Distinctive features of dietary phosphate supply

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Although phosphate is an indispensable dietary component, the effects of its acute (e.g., few days) omission on body growth and food intake have scarcely been addressed. We recently showed that the restoration of growth and food intake by the addition of a limited amount of phosphate in the drinking water was not accompanied by a change in serum phosphate concentration (15). Thus we suggested that phosphate signals, humoral and/or neuronal, emanate from the digestive system (including the hepatoportal system). It was also proposed that analogous humoral intestinal signals affect the sensitivity of insulin-secreting cells to plasma glucose concentration. It was later established that these “incretins,” such as the gastric inhibitory peptide and the glucagon-like peptide-1, are intestinally secreted hormones (13, 29). Intestinal sodium signals, humoral and/or neuronal, which affect natriuresis, are also implicated (17, 18). The effects of dietary phosphate on renal handling of the compound were established in rats (30) and dogs (31) many years ago. These studies showed that animals grown on a low- or high-phosphate diet react by decreasing or increasing urinary phosphate excretion. This, in turn, results from an increase and decrease, respectively, in sodium-driven phosphate reabsorption in the proximal tubules. These effects appear to be independent of changes in plasma phosphate concentration (31). Thus the postulated dietary phosphate intestinal signals may operate both on growth or appetite control and on the renal handling of phosphate.

In this study we demonstrate that 1) dietary phosphate depletion has a direct effect on appetite, in addition to its influence on growth; 2) depletion/repletion of dietary phosphate affects specifically the liver and fat tissues; and 3) alterations in dietary phosphate determine the serum concentration of an inhibitor of the sodium-phosphate (NaPi) transporter.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 100–140 g (aged ~5 wk) were purchased from Harlan Laboratories, Rehovot, Israel. The rats were individually housed in cages under standard animal house conditions of 12:12-h light-dark cycles. All procedures were approved and carried out according to the guidelines of the Hebrew University-Hadassah Medical School Animal Care Committee for the use and care of laboratory animals.

Diet. The fodder consisted of a low-phosphate diet (LPD; phosphorus content 0.02–0.04%) obtained from ICN Pharmaceutical (Costa Mesa, CA). This fodder contained 20% bovine blood fibrin as

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protein source, 0.41% calcium (with no phytic acid) and vitamin D at 325 μg/kg, and ~10% (wt/wt) water. Other essential minerals were prepared from a phosphate-free salt mixture and are specified in the ICN Pharmaceutical catalog. The drinking water was prepared from demineralized water and contained either NaCl and KCl, 22.5 mM each, or phosphate at 25 mM, pH 7.4, with Na+ and K+ as cations at 22.5 mM each. Thus the drinking water always contained equal concentrations of Na+ and K+ and differed only in the anionic content. These solutions are referred to as chloride- or phosphate-containing drinking water. Water intake varied between 34 and 45 ml/100 g body weight. All the variables relate to the last day of the indicated period. *Significance of the differences between two regression lines, both for ple comparisons were made. **Significance was determined by the one-tailed t-test, and the P value is indicated. The Bonferroni correction was applied whenever multiple comparisons were made. P < 0.05 was considered significant. The significance of the differences between two regression lines, both for the slopes and for the intercepts, was determined as described by Brownley (3).

**RESULTS**

Effect of phosphate depletion and repletion on growth and visceral weight. As shown in Table 1 and in agreement with our previous study (15), addition of phosphate to the drinking water of rats raised on LPD elicited a significant increase in growth rate (from 1.75 to 7.50 g/day) and in food intake (from 7.27 to 8.35 g·100 g−1·day−1). Growth was affected immediately, i.e., within 1 day after phosphate repletion (from 1.75 to ~13.19 g/day) or depletion (from 7.5 to 1.63 g/day). The growth rate during the first day after phosphate repletion was more robust than that during the following days (compare columns 1 and 3). On the other hand, phosphate repletion and deprivation affected the visceral weight.

Table 1. Effect of dietary phosphate on growth, food intake, and alimentary tract content

<table>
<thead>
<tr>
<th>Variable Tested</th>
<th>Cl− 4 days (n = 12)</th>
<th>Cl− 4 days followed by P1 1 day (n = 12)</th>
<th>Cl− 4 days followed by P1 5 days (n = 5)</th>
<th>P1, 4 days followed by Cl− 1 day (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rate, g/day</td>
<td>1.75 ± 0.51</td>
<td>15.51 ± 1.64†</td>
<td>7.50 ± 1.46‡</td>
<td>1.63 ± 0.70‡§</td>
</tr>
<tr>
<td>Net growth rate, g/day</td>
<td>1.75</td>
<td>12.19</td>
<td>7.50</td>
<td>1.63</td>
</tr>
<tr>
<td>FI, g 100 g−1·day−1</td>
<td>7.27 ± 0.22</td>
<td>8.94 ± 0.30‡</td>
<td>8.35 ± 0.44*</td>
<td>9.38 ± 0.32†</td>
</tr>
<tr>
<td>Stomach + bowel, % of BW</td>
<td>6.59 ± 0.12</td>
<td>8.23 ± 0.25‡</td>
<td>9.07 ± 0.42†</td>
<td>8.21 ± 0.30‡</td>
</tr>
<tr>
<td>BW, g</td>
<td>120 ± 5.1</td>
<td>129 ± 4.6</td>
<td>186 ± 7.1</td>
<td>152 ± 3.7</td>
</tr>
</tbody>
</table>

Growth rate, food intake (FI), percentage of stomach + bowel weight, and body weight (BW) of rats kept for 4 days on a low-phosphate diet (LPD) and chloride-containing drinking water (Cl− 4 days). Some of the rats were then kept for 1 day or 5 days on LPD and phosphate-containing drinking water (Cl− 4 days followed by P1, 1 day and Cl− 4 days followed by P1, 5 days, respectively). An additional group was kept for 4 days on LPD and phosphate-containing drinking water (P1, 4 days followed by Cl− 1 day). Values are means ± SE. Net mean growth rate was calculated by subtracting the estimated average increment in weight of the crude stomach plus bowel content from the measured average increase in body weight. All the variables relate to the last day of the indicated period. *P < 0.05 vs. first column. †P < 0.01 vs. first column. ‡P < 0.01 vs. second column. §P < 0.01 vs. third column.

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depletion affected food intake asymmetrically: the increase in food intake was evident immediately (within 1 day) after the resupply of phosphate (from 7.27 to 8.94 g·100 g body weight−1 day−1), although there was no significant change in food intake during the first day after phosphate withdrawal (compare columns 3 and 4). To explore the possibility that the decrease in food intake represented not only reduced nutrient utilization but also a drop in appetite, changes in visceral weight were measured before and after dietary phosphate supply or withdrawal. We reasoned that if the decrease in food intake were due only to a lower need of nutrients, the extent of visceral filling or distention would not change by alterations in the dietary phosphate content. As seen in Table 1, the change in food intake was accompanied by a change in bulk stomach plus bowel content, which decreased by at least 27% (compare column 1 with the other three) in the dietary phosphate-depleted rats. Thus it seems that the reduction in food intake upon depletion of dietary phosphate results also from a reduction in appetite. Notably, in spite of the immediate increment in stomach and bowel weight after the resupply of phosphate, the net growth 1 day later was still greater than in the following days (13.19 vs. 7.5 g/day).

Relative organ weight in rats before and after dietary phosphate repletion. To explore the possible effects of dietary phosphate on specific organ weight, the weight of several organs was measured under conditions of phosphate deprivation and after phosphate repletion (Table 2, Fig. 1). The change in relative organ weight after growth after phosphate repletion fell into three general groups: 1) organs whose relative weight decreased with increased body weight (e.g., brain, heart, and spleen), 2) an organ (kidneys) with no significant change in relative weight, and 3) organs whose relative weight increased after phosphate repletion (e.g., liver and epididymal fat).

A more detailed study of the specific organ-growth effects was provided by analyzing the relationship between body and organ weight for phosphate-depleted and -supplied rats (Fig. 1). Statistical analysis of these data revealed that only the epididymal fat (P < 0.02) and the liver (P < 0.07) showed a significantly steeper slope in the relationship between organ weight and body weight. Liver (A), epididymal fat (B), and spleen (C) weight as function of body weight are shown. Rats were kept for 4 days on a low-phosphate diet (LPD) and chloride-containing drinking water (Cl− group, ●), and some were subsequently transferred to phosphate-containing drinking water for 1 or 5 days (P, group, ○). Linear regression lines (broken line for the Cl− group and continuous line for the P group) and their equations are shown. *Significantly different from the Cl− curve in slope (P < 0.07) and intercept (P < 0.02). **Significantly different from the Cl− curve in slope (P < 0.02). †Not significantly different from the Cl− curve.

Table 2. Effect of dietary phosphate on relative organ weight

<table>
<thead>
<tr>
<th>Anatomic Content of Drinking Water</th>
<th>Cl− 4 days followed by P, 1 day (n = 12)</th>
<th>Cl− 4 days followed by P, 5 days (n = 5)</th>
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</thead>
<tbody>
<tr>
<td>Liver</td>
<td>4.51 ± 0.21</td>
<td>5.16 ± 0.21</td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>0.567 ± 0.039</td>
<td>0.607 ± 0.039</td>
</tr>
<tr>
<td>Brain</td>
<td>1.26 ± 0.046</td>
<td>1.102 ± 0.030</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.349 ± 0.014</td>
<td>0.347 ± 0.012</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.891 ± 0.027</td>
<td>0.882 ± 0.022</td>
</tr>
<tr>
<td>Heart</td>
<td>0.449 ± 0.017</td>
<td>0.418 ± 0.011</td>
</tr>
<tr>
<td>Tibia (left)</td>
<td>0.351 ± 0.023</td>
<td>0.347 ± 0.014</td>
</tr>
<tr>
<td>BW, g</td>
<td>120 ± 5.1</td>
<td>129 ± 4.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. BW and percent organ weight of rats kept for 4 days on LPD and chloride-containing drinking water (Cl− 4 days). Some of the rats were then kept for 1 day or 5 days on LPD and phosphate-containing drinking water (Cl− 4 days followed by P, 1 day and Cl− 4 days followed by P, 5 days, respectively). *P < 0.05 vs. first column. †P < 0.001 vs. first column. ‡P < 0.05 vs. second column. §P < 0.001 vs. second column.

and body weight for the phosphate-supplied group. The liver showed also a significant decrease in the intercept of the regression line representing the phosphate-supplied group (P < 0.02). There was no significant difference between the constants of the regression lines of all the other tested tissues: spleen (data shown), tibia, brain, kidneys, and heart (data not shown).

To exclude the possibility that a significant proportion of the increased body weight was due to greater hydration, the water content of the liver was measured before and after resupply of phosphate. Although the change in liver weight was quite pronounced, the percent water content of the liver did not change [69.7 ± 0.60% (n = 6) after 4 days of P, depletion vs. 69.5 ± 0.36% (n = 6) after 5 days of P, resupply].

Alterations in dietary phosphate determine the level of a NaPi-transporter inhibitor in rat serum. As mentioned above, this and our previous study imply the presence of a phosphate signal that emanates from the digestive system. It is well established that an increase in dietary phosphate elicits an increase in renal phosphate excretion brought about by a decrease in Na+-dependent Pi reabsorption (4). The effect of dietary phosphate on the renal handling of phosphate might also be mediated by a humoral phosphate intestinal signal. The presence of such a signal could be assayed by measuring the ability of plasma to inhibit the activity of NaPi transport in a

Fig. 1. Effect of dietary phosphate on the relationship between organ and body weight. Liver (A), epididymal fat (B), and spleen (C) weight as function of body weight are shown. Rats were kept for 4 days on a low-phosphate diet (LPD) and chloride-containing drinking water (Cl− group, ●), and some were subsequently transferred to phosphate-containing drinking water for 1 or 5 days (P group, ○). Linear regression lines (broken line for the Cl− group and continuous line for the P group) and their equations are shown. *Significantly different from the Cl− curve in slope (P < 0.07) and intercept (P < 0.02). **Significantly different from the Cl− curve in slope (P < 0.02). †Not significantly different from the Cl− curve.
model of renal proximal cells. Thus we hypothesized that resupply of phosphate to rats raised on LPD would increase the levels of this putative NaPi inhibitor in the plasma. To this end, a line of renal proximal cells, OK cells, was grown for 18 h in culture media including 10% rat serum. Serum samples were collected from rats fed for 24 h on a LPD and Cl\(^-\)- or P\(_i\)-containing drinking water (see MATERIALS AND METHODS). Because cAMP is a known inhibitor of the NaPi transporter, the activity of the transporter in the OK cells was determined in the absence and presence of dBcAMP in the incubation medium. The results show (Fig. 2) that cells grown in the presence of plasma from rats that received phosphate in their drinking water exhibited a lower Na\(^+\)-dependent phosphate transport activity (~17%). This decrease was statistically significant both in the presence of dBcAMP (P < 0.01) and in its absence (P < 0.05). The \(\chi^2\) test for combined probability showed that the difference between the Cl\(^-\) and the P\(_i\) groups was highly significant (P < 0.0002). These results show that the plasma of phosphate-supplied rats contains a humoral factor that inhibits the Na\(^+\)-dependent P\(_i\) transporter.

**DISCUSSION**

We showed previously that the dietary phosphate supply affects both growth and food intake. In this study we investigated whether the primary (or sole) effect of phosphate is on growth or whether it also affects appetite. Our results unequivocally show that phosphate depletion and repletion are associated also with the extent of intestinal distention. This indicates that the dietary phosphate level exerts a real effect, direct or indirect (e.g., through change in other plasma nutrients) on appetite. This effect is analogous to the influence of leptin and insulin on the central nervous system, augmenting the sensitivity to satiety signals and leading to a decrease in meal size (32).

Our findings rule out the possibility that the primary and sole effect of dietary phosphate withdrawal or supplementation is on appetite and that the influence on growth is secondary to the alterations in food intake. This conclusion is based on the observation that removal of phosphate from the diet was associated with an immediate (<1 day) decrease in growth rate without a concomitant decrease in food intake or gastrointestinal distention (Table 1). The reduced food intake was evident only after 2–3 days of phosphate withdrawal (15).

Analogous effects on growth rate and food intake were observed also upon dietary Mg\(^{2+}\) or K\(^+\) depletion (6, 10), although the relationship between growth rate and food intake was not addressed. Upon dietary Zn depletion, there was an apparently major effect on appetite (23). Another, more pronounced example of a major effect on appetite upon withdrawal of a single essential nutrient from the diet was demonstrated for threonine (7, 14). Dietary threonine depletion, which, unlike dietary phosphate depletion, resulted in a substantial and rapid decrease in plasma threonine concentration, caused an anorectic response. Surprisingly, this response was elicited via local specific effects on the anterior piriform cortex, because local injection of threonine into this area abolished the anorectic response (20). Thus dietary phosphate withdrawal is the only known example in which it is conclusively evident that the primary effect of the elimination of a single nutrient is on growth rate.

Recent studies in rats indicated that phosphate depletion induces also a specific phosphate appetite (5, 25). Notably, this response is acute; i.e., similar responses were observed after 2 days or 7 days of phosphate removal (25). It is plausible that, like its well-established analog the sodium appetite, the phosphate appetite developed after phosphate restriction is caused by signals that arise in the central nervous system and by peripheral hormones that affect elements in the brain (9).

The present study analyzed the effect of dietary phosphate on tissue-specific changes in growth. One of the main conclusions is that phosphate deprivation, unlike potassium deprivation, does not lead to “paradoxical” renal hypertrophy (8, 11). The underlying mechanism and the physiological significance of these differences merit further investigation.

In our study most of the tested organs, heart, spleen, and brain, showed a decrease in their relative weight with increase in body weight (Table 2). This phenomenon corresponds to the “theoretical” pattern observed under normal growth as shown, for example, in the detailed study performed by Schoeffner et al. (22) in rats. Our analysis of the relationship between organ and body weight (Fig. 1) also indicates that there was no significant difference for the above mentioned organs between phosphate-depleted and phosphate-supplied rats. However, our results show that the liver was unique in its response: relative weight increasing with the increase in body weight in the phosphate-supplied group compared with the phosphate-deprived group. The same was true for epididymal fat, which, however, qualitatively followed the general pattern of fat weight change in “normal” growth (22). Thus the main conclusion from this analysis is that during dietary phosphate deprivation there is a specific decrease in the weight of liver and fat tissue and/or, although less plausible, upon resupply of phosphate there is a specific increase in liver and fat weight. In this respect, the phenomenon is similar to the effects of starvation and protein deprivation, in which liver and fat weights were shown to decrease specifically under normal growth hormone levels (1). Notably, the acute dietary phosphate-dependent growth did not change specific bone (tibia) growth (Table 2 and Fig. 1), a finding in contrast to the robust chronic effects of phosphate-wasting diseases on bone (28).
Plasma phosphate concentrations are maintained within a defined range by processes that regulate the intestinal absorption and renal excretion of inorganic phosphate. The hormones considered to influence these processes are parathyroid hormone (PTH) and the active metabolite of vitamin D, 1α,25-dihydroxyvitamin D (12, 19, 27). A new class of phosphate-regulating factors, collectively known as the phosphatonin, has been shown to be associated with various hypophosphatemic diseases (21). These factors, which include fibroblast growth factor 23 (FGF-23), inhibit the renal NaPi cotransporter (21, 26). The roles of these substances under normal conditions are not known (21).

Our results show that rat plasma contains a soluble factor that inhibits the sodium-driven phosphate transport in kidney cells (Fig. 2). A well-known serum constituent that inhibits the Na\(^+\)-dependent Pi transport is PTH (19). However, the factor we discovered is not PTH, because the PTH serum level was not affected by switching from Cl\(^-\) to P\(_2\)-containing drinking water (15).

It is tempting to suggest that the same factor that inhibits active phosphate uptake in the kidney is also the putative phosphate-intestinal signal that stimulates growth after phosphate supplementation. A likely candidate could be the FGF-23 (33), which, by virtue of being a member of the FGF family, may also exert growth promoting effects. Indeed, compared with heterozygous FGF-23 (+/+ ) mice, their homozygous FGF-23 (−/−) counterparts presented remarkable postnatal growth retardation, in addition to a disruption in phosphate homeostasis (24). Recently, however, a study on human volunteers showed no change in serum FGF-23 level after alteration in phosphate intake (16). This observation contradicts the notion that FGF-23 is the intestinal phosphate signal in humans. It should be stressed that the effect of dietary phosphate on phosphaturia (or on growth rate) has not been demonstrated in humans. Thus the possibility that intestinal phosphate signaling is species specific should also be considered.

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GRANTS

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REFERENCES


