Induction of a Phosphate Appetite in Adult Male and Female Rats

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We have reported that dietary inorganic phosphate (Pi) deprivation induces a Pi-seeking behavior in juvenile male rats. The purpose of the present study was to determine whether the Pi appetite is present in adult animals, and if so, whether it is altered during times of increased demand for Pi, such as pregnancy and lactation. Both male and female animals fed a low-phosphate diet (LPD) ingested significantly greater amounts of PiH_2O daily than their normal phosphate diet (NPD) controls, and per 100 g of body weight (BW), the female animals fed LPD tended to ingest greater amounts of PiH_2O than male rats fed LPD. Pregnant and lactating rats fed LPD ingested significantly more PiH_2O than those fed NPD, however, neither group displayed a Pi appetite different than virgin females. However, lactation further reduced Pi levels in plasma and cerebral spinal fluid compared with control values. Despite the additional Pi from the PiH_2O in the mothers fed LPD, pup birth weight was significantly lower than in NPD litters, and this was exacerbated 9 days after birth. This attenuated BW gain was associated with lower plasma Pi levels in the pups. In conclusion, a mild but consistent Pi-seeking behavior is induced in adult male and female rats after only 2 days of dietary Pi restriction. On a relative basis, the amount of PiH_2O ingested is greater in female than in male animals, but does not increase further during pregnancy and lactation. Exp Biol Med 229:914–919, 2004

Key words: gender; pregnancy; nutrition; behavior; lactation

Introduction

Inorganic phosphate (Pi) homeostasis is crucial to bone mineralization and muscle growth, it is a constituent of membrane phospholipids, and it is used in high-energy organic compounds such as ATP, ADP, and AMP (1). Thus, a deficiency in this mineral can cause severe bone abnormalities, stunted growth, and many other metabolic and physiological disorders (2). Under normal conditions, the demand for Pi in an organism is satisfied through an adequate dietary intake of Pi. However, when the dietary supply is inadequate, or demands for growth are high, the kidneys have the ability to adapt by upregulating the reabsorption of Pi (3–6), limiting the loss of this electrolyte. It is now clear that the renal adaptations are only one of the changes occurring during Pi deprivation.

Previous investigators have determined that a diet deficient in Pi stimulates an ingestive behavior to seeks sources of Pi (7). In the early 1980s, Denton and colleagues (8) reported that a phosphate-seeking behavior could be suppressed by raising plasma Pi concentrations through rapid iv infusions of a buffered Pi solution. These findings, as well as those of Blair-West *et al.*, indicated that the behavioral response (i.e., Pi appetite), was linked to phosphorus deficiency (9), and could be controlled by plasma Pi levels. Of interest was that, in herbivores, the Pi appetite was stimulated after weeks to months of Pi deprivation. Whether this was specific to herbivores was unknown.

We have expanded on the earlier studies, and demonstrated that similar behavioral responses could be elicited from juvenile rats within 2 days, and was specific for Pi (10). This rapid response to Pi restriction coincided with the renal adaptation to enhance Pi reabsorption, and thus appears to be part of a general physiologic-behavioral series of adaptive responses to limit Pi losses while seeking Pi sources. Of interest was that plasma and cerebrospinal fluid (CSF) Pi levels were significantly reduced after 2 days of reduced Pi intake, indicating a potential central mechanism controlling the Pi appetite, as observed with the sodium appetite (11–14). It was unknown, however, whether this appetitive behavior is a function of the high demand for Pi in the growing juvenile rat, or whether it would also be observed in the adult animal.

The purpose of the present study was to determine whether a phosphate appetite could be induced in adult animals, and if so, whether sex differences were present,

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especially in females during pregnancy and lactation, when the demand for Pi is elevated. The effect of maternal dietary Pi ingestion was also assessed in offspring.

Methods

Animals, Diets, and Fluids. Experiments were performed using adult male and female Wistar rats (13–16 weeks of age), and were approved by the Georgetown University Animal Care and Use Committee. A separate group of juvenile (6-week-old) male rats was also used. The animals were initially fed standard rodent laboratory chow pellets to acclimate them to laboratory conditions. Each rat was individually housed in an 18 × 12 × 10 cm hard plastic cage, with a stainless steel cage top, and a bedding of wood chips 1 inch deep. All animals were maintained at room temperature and subjected to 12-hour light/dark cycles (light from 0700 to 1900 hrs). For each study, the animals were divided into two groups. The experimental group received a low-phosphate diet (LPD, 0.02% Pi) consisting of a basic, low-Pi rodent diet (Research Diets 93082402; Brunswick, NJ) supplemented with 9.7 g/kg NaCl and 14.76 g/kg KCl. The control group received a normal phosphate diet (NPD, 0.6% phosphate) consisting of the basic LPD supplemented with 3.02 g/kg KH_2PO_4, 15.46 g/kg K_2HPO_4, and 2.28 g/kg NaH_2PO_4 salt additives. Because the rodent diet is powdered, the food was placed in the right hand corner of every cage in a stainless steel bowl to avoid spillage. Care was taken to ensure that fasting did not occur.

Throughout the experiments the rats had continuous access to 300 ml of tap water provided in inverted plastic bottles (500 ml). After 2 days of LPD or NPD, all animals were allowed free access to a 0.3 M solution of KH_2PO_4 water (PiH_2O) at physiologic pH that was slightly sour yet palatable to the human taste (10). PiH_2O ingestion was monitored for 5 additional days. The concentration of PiH_2O was selected in an attempt to provide a solution that is aversive to humans, and not readily ingested by rats. The PiH_2O was provided in 100-ml inverted plastic bottles. The spouts dispensing the water were placed at identical levels in the cage (~10 cm from the floor), and ~7 cm apart from each other. Their relative positions were maintained daily. In each experiment, body weight (BW), food and water intake were measured at the same time daily. After each measurement, both food and water were replenished.

Effect of Pi Deprivation on Pi Appetite in Adult Female and Male Rats. Male and female adult rats were placed on either an LPD (n = 6, each sex) or NPD (n = 6, each sex) for 2 days and provided with unlimited access to distilled water. After 2 days on LPD or NPD, all the rats were given access to PiH_2O for 5 days. Body weight, food intake, and water intake were monitored daily. Plasma and CSF phosphate levels were obtained before sacrifice, as previously described (10). Comparisons were made between absolute PiH_2O ingestion, as well as PiH_2O normalized per 100 g of BW. This allowed evaluation of the relative intake in different sized animals. To complete this assessment, an additional group of juvenile male rats underwent the experimental conditions, and the relative PiH_2O intake normalized per 100 g of BW was determined.

Time Course for the Decrease in Plasma and CSF Pi Concentrations during Pi Deprivation. To determine the time course for the decrease in plasma and CSF Pi levels, juvenile male rats (6 wks) were placed on LPD; plasma and CSF levels were obtained 0 (n = 3), 24 (n = 3), 30 (n = 3), and 48 (n = 3) hrs later. The levels of Pi in plasma and CSF were compared to the Time 0 point.

Pi Appetite in Pregnant and Lactating Rats. These experiments were performed to determine the effect of pregnancy and lactation on the Pi appetite. On about the 16th day (in the third trimester) of pregnancy, females were divided into two groups, with one group fed NPD (n = 3) and the other fed LPD (n = 4). Two days later, all rats were given access to PiH_2O in addition to tap water. The rats remained on their respective diets throughout the pregnancy and for 9 days after parturition. The animals had continual access to the PiH_2O in addition to tap water. At parturition, pups were weighed, and the litters were culled to eight pups per mother so that milk production between mothers would be consistent. Daily BWs, tap water intake, PiH_2O intake, and food consumption were monitored. After 9 days, plasma and CSF samples were obtained from the mothers. The pups were weighed, decapitated, and plasma samples were collected for Pi analysis (CSF samples were not collected from pups).

Plasma and CSF Pi Concentrations. At the end of each experiment, Pi levels in plasma and CSF from Pi-deprived and replete animals were determined. Rats were anesthetized with an injection of pentobarbital (100 mg/kg BW). To collect CSF, a 23-gauge stainless steel guide cannula was implanted stereotaxically into the third ventricle of each rat. Injectors were placed in the guide cannula, and CSF was drawn into PE-10 tubing. The tubing was sealed at both ends by flame, and stored at 4°C until microanalysis. Analysis was performed using a flow-through microspectrophotometer (15), and a modified phosphomolybdate method described by Chen *et al.* (16). CSF samples (100 nl), or phosphate standards (50 nl) were transferred to separate tubing containing 2 µl of reagent (10% ascorbic acid, 10% [8 M] H_2SO_4, 10% [2.5%] ammonium molybdate, in distilled water). The samples were then sealed, mixed, and incubated in a 37°C water bath for 90 mins. After incubation, the samples were injected into the spectrophotometer port, and the absorbance was read as a change in voltage. All samples were run in duplicate in two separate assays, and the phosphate concentration was determined against a known standard curve.

Following CSF sample collection, a 1.5-ml blood sample was obtained directly from the heart of each rat, and plasma was harvested and frozen at 70°C. Plasma

phosphate concentration was measured by the phosphomolybdate method described by Chen *et al.* (16).

Statistical Analysis. Comparisons between adult and juvenile groups, and normal, pregnant, and lactating female groups were performed by analysis of variance, with Tukey-Kramer posthoc analysis, for the most conservative analysis of overall error between multiple groups. Comparisons in Figures 1 and 2 were made on Pi intake adjusted for individual BWs, and are expressed in ml/day/100 g BW. This was performed prior to analysis. Data are expressed as means ± SEM, and significance is designated as $P \leq 0.05$.

Results

Pi Appetite in Adult Male and Female Rats. Two days of dietary phosphate deprivation significantly ($P \leq 0.05$) increased PiH_2O consumption in both female and male adult rats fed LPD (3.9 ± 0.1 and 4.2 ± 0.3 ml/day, respectively) compared with NPD controls (2.6 ± 0.2 and 2.7 ± 0.2 ml/day, respectively). The absolute amount of PiH_2O ingested by adult rats fed LPD was comparable to that consumed by juvenile rats fed LPD (3.4 ± 0.4 ml/day, n.s.). Absolute ingestion of PiH_2O by juvenile rats fed NPD (1.7 ± 0.2 ml/day) was significantly less than that observed in adult male (2.6 ± 0.2 ml/day, $P \leq 0.05$) or female (2.6 ± 0.1 ml/day, $P \leq 0.05$) rats fed NPD. Thus, juvenile rats fed LPD increased their PiH_2O intake by 100% over control NPD values, whereas adult male and female rats fed LPD had comparable increases in PiH_2O intake of 59% and 52%, respectively.

Although the absolute amounts of PiH_2O ingested by rats fed NPD or LPD were not significantly different, absolute intakes do not account for the general differences in size of the animals. As noted in Table 1, food and water intake was significantly lower in the juvenile rats compared with adult rats, and thus, it is possible that on a relative basis, there may be differences in PiH_2O intake. Indeed, when the data were adjusted per 100 g of BW, age differences were evident. The amount of PiH_2O ingested per 100 g of BW was greater in juvenile rats compared with male and female adult rats on both normal and low-Pi diets ($P < 0.05$; Fig. 1). This demonstrates that regardless of age, rats fed LPD consume more PiH_2O than those fed NPD, and that the PiH_2O intake in juvenile rats is significantly higher than both male and female adult rats when adjusted per 100 g of BW.

The increase in PiH_2O consumption in male and female rats fed LPD was associated with an increase in daily BW gain over Days 3–7 compared with Days 1–2 (Table 1), indicating that the additional Pi ingested facilitated the growth process. This was not observed in rats fed NPD. In general, average distilled water and food intakes were less in the female rats fed NPD or LPD compared with the values obtained in male rats (Table 1), which was consistent with the significantly lower BW in the females. Plasma Pi concentrations were comparable in adult rats fed NPD (2.11 ± 0.06 in males and 2.03 ± 0.08 mM in females) and LPD (1.59 ± 0.12 in males and 1.51 ± 0.08 mM in females), whereas plasma Pi levels were higher in juvenile animals (3.11 ± 0.03 mM in NPD, and 1.77 ± 0.10 mM in LPD).

Time Course for Plasma and CSF Pi Concentrations in Juvenile Male Rats. Because a Pi appetite was evident in these experiments after 2 days of Pi deprivation, plasma and CSF Pi concentrations were measured at intervals from 0 to 48 hrs in juvenile male rats. Table 2 illustrates the time course for changes in plasma and CSF Pi levels over the experimental period. Plasma Pi levels fell significantly by 24 hrs and remained low. CSF Pi concentrations decreased after 24 and 30 hrs, and reached significance by 48 hrs of Pi deprivation. This suggests that the decrease in plasma Pi eventually lowered CSF Pi levels. There was also a disproportionate decrease in CSF Pi (-50%) in response to the 20% decrease in plasma Pi after 48 hrs of Pi deprivation.

Effects of Pi Deprivation on Pi Appetite in

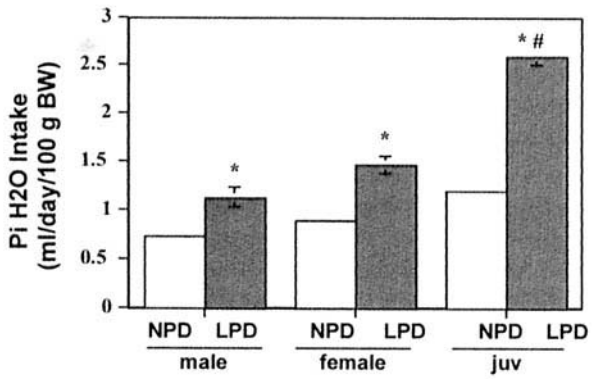

Figure 1. Average daily inorganic phosphate (Pi)H_2O ingestion adjusted per 100 g body weight (BW) in adult male and female rats and juvenile male rats fed either normal phosphate diet (NPD) or low phosphate diet (LPD). All animals were Pi-deprived for 2 days. *$P \leq 0.05$ vs. NPD in same group, #$P \leq 0.05$ vs. LPD females and males. (Some error bars are too small to visualize on the figure at this resolution.)

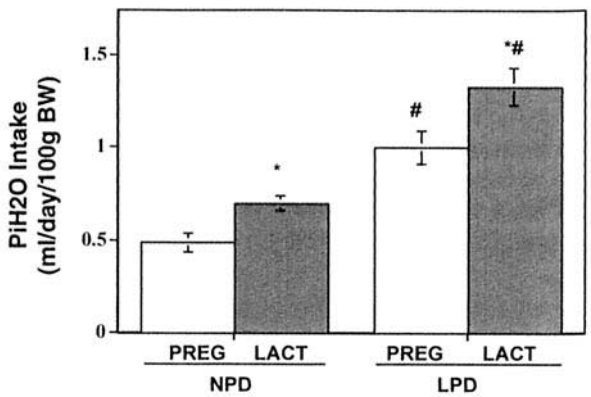

Figure 2. Average daily inorganic phosphate (Pi)H_2O ingestion in pregnant and lactating rats adjusted per 100 g body weight (BW) fed either normal phosphate diet (NPD) or low phosphate diet (LPD).*$P \leq 0.05$ vs. pregnant, #$P \leq 0.001$ vs. NPD controls.

Table 1. Growth and Intake Values for Animals Fed Normal Phosphate Diets (NPD) and Low Phosphate Diets (LPD) Diets

	Avg. daily BW gain (g) (Days 1–2)	Avg. daily BW gain (g) (Days 3–7)	Avg. dH_2O intake (ml/day)	Avg. food intake (g/day)	Final BW (g)
Adult male					
NPD ($n = 5$)	2.6 ± 0.8	2.9 ± 0.5	41 ± 2	22 ± 0	363 ± 3
LPD ($n = 6$)	-1.8 ± 0.8*	2.8 ± 0.4^{abd}	43 ± 2	25 ± 2	349 ± 4
Adult Female					
NPD ($n = 3$)	1.7 ± 3.1	2.2 ± 0.3^{ab}	33 ± 2*^{a}	14 ± 0*^{a}	286 ± 14*^{a}
LPD ($n = 6$)	0.7 ± 0.7^{a}	1.1 ± 0.3^{ab}	32 ± 3*^{a}	15 ± 1*^{a}	268 ± 6*^{a}
Juvenile Male					
NPD ($n = 6$)	4.7 ± 0.6*^{ac}	5.9 ± 0.3*^{abc}	27 ± 1^{a}	12 ± 1*^{a}	176 ± 2*^{abc}
LPD ($n = 6$)	2.0 ± 0.3^{a}	6.4 ± 0.9*^{abcd}	32 ± 3*^{a}	13 ± 0*^{a}	164±3*^{abc}

* $P \leq 0.05$ vs. adult male NPD; ^{a} $P \leq 0.05$ vs, adult male LPD; ^{b} $P \leq 0.05$ vs. female NPD; ^{c} $P \leq 0.05$ vs. female LPD; ^{d} $P \leq 0.05$ vs. body weight (BW) gain in Days 1–2.

Pregnant and Lactating Rats. Pregnancy and lactation did not stimulate PiH_2O ingestion in rats fed NPD compared with virgin controls (1.9 ± 0.2, 2.1 ± 0.2, and 2.5 + 0.2 ml/day, respectively, n.s.). However, pregnant rats fed LPD ingested significantly more PiH_2O than those fed NPD (3.6 ± 0.3 vs. 1.9 ± 0.2 ml/day, respectively, $P \leq 0.05$), indicating induction of the appetitive behavior. PiH_2O ingestion in lactating rats fed LPD was also higher than in lactating rats fed NPD (3.5 ± 0.3 vs. 1.9 ± 0.3 ml/day, $P < 0.051$). Again, when PiH_2O ingestion was adjusted by BW, the lactating rats on either respective diet displayed an increase in PiH_2O ingestion compared with the pregnant rats (due to increased BW with pregnancy; NPD, 0.49 ± 0.05 vs. 0.70 ± 0.04 ml/100 g BW in pregnant and lactating rats; [$P < 0.05$]; LPD, 1.0 ± 0.09 vs. 1.3 ± 0.10 ml/100 g BW in pregnant and lactating rats [$P < 0.05$]; Fig. 2).

Figure 3 indicates that plasma Pi levels were very low in the mothers fed LPD compared with mothers fed NPD, or male and female rats fed LPD. The plasma Pi levels in lactating mothers fed LPD were also, interestingly, significantly lower than those observed in nonlactating female rats fed LPD. Furthermore, as can be seen in Figure 3, CSF Pi levels were significantly reduced in the lactating mothers fed LPD (0.095 ± 0.017 vs. 0.362 ± 0.005 mM in NPD mothers, $P < 0.05$). The 70% reduction in CSF Pi was again disproportionately lower than the decrease in plasma Pi (-50%). This strongly suggests that the demand for Pi was so high during lactation that Pi was taken from the plasma pool for milk production, reducing the plasma and hence the CSF Pi lower than normally observed. In addition, the utilization of Pi in the brain may also contribute to the lower CSF Pi levels.

Of note was that the Pi-deprived lactating mothers continued to lose significant amounts of weight (-3 ± 1 g/day) despite ingestion of PiH_2O. Although they ate comparable amounts of food as the mothers fed NPD, their weight loss was notable, and they did not increase their distilled water intake to the same extent as the NPD mothers.

Effects of Pi Deprivation on Rat Pups. The offspring of the pregnant female rats fed NPD and LPD were monitored for 9 days postpartum, during which time the mothers were maintained on their respective diets with access to PiH_2O. At birth, the pups from mothers fed LPD weighed significantly less than the pups of the control mothers ($P < 0.05$; Fig 4). After 9 days, the difference in BW was more pronounced in the LPD pups compared with the pups from mothers fed NPD ($P < 0.05$; Fig 4).

Plasma Pi concentrations in the pups was also determined 9 days after birth. Pups from mothers fed LPD had significantly lower plasma Pi concentrations (3.62 ± 0.05 mM) than the pups from mothers fed NPD (4.09 ± 0.08 mM; $P < 0.05$). CSF Pi concentrations could not be determined because of the small size of the pups. Anecdotally, one lactating mother fed LPD ate three of her pups the evening before sacrifice on Day 9. Analysis of her plasma and CSF Pi showed supraphysiologic levels of Pi, elaborated from the bones and tissue of the pups (plasma Pi, 4.49 mM; CSF Pi, 0.89 mM). Her PiH_2O intake (2.2 ml)

Table 2. Time Course for Changes in Plasma and CSF Pi Levels[a]

	Time after access to low-phosphate diet			
	Basal (0 h)	24 hrs	30 hrs	48 hrs
Plasma Pi (mM)	3.87 ± 0.25	2.62 ± 0.11*	2.41 ± 0.19*	2.56 ± 0.06*
CSF Pi (mM)	0.44 ± 0.03	0.42 ± 0.06	0.36 ± 0.06	0.29 ± 0.05*

[a] CSF, cerebrospinal fluid; Pi, phosphate; * $P \leq 0.05$ vs. respective basal value.

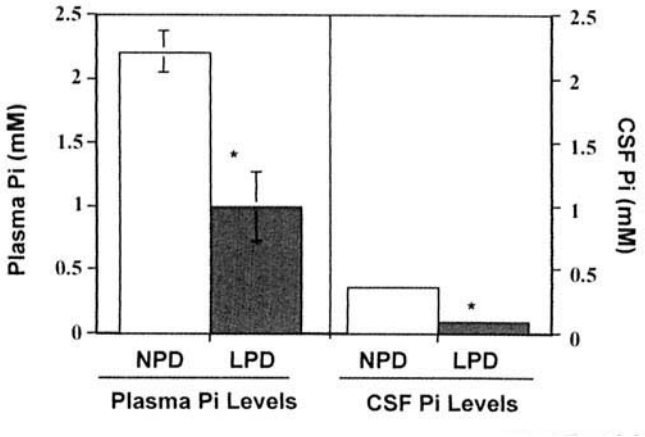

Figure 3. Average plasma (left) and cerebrospinal fluid (CSF) (right) inorganic phosphate (Pi) concentrations from lactating rats 9 days postparturition, fed either normal phosphate diet (NPD) or low phosphate diet (LPD). After 2 days of Pi restriction, the animals had access to PiH_2O until 9 days postparturition. $^*P \leq 0.05$ vs. NPD. (Some error bars are too small to visualize on the figure at this resolution.)

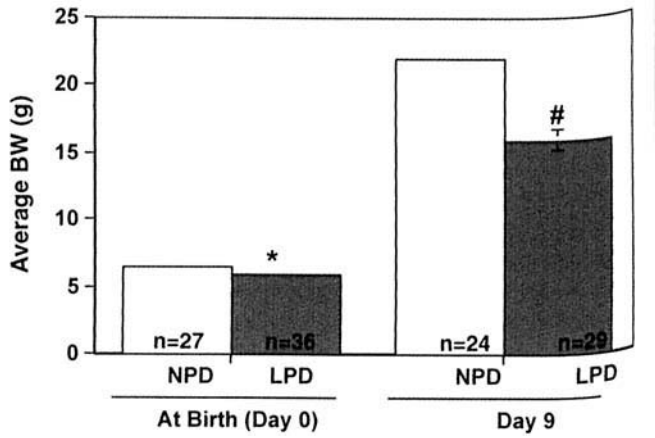

Figure 4. Average body weight (BW) of pups from mothers fed either normal phosphate diet (NPD) or low phosphate diet (LPD) at birth and 9 days postparturition. $^*P \leq 0.025$ vs. NPD, $\#P \leq 0.001$ vs. NPD. (Some error bars are too small to visualize on the figure at this resolution.)

also fell to normal levels, indicating a rapid quenching of the Pi appetite, as previously reported in juvenile rats (10).

Discussion

This study demonstrates that adult rats exhibit Pi appetitive behavior when they are deprived of phosphate in their diet. Plasma and CSF levels decrease rapidly, and the consumption of PiH_2O is significantly increased within 1 day. These findings complement and extend our previous report in juvenile rats (10).

Both the male and female adult rats fed LPD ingested significantly more PiH_2O than animals fed NPD, and the absolute amount of PiH_2O ingested by the male and female LPD animals was almost equivalent. In general, the male animals weighed significantly more than the female animals, and ingested more food and water than the females on a daily basis, and when the data were adjusted for BWs, there was the tendency for female PiH_2O ingestion to be greater. This is consistent with findings that female rats have the tendency to ingest, or absorb, more sodium (17) and calcium (18, 19) under a variety of conditions. In addition, when PiH_2O intake is adjusted for BW in juvenile animals, the juvenile male rat consumed significantly more PiH_2O than either the male or female adult rats. We hypothesize that this phenomenon reflects the greater demand for Pi in the juvenile, due to the rapid growth process.

Of potential importance is that the ratio of plasma:CSF Pi is disproportionately higher in rats fed LPD, especially in the lactating mothers 9 days postpartum. Our hypothesis is that a decrease in plasma and CSF Pi levels initiates the behavioral response in the LPD animals. Pi-sensitive cells in the brain may be stimulated by decreases in CSF Pi level and induce the behavioral response to seek Pi. Indeed, we have reported that rat type IIa sodium-Pi transporter (NaPi-2)-like activity, first described in the kidney, is not only found in areas of the brain associated with behavioral signals, but their expression is regulated by changes in dietary Pi (20). Furthermore, these potential "Pi-sensors" in the brain also control renal NaPi-2 transporters, enhancing Pi reabsorption during dietary Pi deprivation (20). We believe that the Pi appetitive behavior and renal adaptations may be stimulated through this central mechanism, which may serve to regulate overall Pi homeostasis.

Phosphate is an important nutritional requirement during pregnancy (21), and a Pi appetite was inducible in the pregnant and lactating rat. However, contrary to our original hypothesis, the increased demands for Pi associated with pregnancy and lactation did not elevate the Pi appetite above that in virgin rats fed LPD. Pi appetitive behavior in humans is supported by a study of women in The Gambia showing an 11% increase in Pi consumption while lactating (22). It is also recognized that levels of calcium and phosphorus fluctuate greatly during lactation (19, 23, 24). In our study, Pi deprivation of the mothers also had a significant effect on the litters. Birth weights were significantly decreased in the pups from mothers fed LPD. They also displayed a stunted growth rate over the 9 days after birth compared with pups from mothers fed NPD. Thus, the PiH_2O consumed by the mothers was not enough to satisfy the great demand presented by the pups. Additionally, the plasma Pi levels in the Pi-deprived pups was also significantly lower than that seen in the controls, indicating the profound affect of dietary Pi deprivation on developing animals. This was a significant observation, because the plasma levels in the LPD pups would still be considered very high (3.62 ± 0.05 vs. 4.09 ± 0.08 mM in NPD pups, $P < 0.05$); it highlights the concept that specific levels of plasma and CSF Pi are necessary for normal growth and development. Interfering with these levels, even subtly, may have profound effects on neonatal development.

Whether this plasma Pi level also has an effect on Pi-seeking behavior in the pups is unknown.

Of anecdotal interest, one of the Pi-deprived lactating rats ate three of her pups the night before the end of the experiment. Whether this was an attempt to gain Pi is unknown, although the bones and tissues of the pups provided a tremendous source of Pi. Consequently, this mother's plasma and CSF Pi level were exceedingly high and her PiH_2O water intake decreased to control levels. The rapid increase the CSF Pi and quenching of the Pi appetite are further links between the brain, CSF, and appetitive behavior. Also, the cannibalistic behavior may indicate an instinctual method to compensate for Pi-deprivation in nature.

In conclusion, the Pi appetite is a behavioral response to seek a source of phosphate to compensate for dietary Pi deprivation, and is inducible in adult animals. Although lactating rats have a high demand for phosphate necessary to support their offspring, they did not demonstrate an enhanced Pi appetite compared with nonpregnant female rats. The normal growth of pups was severely affected by maternal dietary Pi deprivation, as illustrated by their lower birth weights, stunted growth, and reduced plasma Pi levels. Overall, appetitive behaviors are integral to the maintenance of the *milieu interieur*, and it is evident that although the Pi appetite is not as pronounced as the drive to ingest sodium, it does serve to compensate for an imbalance in the supply and demand for Pi in both young and adult animals. One of the most intriguing aspects of both the behavioral and functional adaptations (appetite and increase in renal Pi transporters) to Pi deprivation is that it appears to be dependent on central (CSF) Pi concentrations. Considering there is great focus on discovering ways to slow cellular aging (which includes a reduction in high energy phosphates), understanding the central mechanisms controlling Pi homeostasis and adaptations may be an important avenue for future work.

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