Effect of Insulin on Urinary Phosphate Excretion in Type II Diabetes Mellitus With or Without Renal Insufficiency

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We investigated the effect of insulin on urinary excretion of phosphate in type II diabetes mellitus (DM) with respect to the absence or presence of renal insufficiency. A euglycemic-hyperinsulinemic clamp was performed in 37 type II DM patients. Subjects were divided into two groups: group A consisted of patients with serum creatinine levels less than 1.5 mg/dL (n = 22), and group B consisted of patients with serum creatinine levels of 1.5 mg/dL or greater (n = 15). Blood and urine samples were collected at the beginning and end of the clamp, and urinary excretion of phosphate was evaluated by calculating fractional excretion (FE-P). Tissue sensitivity to insulin in the whole body was expressed as the glucose infusion rate (M value) and that divided by steady-state plasma insulin levels (M/I ratio) during the last 30 minutes of the clamp. FE-P in group A patients significantly decreased during the clamp (from 9.46 ± 0.67% before the clamp to 7.12 ± 0.73% after the clamp, $P < .004$), whereas FE-P in group B patients did not change significantly during the clamp. The percent decrease of FE-P (decrease of FE-P during the clamp divided by FE-P before the clamp) in group A patients was significantly higher than in group B patients (22.5 ± 7.0% and 2.5 ± 5.1%, respectively, $P < .04$). In all 37 patients, the percent decrease of FE-P was negatively correlated with blood urea nitrogen ([BUN] $r = -.36$, $P < .05$), serum creatinine ($r = -.34$, $P < .05$), and serum β_2-microglobulin ($r = -.44$, $P < .01$) and positively correlated with creatinine clearance ($r = .570$, $P < .004$), but it was not correlated with the M value or M/I ratio. These results showed that the kidneys of diabetic patients with renal insufficiency are insulin-insensitive in terms of phosphate transport, and the insulin insensitivity is related to the glomerular filtration rate but not to systemic insulin insensitivity. The percent decrease of FE-P on clamp study could be useful for assessing the insulin insensitivity of the kidney, which probably occurs primarily in the renal tubules.

INSULIN HAS BEEN REPORTED to have an effect on the renal tubules, enhancing sodium and phosphate reabsorption, and to have a calciuric effect in humans and animals.[1-5] Although the effect of insulin on enhancement of sodium reabsorption was also reported in patients with diabetes mellitus (DM) without uremic nephropathy,[6,7] the renal tubular response to insulin in urinary phosphate excretion is not known. In DM patients, insulin insensitivity is known to be present.[6,8,9] Furthermore, uremia is also known to be a cause of insulin insensitivity.[10-13] We recently reported that the presence of uremia in type II DM patients further accelerates existing insulin resistance.[14] However, little is known about whether insulin resistance in type II DM or in the presence of uremia affects urinary phosphate excretion. In this study, we examined whether the effect of insulin on urinary phosphate excretion is altered in type II DM in the absence or presence of renal insufficiency, using a euglycemic-hyperinsulinemic clamp.

SUBJECTS AND METHODS

Subjects

Thirty-seven patients (21 men and 16 women) with type II DM were included in this study. They were admitted to Osaka City University Hospital for the purpose of blood glucose control and DM education. Ten patients were treated with insulin, 17 with sulfonylureas, and 10 with diet therapy alone. The patients were divided into two groups according to serum creatinine level: group A patients had levels less than 1.5 mg/dL and group B patients had levels of 1.5 mg/dL or greater. Characteristics of the two groups are shown in Table 1. There were no significant differences in age, gender, body mass index, blood glucose levels before the clamp, hemoglobin A_{1c}, or fructosamine between the two groups. In addition to significant differences in serum creatinine, serum β_2-microglobulin levels were higher, creatinine clearance was lower, duration of DM was longer, and mean blood pressure was significantly higher in group B than in group A.

Euglycemic-Hyperinsulinemic Clamp

Two to 4 weeks after admission—when blood glucose was fairly well controlled—a hyperinsulinemic-euglycemic clamp (clamp) was performed according to the method described by DeFronzo et al,[8] using an artificial pancreas (model STG 22; Nikkiso, Tokyo, Japan). Patients with poor blood glucose control were excluded from the study. After having meals at 6 PM, patients were instructed not to eat any food but were allowed to drink tap water ad libitum. The clamp was started at 9 AM the next morning with a 10-minute priming insulin infusion followed by continuous insulin infusion at 1.25 mU/kg/min. Blood glucose levels were maintained at 90 mg/dL by infusion of variable amounts of 20% glucose as needed. A quantitative estimate of insulin sensitivity was provided by the mean glucose infusion rate ([M value] milligrams per kilogram per minute) during the last 30 minutes of the 2-hour clamp.[7,8] The M value represented the insulin-mediated glucose uptake in the whole body, primarily in the muscle.[7,8] The M/I ratio (where I is the steady-state plasma insulin level) is a measure of the quantity of glucose metabolized per unit of plasma insulin concentration.[7,8] After correcting for differences in steady-state plasma insulin levels, M/I was a better index for comparison of possible changes or differences in tissue sensitivity to insulin.[7,8] For convenience, the M/I ratio was multiplied by 100.

Blood and Urine Sampling and Evaluation of Urinary Phosphate Excretion

Patients were instructed to completely void urine early in the morning, but were allowed to drink tap water ad libitum to obtain sufficient urine volume. Immediately before the start of the clamp,

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Table 1. Patient Characteristics (mean ± SD)

Characteristic	Group A (n = 22)	Group B (n = 15)
Gender		
Male	12	9
Female	10	6
Age (yr)	57.8 ± 9.0	61.5 ± 5.7
Duration of DM (yr)	9.7 ± 6.8	17.9 ± 8.1*
Body mass index	22.7 ± 2.7	23.5 ± 2.3
Fasting blood glucose (mg/dL)	127.3 ± 2.7	116.9 ± 38.0
Hemoglobin A_{1C} (%)	7.9 ± 2.2	7.5 ± 1.6
Fructosamine (μmol/L)	317 ± 72	260 ± 69
BUN (mg/dL)	16.3 ± 3.6	43.8 ± 21.0*
Serum creatinine (mg/dL)	0.69 ± 0.12	2.83 ± 1.79*
Serum β_2-microglobulin (μg/L)	1.77 ± 0.32	7.23 ± 3.99*
Creatinine clearance (mL/min)	84.3 ± 28.2	36.7 ± 32.4*
Blood pressure (mm Hg)	85.8 ± 13.2	105.8 ± 19.5*

*$P < .01$ *v* group A.

Table 2. Clamp Characteristics and Changes in Serum Phosphate and During the Clamp

Parameter	Group	Before Clamp	After Clamp
SSPG (mg/dL)	A	—	90.6 ± 0.5
	B	—	91.2 ± 0.6
M value (mg/kg/min)	A	—	5.16 ± 0.33
	B	—	4.44 ± 0.38
SSPI (μU/mL)	A	—	117.9 ± 7.2
	B	—	123.6 ± 7.6
M/I ratio	A	—	5.00 ± 0.55
	B	—	4.09 ± 0.56
P (mg/dL)	A	3.57 ± 0.12	2.83 ± 0.13†
	B	4.41 ± 0.32	3.10 ± 0.24†
FE-P (%)	A	9.46 ± 0.68	7.12 ± 0.73*
	B	33.0 ± 4.4	32.7 ± 4.9

*$P < .01$, †$P < .001$: *v* before clamp.

Abbreviation: SSPI, steady-state plasma insulin concentration during the last 30 minutes of the 2-hour clamp.

blood was drawn from patients in the supine position, and urine was collected. Immediately after the clamp ended, blood was drawn before patients rose from the supine position, and urine was collected. Serum was separated, and Hemoglobin A_{1C} was measured by high-performance liquid chromatography. Serum and urine phosphate and creatinine and blood urea nitrogen (BUN) were assayed using an autoanalyzer. Serum β_2-microglobulin level was measured using the latex agglutination method (LX-Eiken β_2-M kit; Eiken Chemical, Tokyo, Japan). Plasma insulin concentration was measured by radioimmunoassay. Urinary excretion of phosphate was assessed by calculation of fractional excretion of phosphate (FE-P) both before and after the clamp using the equation, FE-P = 100 × (serum-cr × urine-P)/(urine-cr × serum-P), where cr and P denote creatinine and phosphate, respectively.

FE-P was chosen instead of the absolute electrolyte excretion rate because FE-P corrects for changes in glomerular filtration rate (GFR) in including cases with decreased GFR.[7] FE-P could also correct for dead space or incomplete voiding due to the possible presence of diabetic neuropathy.[7] Since FE-P increases extensively in renal insufficiency,[15] the percent decrease of FE-P was calculated as follows to evaluate differences in the effect of insulin on FE-P in patients, including those with low GFR: % decrease FE-P = 100 × [(FE-P before) − (FE-P after)]/(FE-P before), where FE-P before and FE-P after denote FE-P before and after the clamp, respectively. Using this calculation, minute changes of FE-P during the clamp in both group A and group B could be assessed.

Statistical Analysis

Values are expressed as the mean ± SE unless stated otherwise. Statistical analysis was performed with Student's *t* test, and statistical significance was set at $P < .05$. Correlation and linear regression analysis were applied when appropriate.

RESULTS

Blood glucose levels immediately before the clamp, after overnight fasting, were 127.3 ± 7.7 and 116.9 ± 9.8 mg/dL in group A and group B, respectively, since patients with good blood glucose control were selected for this study (Table 1). Clamp characteristics and insulin sensitivity variables are shown in Table 2. Steady-state plasma glucose levels were maintained constant at 90 mg/dL (coefficient of variation, 3.5%) in both groups, and did not differ significantly between the two groups. As previously reported,[14] both the M value and the M/I ratio in group B were lower than in group A, but the differences were not significant ($P = .078$ and $P = .14$, respectively). Steady-state plasma insulin concentration did not differ significantly between the two groups. During the clamp, serum phosphate levels were significantly decreased in both groups ($P < .001$). Serum levels of BUN, creatinine, and β_2-microglobulin did not significantly change during the clamp (data not shown). FE-P in group B was significantly higher than in group A, as previously described by others.[15] In group A, FE-P before the clamp was 9.46 ± 0.68% and FE-P after the clamp was 7.12 ± 0.73%. A significant decrease in FE-P during the clamp was found in group A ($P < .004$), whereas FE-P during the clamp in group B did not change significantly. The percent decrease of FE-P in group A was 22.5 ± 6.9%, and in group B, 2.5 ± 5.0% (Fig 1). The percent decrease of FE-P in group A was significantly higher than in group B ($P < .04$).

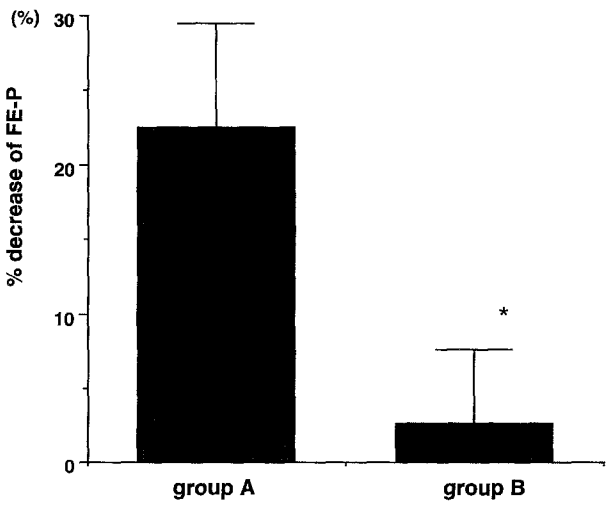

Fig 1. Percent decrease of FE-P in the two groups. *Percent decrease of FE-P in group A was significantly higher than in group B ($P < .04$).

The relationships between the percent decrease of FE-P and several parameters were examined, including age, gender, body mass index, hemoglobin A_{1C}, duration of DM, blood glucose concentration before the clamp, steady-state plasma insulin, M value, M/I ratio, serum calcium, serum phosphate, serum creatinine, BUN, serum β_2-microglobulin, and creatinine clearance. There were no significant relationships between the percent decrease of FE-P and the insulin sensitivity variables, M value and M/I ratio (Fig 2). There was no significant correlation between the percent decrease of FE-P and other parameters, except between the percent decrease of FE-P and parameters related to renal function. A significant negative correlation was seen between the percent decrease of FE-P and BUN ($r = -.36$, $P < .05$), percent decrease of FE-P and serum creatinine ($r = -.34$, $P < .05$), and percent decrease of FE-P and β_2-microglobulin ($r = -.44$, $P < .01$). Furthermore, there was a positive relationship between the percent decrease of FE-P and creatinine clearance ($r = .57, P < .004$; Fig 3).

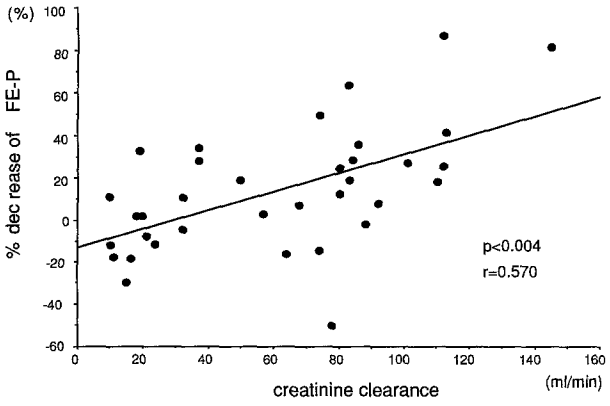

Fig 3. Correlation between percent decrease of FE-P and creatinine clearance. There was a significant positive relationship between percent decrease of FE-P and creatinine clearance ($r = .57, P < .001$).

DISCUSSION

The effect of insulin on renal electrolyte excretion and tubular electrolyte transport has been examined in several studies in humans, dogs, and rats.[1-4,6,7,16-18] These studies have demonstrated that insulin enhances sodium and phosphate reabsorption in the kidney. In patients with DM, in whom insulin insensitivity is present in peripheral tissues (particularly the muscles[6,8,9]), the effect of insulin on mineral transport is not known, except for sodium transport. Nosadini et al[6] and Gans et al[7] reported that insulin enhances sodium reabsorption in the kidney in DM patients without uremic nephropathy. Little is known about the effect of insulin on electrolyte transport in uremia, which is also a case of insulin insensitivity.[10-13] In this study, we had the opportunity to perform a euglycemic-hyperinsulinemic clamp in type II DM patients with or without renal insufficiency, and were able to examine the effect of insulin on urinary phosphate excretion in DM patients in the absence or presence of renal insufficiency. We also examined the relationship between systemic insulin sensitivity and renal phosphate excretion.

In type II DM patients without renal insufficiency (group A), insulin infusion significantly decreased FE-P. The result was similar to that of a previous study, in which insulin showed an effect on proximal tubular phosphate reabsorption.[2,3] The present findings do not seem related to changes in blood glucose levels during the clamp, since the decrease in blood glucose was relatively small during the clamp and, furthermore, a similar decrease in blood glucose in group B did not cause increased urinary phosphate excretion. Although serum phosphate levels decreased significantly and may have contributed to a decrease in FE-P, a similar decrease in serum phosphate in group B did not cause a similar decrease in FE-P. This suggests that the decrease in serum phosphate levels alone could not contribute to decreased FE-P in group A. The renal tubular response to insulin infusion seemed to cause changes in FE-P in this study, as previously directly demonstrated in dogs.[3] Although several tubular functional abnormalities in the early stage of DM without nephropathy have been reported to exist,[19,20] renal tubuli in DM patients without renal insufficiency seem to be responsive to insulin in terms of enhanced phosphate reabsorption, since insulin is reported to enhance sodium reabsorption in DM patients without renal insufficiency.[6,7]

Little is known about the effect of insulin on phosphate transport in the presence of renal insufficiency. Several studies have shown that uremia causes tissue insensitivity to insulin which is improved by therapy for uremia such as hemodialysis, protein food restriction, and vitamin D treat-

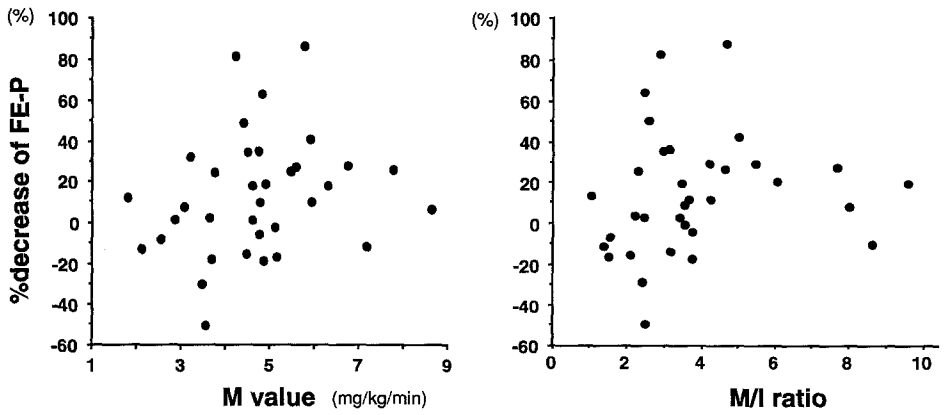

Fig 2. Relationships between percent decrease of FE-P and the insulin sensitivity variables, M value and M/I ratio. No significant correlation was found between percent decrease of FE-P and M value or M/I ratio.

ment.[11-13] In the present study, as we reported previously,[14] the insulin sensitivity variables, M value and M/I ratio, were lower in group B than in group A, although the difference was not significant. The results were similar to a study of nondiabetic uremic patients reported by others.[11-13] Insulin sensitivity expressed by the M value or M/I ratio is related primarily to muscle sensitivity to insulin.[9,11,13] The effect of insulin on changes in FE-P mainly reflects the sensitivity of the renal tubules.[1,2,3] In the present study examining the effect of insulin on urinary phosphate excretion, insulin significantly affected FE-P in group A but not in group B, although steady-state plasma insulin levels were not significantly different between the two groups. In type II DM patients with renal insufficiency, therefore, insulin insensitivity seems to be present in the renal tubules as well as in peripheral tissues such as muscle.

We further evaluated the insulin effect by calculating the percent decrease of FE-P in each case, since FE-P in group B was markedly increased compared with that in group A, as described elsewhere.[15] Using this equation, the percent decrease of FE-P during the clamp was significantly higher in group A than in group B. The result clearly confirms that insulin infusion had a greater effect in group A than in group B. It also suggests that minute changes in the effect of insulin on urinary phosphate excretion can be assessed by this equation. We therefore used the value (percent decrease of FE-P) to determine the relationships between insulin sensitivity of the kidney and several parameters. Significant correlations were seen between the percent decrease of FE-P and parameters related to renal function. The percent decrease of FE-P was significantly negatively correlated with BUN, serum creatinine, and β_2-microglobulin, and positively correlated with creatinine clearance. The results indicate that in terms of urinary phosphate excretion, insulin sensitivity of the kidney decreases as renal function decreases. Interestingly, the insulin sensitivity is not related to systemic insulin sensitivity expressed as the M value and M/I ratio. It is thus suggested that insulin sensitivity variables (M value and M/I ratio) that reflect mainly muscle insulin sensitivity[9,11,13] are different from those for insulin sensitivity of the kidney and probably those for the renal tubules, although both tissues are insensitive to insulin in uremia.

Differences in the mode of insulin insensitivity between the kidney and peripheral tissues were suggested by the results of the present study. It is difficult to speculate about what mechanism underlies insulin insensitivity of the kidney in the uremic state. Under uremic conditions, decreased reactivity of several endocrine hormones has been reported, such as the thyrotropin-releasing hormone–thyroid-stimulating hormone system and the hypophysial-gonadal system.[21] It was reported that middle molecule toxins (1,000 to 2,000 daltons) from uremic sera inhibit glucose metabolism in rat adipocytes.[22] Folli et al[23] showed that uremic sera rendered hepatocytes resistant to insulin, and that intracellular second-messenger of insulin action was markedly reduced. A recent study by Urena et al[24] showed downregulation of mRNA for parathyroid hormone (PTH)-receptor protein and decreased responsiveness of adenyl cyclase activity to PTH in uremic rat kidneys. They also showed that the ratio of PTH mRNA downregulation is related not to PTH levels, but to decreased renal function.[24] Investigation of the intracellular mechanism of insulin insensitivity of renal tubules in uremia, as suggested in the present study, should be required in the future. However, in the histopathology of renal failure in DM, tubuli develop atrophy as glomeruli become sclerotic.[19,20] Structurally atrophied tubuli may be functionally insensitive to several stimuli, including insulin.

In conclusion, using a euglycemic-hyperinsulinemic clamp in humans we demonstrated that the kidney of type II DM patients without renal insufficiency responds to insulin in terms of phosphate reabsorption. Insulin insensitivity of the kidney in phosphate reabsorption is seen in patients with renal insufficiency. This insulin insensitivity is related to the glomerular filtration rate, but not to systemic insulin resistance. The percent decrease of FE-P could be a useful parameter during clamp studies to evaluate insulin sensitivity of the kidney in diabetic nephropathy.

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