Effects of Insulin and Glucose on Renal Phosphate Reabsorption: Interactions With Dietary Phosphate

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ABSTRACT

Both insulin deficiency and glycosuria are known to inhibit the tubular reabsorption of phosphate. This inhibition has previously been evaluated either in the fasted state or on a normal phosphate diet. The goal of this study was to evaluate how dietary phosphate depletion affected the relative effects of insulin deficiency and glycosuria on the tubular reabsorption of phosphate. Rats were maintained on either a low (0.03%) or normal (0.8%) phosphate diet. After 5 days, one half of the animals in each group received streptozotocin to induce short-term insulin deficiency, whereas the other half received vehicle alone. Two days later, sodium-dependent phosphate uptake by renal brush border membrane vesicles (BBMV) was evaluated in each of the four experimental groups. The effect of glucose on phosphate uptake was determined by the addition of varying concentrations of glucose (between 0 and 32 mmol/L) to the extravesicular transport fluid. BBMV phosphate uptake was about threefold higher in the fasted state associated with insulin deficiency, BBMV phosphate transport was also measured in rats fasted for 48 h after the administration of streptozotocin or vehicle. In these fasted rats, streptozotocin treatment had no effect on phosphate uptake, whereas extravesicular glucose inhibited phosphate transport in both treatment groups. These observations suggest that the effects of insulin deficiency and glycosuria on phosphate uptake by the proximal tubule BBMV differ according to the dietary regimen.

Key Words: Phosphate, insulin, glucose, streptozotocin

The major site of the renal reabsorption of phosphate is the proximal tubule (1). Glycosuria and insulin deficiency have both been reported to inhibit sodium-dependent phosphate uptake in this segment. Thus, in vivo experiments (2–6) and studies in isolated perfused proximal tubules (7) have revealed the inhibition of the tubular reabsorption of phosphate by intraluminal glucose. Likewise, extravesicular glucose has been reported to decrease phosphate uptake by renal cortical brush border membrane vesicles (BBMV) (8). Data obtained from both in vivo clearance experiments with euglycemic hyperinsulinemia (9–12) and in vitro incubations of proximal tubules with insulin (13) have established an antiphosphaturic effect of insulin. This effect could be abolished in fasted rats by the inhibition of endogenous insulin release with somatostatin (10).

The majority of studies evaluating the effects of insulin and glucose on the tubular reabsorption of phosphate have involved animals or humans that were either fasted or fed a normal phosphate diet. The interactions of insulinopenia and glycosuria have not been well characterized in the setting of dietary phosphate restriction. Two recent studies demonstrated the role of insulin in the renal phosphate retention associated with phosphate deprivation (14,15). It is not known, however, whether dietary phosphate depletion alters the inhibitory effect of glucose on phosphate transport and whether the effects of insulin and glucose are additive. The aim of this study, therefore, was to examine the relative contributions of insulin and glucose to renal phosphate handling in rats maintained on either a normal phosphate or a low-phosphate diet. The effect of...
Insulin on phosphate uptake under each of these dietary conditions was measured in BBMV obtained from diabetic (streptozotocin-treated) or nondiabetic rats. The effect of glucose on this uptake was evaluated by incubating the BBMV in varying concentrations of glucose.

METHODS

Preparation of Animals

Male Wistar rats (Charles River Breeding Company, Wilmington, MA) weighing 275 to 325 g were used. All animal experiments were conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals. The animals were maintained on standard rat chow containing 0.8% phosphate and 1.0% calcium (ICN Nutritional, Costa Mesa, CA) and ad lib water intake until 1 wk before the acute experiment, at which time the diet was modified. The animals were then divided into two groups, half of which were maintained on a low (0.03%) phosphate diet and half of which were on a normal (0.8%) phosphate diet. The normal phosphate diet was prepared from a low-phosphate diet (ICN Nutritional) by the addition of sodium phosphate (four parts dibasic to one part monobasic) to achieve an 0.8% phosphate content. A sufficient amount of sodium chloride was added to the low-phosphate diet to match the sodium intake in both groups. Both diets included 1.0% calcium, 66% sucrose, and 20% protein. Because fasting has been shown to impair tubular phosphate transport (16, 17), only animals consistently eating more than 10 g of food per day were studied.

After 5 days on their respective diets, half of the animals in each dietary group received an injection of streptozotocin (65 mg/kg i.p.) to induce diabetes, whereas the other half were injected with vehicle. After an additional 48 h on their respective diets, the animal were killed and renal BBMV were prepared for uptake studies. Only the streptozotocin-treated rats whose urine had more than 2% glucose by dipstick of the urine were studied.

Fasting mimics the catabolic state associated with insulin deficiency. To distinguish between the effect of phosphate depletion as compared with that of a total fast, BBMV phosphate transport was also measured in rats fed a normal phosphate diet for 5 days, then injected with streptozotocin or vehicle, and fasted for a 48-h period.

Preparation and Analysis of BBMV

All animals in the first four experimental groups were studied in the fed state, whereas the remaining rats were studied after a 48-h fast. After the induction of anesthesia with sodium pentobarbital (50 mg/kg i.p.), blood was collected by cardiac puncture. Both kidneys were rapidly removed and immediately placed on ice. The BBMV were prepared by the divalent cation and differential centrifugation technique, adapted from Beck and Sacktor (18), with MgCl₂ substituted for CaCl₂. Both kidneys of one rat were used for a single BBMV preparation. The crude homogenate of renal cortex was suspended in 300 mM mannitol–5 mM 2-[N-morpholinojethanesulfonic acid (MES)-Tris (pH = 7.0). All subsequent pellets were loaded in 240 mM mannitol–10 mM KF–10 mM MgCl₂, 5 mM MES-Tris (pH = 7.4).

The adequacy of the BBMV preparation was evaluated by measuring the activities of two enzymes: alkaline phosphatase, a marker of renal brush border membrane, and Na⁺-K⁺-ATPase, a marker of the basolateral membrane. As compared with the enzyme activity in the crude homogenate, it was expected that in the BBMV there would be an enrichment of alkaline phosphatase and a decrease in the Na⁺-K⁺-ATPase activity. In addition, the adequacy of the vesicle preparation was confirmed in a random sample by electron micrography.

Measurement of Phosphate and Glucose Uptake in BBMV

The final BBMV pellet was suspended in 240 mM mannitol, 10 mM KF, 10 mM MgCl₂, and 5 mM MES-Tris (pH = 7.4). The BBMV were distributed in aliquots of 10 μL, which were warmed to 25°C in a water bath. A 40-μL solution containing 150 mM NaCl, 5 mM MES-Tris (pH = 7.4), and 31 μM KH₂PO₄ with radioactive ³²P (25 × 10⁶ cpm/mL) was added to the BBMV to initiate transport. Phosphate transport was measured by the rapid filtration method of Aronson and Sacktor (19). All transport measurements were performed on freshly made vesicle preparations. To reduce experimental variability, the BBMV from diabetic and nondiabetic animals on a given diet were prepared in parallel on the same day, with the uptakes measured concurrently on the same day and under the identical conditions.

Active phosphate transport was stopped at 10 s with ice-cold 150 mM NaCl–5 mM MES-Tris–5 mM sodium arsenate. In addition, the steady-state phosphate uptake was measured after a 90-min incubation. The uptakes were measured in triplicate, and nonspecific binding activity was subtracted from the total radioactivity. Sodium-independent phosphate transport was measured by substituting KCl for NaCl in the transport solution. The sodium-dependent phosphate transport was calculated as the difference between total transport and sodium-independent transport. Uptakes were expressed as picomoles of phosphate per milligrams of protein. To evaluate the effect of glucose on phosphate uptake, the glucose concentration in the transport solution was varied between 0 and 32 mmol/L. In addition, the phosphate
transport was evaluated in some vesicles after the addition of 0.1 mM phlorizin, a competitive inhibitor of sodium-dependent glucose transport (19), to the transport solutions.

To evaluate whether streptozotocin might have a nonspecific effect on sodium-dependent transport, the uptake of glucose was also measured in the BBMV. This was done by substituting 50 μM \( \text{D-}[2\text{H}]\text{-glucose} \) instead of KH\(_2\)PO\(_4\) in the transport solution. The glucose uptake was measured at 15 s and at 90 min (steady state).

Biochemical Assays

Plasma samples were assayed for phosphate, calcium, glucose, insulin, and bicarbonate. Phosphate was determined colorimetrically by the method of Daly and Ertinghausen (20), calcium was determined by atomic absorption spectrophotometry, glucose was determined by an automated Beckman analyzer (Beckman Instruments, Inc., Fullerton, CA), and insulin was determined by RIA. Protein concentrations were determined by the method of Lowry et al. (21). The enzyme activities of alkaline phosphatase and Na\(^+\)-K\(^+\)-ATPase in the crude homogenate and in the BBMV were measured colorimetrically (22,23).

### Table 1. Weight gain of the experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Weight Gain (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPD</td>
<td>Control 333 ± 5</td>
<td>370 ± 9</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>STZ 323 ± 3</td>
<td>341 ± 8(^b)</td>
<td>2.6 ± 0.7(^b)</td>
</tr>
<tr>
<td>LPD</td>
<td>Control 315 ± 6</td>
<td>329 ± 5</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>STZ 314 ± 3</td>
<td>310 ± 10</td>
<td>-0.6 ± 1.7</td>
</tr>
</tbody>
</table>

\(^a\) NPD, normal phosphate diet; LPD, low-phosphate diet; STZ, streptozotocin.
\(^b\) \(P < 0.05\) versus the corresponding control group.

### Table 2. Plasma phosphate, calcium, bicarbonate, insulin and glucose concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Phosphate (mmol/L)</th>
<th>Calcium (mmol/L)</th>
<th>Bicarbonate (pmol/L)</th>
<th>Insulin (pmol/L)</th>
<th>Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPD</td>
<td>Control 2.59 ± 0.10</td>
<td>2.71 ± 0.02</td>
<td>29.9 ± 1.4</td>
<td>1062 ± 172</td>
<td>13.7 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>STZ 2.26 ± 0.09</td>
<td>2.74 ± 0.05</td>
<td>30.8 ± 0.6</td>
<td>103 ± 26(^b)</td>
<td>31.9 ± 3.7(^b)</td>
</tr>
<tr>
<td>LPD</td>
<td>Control 1.53 ± 0.15(^c)</td>
<td>3.08 ± 0.04(^c)</td>
<td>29.0 ± 2.3</td>
<td>1048 ± 179</td>
<td>11.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>STZ 2.14 ± 0.11(^c)</td>
<td>2.80 ± 0.12(^c)</td>
<td>24.2 ± 3.0</td>
<td>85 ± 36(^c)</td>
<td>24.9 ± 1.3(^c)</td>
</tr>
</tbody>
</table>

\(^a\) NPD, normal phosphate diet; LPD, low-phosphate diet; STZ, streptozotocin.
\(^b\) \(P < 0.05\) versus the corresponding control group.
\(^c\) \(P < 0.05\) versus NPD control.

### Statistical Analysis

All results were expressed as means ± SE. The effect of varying glucose concentrations on phosphate transport by BBMV within an experimental group was evaluated by analysis of variance for repeated measures, followed by paired t tests. Comparisons between diabetic and nondiabetic rats on a given diet were performed by analysis of variance followed by the Newman-Keuls test. A \(P\) value <0.05 was considered statistically significant.

RESULTS

Before the rats were placed on their respective dietary regimens, their weights did not differ among the four experimental groups that were fed (Table 1). On either diet, the weight gain tended to be lower in the diabetic rats, as compared with that in their respective nondiabetic controls.

The plasma phosphate concentration was significantly lower in the control rats on the low-phosphate diet than in the nondiabetic animals maintained on a normal phosphate diet (Table 2). Streptozotocin administration did not significantly change the plasma phosphate concentration in rats maintained on a normal phosphate diet. In contrast, the phosphate concentration was significantly higher in the rats on a low-phosphate diet receiving streptozotocin than in the corresponding control group.

The plasma calcium concentration was significantly higher in the control animals on the low-phosphate diet than in the nondiabetic groups maintained on a normal phosphate diet (Table 2). Streptozotocin administration had no significant effect on the calcium concentration in the animals fed a normal diet. In contrast, the calcium concentration was significantly decreased in rats on a low-phosphate diet receiving streptozotocin as compared with that in the corresponding control group.

The terminal plasma bicarbonate concentrations were not significantly different among the four ex-
TABLE 3. Characteristics of the BBMV preparations

<table>
<thead>
<tr>
<th>Group</th>
<th>BBMV (Protein) (mg/mL)</th>
<th>Alkaline Phosphatase Activity (BBMV/homogenate)</th>
<th>Na⁺-K⁺-ATPase Activity (BBMV/homogenate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.4 ± 0.4</td>
<td>11.01 ± 0.22</td>
<td>0.47 ± 0.08</td>
</tr>
<tr>
<td>STZ</td>
<td>12.1 ± 0.4</td>
<td>10.98 ± 0.22</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td>LPD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.3 ± 0.2</td>
<td>10.95 ± 0.24</td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td>STZ</td>
<td>11.6 ± 0.7</td>
<td>10.88 ± 0.53</td>
<td>0.61 ± 0.10</td>
</tr>
</tbody>
</table>

* NPD, normal phosphate diet; LPD, low-phosphate diet; STZ, streptozotocin.

Experimental groups (Table 2), suggesting that short-term streptozotocin-induced diabetes did not alter the acid-base status. The administration of streptozotocin to rats on either dietary regimen (normal phosphate and low-phosphate diet) resulted in marked hyperglycemia, as compared with the corresponding nondiabetic controls (Table 2). The plasma insulin concentrations were markedly lower in the streptozotocin-treated rats, as compared with those in their respective controls.

The protein concentrations of the BBMV preparations were not different among the experimental groups (Table 3). Likewise, the ratios of both alkaline phosphatase activity and Na⁺-K⁺-ATPase activity of the BBMV as compared with the crude homogenate was not significantly different among the four experimental groups, suggesting similar vesicle preparations.

Phosphate uptake by the renal cortical BBMV was approximately threefold higher in the nondiabetic rats fed a low-phosphate diet than in the control animals maintained on a normal phosphate diet ($P < 0.001$) (Figure 1). Streptozotocin-induced diabetes did not affect BBMV phosphate uptake in rats fed a normal phosphate diet or in rats fasted for 48 h. In contrast, streptozotocin treatment of rats maintained on a low-phosphate diet prevented the stimulation of phosphate uptake as compared with that of the control animals fed this diet ($P < 0.001$).

Progressively higher concentrations of glucose produced progressive decreases in BBMV phosphate uptake in the four fed experimental groups (Figures 2 and 3). For any given glucose concentration between 0 and 32 mmol/L, there was no significant difference in the sodium-dependent phosphate transport between streptozotocin-treated and control rats maintained on a normal phosphate diet (Figure 2). In contrast, for any glucose concentration, BBMV phosphate uptake was consistently lower in the diabetic rats on a low-phosphate diet than in the nondiabetic animals on the same diet (Figure 3). The addition of phlorizin, a competitive inhibitor of glucose transport, to the extravesicular fluid attenuated the glucose-induced inhibition of BBMV sodium-dependent phosphate transport (Figure 4). Steady-state rates of sodium-dependent phosphate transport (measured after 90 min of incubation) were not different among the four experimental groups (101 ± 13, 111 ± 12, 82 ± 16, 112 ± 8 pmol/mg of protein, respectively). Sodium-independent phosphate transport did not differ among the four groups.

Sodium-dependent glucose transport at 15 s was similar among the four experimental groups (544 ± 28, 640 ± 28, 570 ± 23, and 668 ± 33 pmol/mg of protein, respectively). Similarly, the steady-state BBMV glucose uptakes did not differ among the groups (65 ± 4, 62 ± 3, 65 ± 3, and 65 ± 3 pmol/mg of protein, respectively).

Two additional experimental groups were studied to distinguish the effects on phosphate transport due to the catabolic state of fasting, as compared with those caused by the catabolic state associated with...
insulin deficiency. These animals were fed a normal phosphate diet for 5 days and then were injected with streptozotocin or vehicle, followed by a 48-h fast. The 2-day fast in these two experimental groups resulted in a mean weight loss of 25 ± 2 and 36 ± 4 g, respectively, in the rats injected with vehicle and streptozotocin. There was no significant difference between the BBMV phosphate uptake in these two groups (Figure 1). However, extravesicular glucose produced a similar decrease in phosphate transport in fasted animals pretreated with streptozotocin or vehicle (Figure 5).

DISCUSSION

The effects of insulin deficiency and extravesicular glucose on phosphate uptake by renal BBMV differed according to the dietary regimen. Insulin deficiency significantly decreased phosphate uptake in rats maintained on a low-phosphate diet but had no effect in animals fed a normal phosphate diet. In contrast, extravesicular glucose invariably inhibited BBMV phosphate transport in a dose-related manner, regardless of dietary phosphate or insulin status. Thus, insulin deficiency and extravesicular glucose had an additive inhibitory effect on phosphate uptake in dietary phosphate-restricted rats.

The observed increase in BBMV phosphate uptake after dietary phosphate deprivation is consistent with that described in previous reports (15,24–26). The inhibition of this adaptation in streptozotocin-
treated rats suggests that during phosphate depletion, insulin is required for renal phosphate retention to occur. It is also in agreement with in vivo observations in which the prevention of insulin secretion after a single low-phosphate meal abolished the expected antiphosphaturic response (14). The reason for the discrepancy between the effects of streptozotocin treatment on phosphate uptake in phosphate-depleted and phosphate-replete animals is not clear. It has previously been observed that plasma parathyroid hormone (PTH) concentrations are significantly higher in streptozotocin-treated rats on a low-phosphate diet, as compared with those in nondiabetic, phosphate-depleted animals (15). Insulin antagonizes the phosphaturic effect of PTH (12). Thus, in the setting of insulin deficiency, the increased levels of PTH would be unopposed and might promote significant phosphaturia.

The inhibition of BBMV phosphate uptake in streptozotocin-treated, phosphate-depleted rats might not be a direct effect of insulin deficiency. Because insulin deficiency may sometimes produce ketoacidosis, the effect of insulin deficiency on phosphate transport might be indirectly mediated by metabolic acidosis, rather than a direct effect of insulin. Metabolic acidosis has previously been shown to impair the tubular reabsorption of phosphate (27,28). In the experimental model presented here, this is unlikely, because the plasma bicarbonate concentrations did not differ among the experimental groups.

It is also possible that the inhibition of phosphate transport in BBMV from phosphate-depleted, diabetic rats could be due to a nephrotoxic effect of streptozotocin, rather than to insulin deficiency. Streptozotocin may cause a generalized defect in proximal tubular function (29). Two lines of evidence in this study would argue against this possibility. First, sodium-dependent glucose uptake was not inhibited in the streptozotocin-treated animals, suggesting a specific effect on sodium-dependent phosphate uptake. Second, it has been previously reported that exogenous insulin corrects the defect in phosphate transport in phosphate-depleted, streptozotocin-treated rats (15).

The streptozotocin-treated rats gained less weight than their respective controls on the same diet, in agreement with previous observations (30). Fasting can inhibit phosphate uptake by the renal tubule (16,17), but this potentially confounding variable was eliminated by studying only animals that ate consistently. Moreover, in rats that were fasted for 48 h, BBMV phosphate uptake was not decreased in streptozotocin-treated animals, as compared with that in the vehicle-treated controls. Thus, in this experimental model, the effect of insulin deficiency on BBMV phosphate transport was specific for animals maintained on a low-phosphate diet.

El-Seifi et al. (31) observed a decrease in BBMV phosphate uptake in diabetic rats fed a normal phosphate diet, which is in contrast to observations in this study. It should be noted, however, that in the former study the animals were studied 12 days after streptozotocin injection, as compared with 2 days in this study. The prolonged diabetes was associated with a generalized defect in sodium-dependent transport, including transport of protons, glucose, and amino acids. In contrast, in this study, the defect was specific for sodium-dependent phosphate transport and was only observed in rats on a low-phosphate diet.

The inhibition of the tubular reabsorption of phosphate by glucose is consistent with previous reports in intact animals and in BBMV (2-8). This action of glucose could conceivably be due to an osmotic effect (32). However, this possibility seems unlikely for two reasons. First, the inhibitory effect was noted even at the lowest concentration of glucose, at which the osmolality of the transport solution would be increased by only 2 mmol/L. Second, the effect of glucose was markedly attenuated by the addition of phlorizin to the transport solution.

A zero glucose concentration approximates that measured in tubular fluid in the S2 segment of the proximal tubule of normoglycemic rats (33). Glucose concentrations between 2 and 32 mmol/L approxi-
mate those present in the S2 segment during moderate to severe hyperglycemia in rats (34). Thus, glycosuria in the range present in poorly controlled diabetes significantly inhibits sodium-dependent phosphate transport by the proximal tubule, independently of insulin status. The inhibitory effect of glucose on BBMV phosphate transport is likely due to a common dependence of both the phosphate and glucose transport systems on the generation of an electrochemical sodium gradient. The effect of glycosuria on phosphate transport, as opposed to the effect of extravascular glucose, was not apparent in the diabetic rats fed a normal phosphate diet. It would appear that in contrast to insulin, which affects the kinetics of the sodium-dependent phosphate transporter (13), glucose does not affect the transporter directly. Thus, preexisting glycosuria does not produce a permanent change that would be retained in the isolated vesicles. However, the effect of intratubular glucose in vivo can be reproduced in vitro by the addition of glucose to the extravascular fluid.

Dietary phosphate restriction in nondiabetic rats was associated with hypophosphatemia, as has been reported previously (15,24,25). This effect appeared to be blunted by insulin deficiency, as streptozotocin-treated animals on a low-phosphate diet had higher plasma phosphate concentrations as compared with their nondiabetic controls (Table 2). At first glance, this observation may seem paradoxical, because the phosphaturia stemming from insulinopenia might be expected to promote hypophosphatemia. However, insulin not only stimulates the tubular reabsorption of phosphate, but it also promotes the intracellular shift of phosphate (35). Insulinopenia, therefore, would result in both an increase in phosphaturia, as well as a shift of phosphate back into the plasma. The net effect on plasma phosphate would, thus, depend on the relative magnitude of these two opposing effects. Matsumoto et al. (30) reported similar plasma phosphate concentrations in streptozotocin-treated and control rats on a low-phosphate diet. The duration of insulinopenia in that study was 7 days, however, as compared with 48 h in this investigation.

The development of hypercalcemia in the rats on a low-phosphate diet is consistent with that described in previous reports (15,25,30). The lack of hypercalcemia in the streptozotocin-treated rats on dietary phosphate restriction is also in agreement with that described in previous reports (15,30). Those investigators reported an increase in plasma calcitriol concentrations in normal rats on a low-phosphate diet that was absent in streptozotocin-treated animals on the same diet. Thus, insulinopenia may indirectly prevent the hypercalcemia by abolishing the stimulation of calcitriol synthesis during dietary phosphate deprivation, thereby limiting the mobilization of calcium from the gut and bone.

The investigation presented here suggests that insulin deficiency and glycosuria have independent and additive effects that inhibit the tubular reabsorption of phosphate during phosphate depletion. This observation may have clinical relevance in the phosphate depletion of poorly controlled diabetes. Thus, although Type I and Type II diabetics both have glycosuria, the former suffer from insulin deficiency, and the latter exhibit hyperinsulinemia. This difference may predispose Type I diabetics to greater degrees of phosphate depletion than that seen in Type II diabetics.

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**REFERENCES**