 1 hr after mixed meal ingestion was 96 ± 5 and 101 ± 7 mg/dl, respectively. This change is not statistically significant. S-IRI and S-CPR reached their peaks of 25.1 ± 1.7 μU/ml and 4.47 ± 0.27 ng/ml respectively in 60 min. Maximal U-CPR excretion was seen at 2nd hour. U-CPR excretion returned to the basal level by the 4th hour.

U-CPR excretion was not affected by the hourly urine volume in any test meal described above. (Fig. 2–6)

6) Five Hour U-CPR Excretion after Test Meal Ingestion (Table 2)

Basal U-CPR secretion was 0.75 ± 0.11 μg/hour. Five hour U-CPR excretion after glucose ingestions was 9.5 ± 1.3 μg, which is 3 times as great as the basal excretion. The starch meal ingestion induced the largest response of the U-CPR (13.7 ± 1.9 μg), approximately 4 times as much as the basal excretion. After the protein meal ingestion, U-CPR was 7.4 ± 0.9 μg, being twice that of the basal level, while the fat meal ingestion did not induce any rise. The mixed meal ingestion resulted in a rise in U-CPR excretion, the magnitude of which was approximately equal to the mathematical mean of the U-CPR levels on the 3 kinds of test meal, (starch, protein and fat meal).

Table 2. Five Hour U-CPR Excretion after Various Nutrient Loadings

<table>
<thead>
<tr>
<th>5-hour: U-CPR (μg)</th>
<th>Mean ± SE</th>
<th>Ratio to Basal</th>
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</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>9.5 ± 1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Starch</td>
<td>13.7 ± 1.9</td>
<td>3.7</td>
</tr>
<tr>
<td>Protein</td>
<td>7.4 ± 0.9</td>
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</tr>
<tr>
<td>Fat</td>
<td>3.5 ± 0.4</td>
<td>0.95</td>
</tr>
<tr>
<td>Mixed Meal</td>
<td>8.2 ± 0.6</td>
<td>2.2</td>
</tr>
<tr>
<td>*Basal</td>
<td>3.7 ± 0.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* U-CPR excretion without food loading.

Discussion

It was reported that about 4–20% of total C-peptide released from the pancreas was excreted in the urine and the pattern of U-CPR excretion could well reflect the serum CPR response in the glucose tolerance test (Kaneko et al., 1975, Kuzuya et al., 1976a, b, Block et al., 1972). Accordingly U-CPR measurement could provide a useful index of pancreatic B-cell function, and thereby could be used for establishing the classification of diabetes mellitus. For this purpose, the reproducibility of U-CPR measurement should be analyzed. In the present study, consecutive measurements of daily U-CPR disclosed a considerable day-to-day variation. This could be ascribed to, firstly, qualitative and quantitative changes in ingested food. Naturally, food quantity affects the U-CPR amount. However, even under the isocaloric conditions, U-CPR fluctuates in a wide range as shown in the present study. After 300 kcal of fat ingestion, isocaloric to 75 g glucose, U-CPR excretion did not show any increase, while starch ingestion of the caloric equivalent induced a large increase, approximately 4 times as much as the basal excretion. After ingestion of the isocaloric protein meal, an increase in urinary excretion of CPR lasted for a longer period, though the increase was only twice the basal excretion. In this context, the role of the enteroinsular axis might be another factor responsible for the variation, but this has not yet been established. On the other hand, it was reported that insulin response to ingested protein was more marked in diabetic patients than in normal subjects (Berger and Vongaraya, 1966). Therefore, a little change in food nutrient could cause wide variations in U-CPR excretion even when a isocaloric diet was consumed by either normal or diabetic subjects.

Secondly, as to the fluctuation of daily
U-CPR, we must consider the effect of exercise. In this study, we measured the 24-hour U-CPR quantity without any restriction on exercise. Blix et al. (1982) reported that urine C-peptide excretion was decreased during exercise. LeBlanc et al. (1981) described a sparing effect of exercise on insulin requirements in human subjects as well. We must therefore consider the possibility that daily variation of exercise intensity causes some fluctuations of urine C-peptide.

Thirdly, we must pay attention to CPR clearance. We measured CPR clearance during fasting in eight healthy subjects. It ranged from 5.0 to 14.7 ml/min, with a mean level of $8.7\pm1.3$ ml/min. It coincided closely with the value of 7–12 ml/min, reported by Horwitz et al. (1975) and 7.1 ml/min by Kuzuya et al. (1976 b). However, the mean level of C.V. of CPR clearance in eight subjects, was 47.5%. And the daily variation of CPR clearance was also very large. The variation of CPR clearance could be induced by daily changes in exercise and diet. The kidney size and glomerular filtration rate (GFR) are increased in diabetic patients at least at the onset of diabetes (Rash 1979). So the diabetic stage could affect CPR clearance.

Furthermore, there was a possibility that the hourly urine volume could influence the hourly U-CPR levels. However, the results shown in Fig. 2–6 were not along these lines. There was no correlation between these two variables.

Therefore, in order to obtain useful information on the pancreatic B-cell function by measuring U-CPR, all the factors mentioned above should be taken into consideration. Among them, food constituents are most crucial. For the assessment of maximal B-cell secretory capacity which is one of our primary purposes, one random measurement of U-CPR may underestimate or overestimate the B-cell capacity. To avoid this error while preserving the simplicity and applicability of U-CPR measurement it is practical to collect urine samples for 3 to 5 days to find average U-CPR excretion rather than to put the patients under certain standardized conditions. In this way, we could obtain useful information on the B-cell function of a patient leading an undisturbed, normal daily life.

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