PROCEEDING

Control of phosphate appetite in young rats

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Abstract : In the present study, we investigated whether a diet deficient in inorganic phosphate (Pi) stimulates an ingestive behavior to seek sources of Pi. Male Wistar rats were placed in individual cages with unrestricted access to tap water and a low (LPD, 0.02% Pi) or normal (NPD, 0.6% Pi) Pi diet for 6 days. On day 7, LDP rats were given unlimited access to a solution of 25 mM potassium phosphate water (Pi-water) for 9 additional days. Rats fed LPD consumed 70-100% more Pi-water then those fed NPD. The increase in Pi-water intake resulted in a marked rise in the growth rate of rats fed LPD during day 9. A similar intake of Pi was induced after only 2 days of LPD and was associated with significant reductions in both plasma and cerebrospinal fluid (CSF) levels of Pi ; these levels remained low throughout Pi restriction, despite a significant intake of Pi-water. Replenishment with a high-Pi diet rapidly quenched the appetite for Pi-water and was associated with restoration of both plasma and CSF Pi levels. These findings suggest that an appetite for Pi can be induced in rats, perhaps through lowered plasma and CSF Pi levels. J. Med. Invest. 54 : 366-369, August, 2007

Keywords **:** *dietary phosphate, growth, and cerebrospinal fluid*

INTRODUCTION

Dietary phosphate restriction is one of the means of controlling phosphatemia in dialysis patients. A patient subjected to limited dietary phosphate appears to seek food containing this mineral (1). It is unknown, however, whether dietary Pi restriction has a direct effect on appetite. Omission of a single essential component from the diet of animals affects two prominent physiological processes : body growth and food intake (2). These effects have been shown for diets deficient in zinc, magnesium, potassium, and single essential amino acids such as threonine (2).

Inorganic phosphate (Pi) homeostasis is crucial to bone mineralization and muscle growth ; Pi is a constituent of membrane phospholipids and is used in high-energy organic compounds such as ATP, ADP, and AMP (3). A deficiency in this mineral can cause sever bone abnormalities, stunted growth, and many other metabolic and physiological disorders (3). Although mineral deficiencies (e.g., potassium, iron, and calcium) result in sodium ingestion, Pi deficiency does not (2). This suggests that the motivation to obtain Pi in the rat may be specific to Pi and may in fact be an intrinsic response (1-4). Recently, several groups demonstrated that dietary Pi has a direct effect on appetite (1-5). In the present study, we investigated whether an appetite for Pi can be induced in young rats through lowered plasma and CSF levels of Pi.

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MATERIALS AND METHODS

All experiments were performed using male Wistar rats ranging from 150 to 153 g at the onset of testing. Animals were housed under a 12 : 12-h light-dark cycle. Dependent on the experiment, rats were given access to a 25 mM solution of KH_2PO_4 (Pi-water) at physiological pH that was slightly sour to human taste (2). A control solution of 25 mM Ca-water was also prepared at physiological pH and had a bitter taste to humans. To collect cerebrospinal fluid (CSF), 23-guage stainless steel guide cannulas were implanted stereotaxically into the third ventricle in rats fed either low (LPD ; 0.02% Pi) or normal (0.6% Pi ; NPD) Pi diet for 3 days.

RESULTS

Rats fed LPD demonstrated a significant decrease in growth rate during the first 6 days of the experiment as compared with animals fed NPD (Fig. 1a). From days 0 to 6, the average daily gain in body weight in NPD rats was two times higher than that in rats fed LPD. On provision of the Pi-water to both NPD and LPD rats after day 6, the LPD rats showed an increase in daily body weight gain for the remaining 9 days that matched that observed in controls

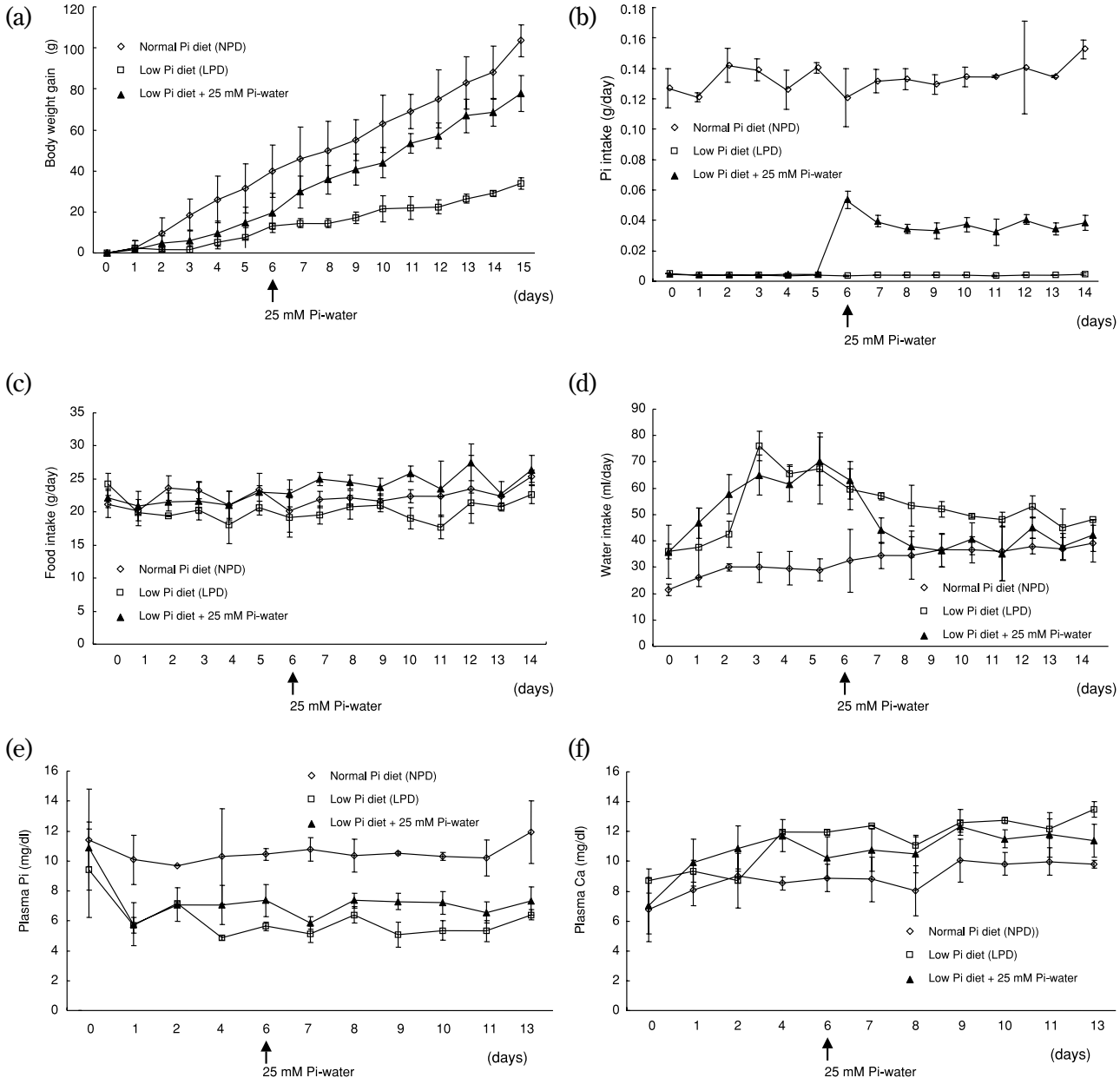

Fig. 1 Effects of phosphate supplement on growth (a), food (b) and water (c) intake, and plasma Pi (d) and calcium (e) levels of rats raised on a low-Pi diet, normal Pi diet or a low-Pi diet plus Pi-water. On day 6, the drinking water was changed to Pi-water for all groups. Values are means with their standard errors represented by vertical bars.

(Fig. 1b). The slope for days 7-15 reflects the increase in growth rate of rats fed LPD plus Pi-water (Fig. 1a). Ingestion of Pi-water was significantly greater in the rats fed LPD as compared with the NPD controls over days 7-15 (Fig. 1b). 'The Pi taken up by water ingestion was less than one-half of the amount of Pi ingested by the animals eating NPD (Fig. 1b). When Pi-water was provided on day 7, overall water consumption increased in the LPD-fed rats (Fig. 1c).

Because the appetite for Pi was evident early (after 2 days of Pi deprivation), we assessed the time course of changes in plasma Pi concentration (Fig. 1e). The plasma Pi and calcium levels after Pi restriction are shown in Fig. 1e and f, respectively. By day 1 of Pi restriction, the plasma Pi levels were significantly decreased from control levels (Fig. 1e). This reduction in plasma Pi did not change after Pi-water ingestion (Fig. 1d). By contrast, plasma calcium levels were unchanged throughout the experimental periods (Fig. 1f). Furthermore, CSF Pi levels in rats fed LPD for 3 days were significantly reduced as compared with controls (Fig. 2).

Ingestion of Pi-water was stimulated in rats fed LPD after only 2 days of dietary Pi deprivation. At this time, the plasma and CSF Pi levels remained significantly reduced despite 7 days of ingestion of Pi-water (Fig. 2). The ingestion of Pi-water was associated with a significant increase in growth rate in the rats fed LPD as compared with their growth rate over the 2 days of LPD only (Fig. 2). The increase in growth rate in rats fed LPD and ingesting Pi-water was not due to alterations in food intake. Replenishment of Pi in the diet did not change food intake in the animals initially fed LPD (Fig. 2) ; however, there was a further, significant increase in body weight gain. Finally, rats fed NPD and LPD for 2 days exhibited a preferential intake of Pi-water when given a choice between equimolar Pi-water and Ca-water (data not shown).

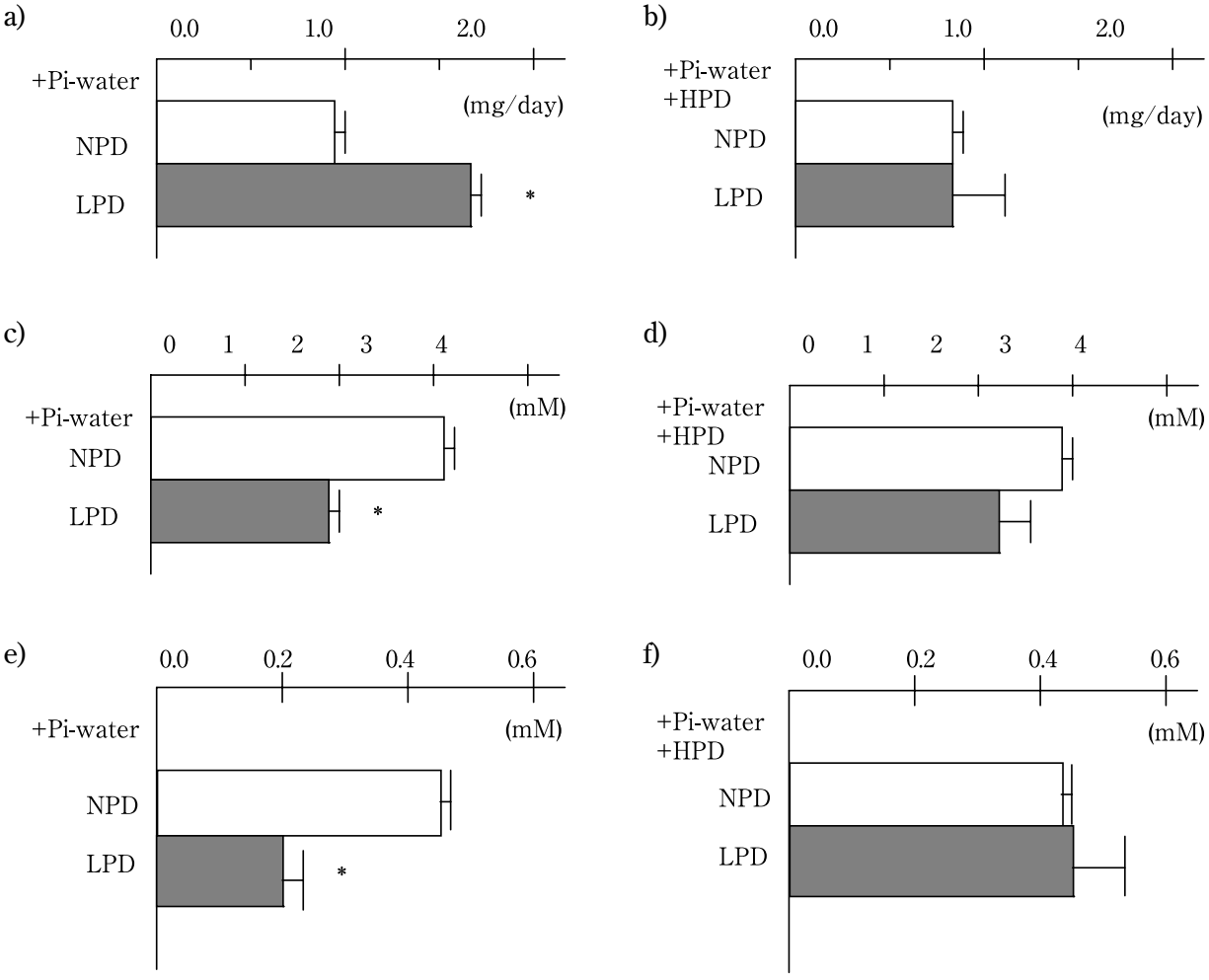

Fig. 2 Plasma Pi and CSF Pi levels in rats fed NPD or LPD
Plasma Pi (c and d) and CSF Pi (e and f) levels in rats fed NPD or LPD after 5 days of 25 mM Pi-water ingestion (c, e) or 2 days of Pi-water and further replenishment with HPD (d, f). ($P < 0.05$ vs.NPD). (a, b) Pi-water intake (mg/day), (c, d) Plasma Pi levels (mM), (d, f) CSF Pi levels (mM)

DISCUSSION

The present study indicates that when young rats are deprived of phosphate in their diet, plasma and CSF Pi levels decrease rapidly and the consumption of Pi-water is significantly increased. Interestingly, the ingestive response did not differ between acute (2-day) and longer (9-day) Pi restriction. The Pi deficiency induced in this study resulted in the ingestion of Pi-water in preference to a solution of calcium. Moreover, the reduction in plasma and CSF Pi levels may be part of the mechanism by which the behavioral response is elicited, because restoration of plasma and CSF Pi levels was associated with quenching of the Pi appetite. Thus, stimulation of an appetite for Pi may provide a behavioral mechanism, in addition to the intrinsic increase in renal Pi transport, by which a young animal can increase Pi supply during periods of phosphate depletion (5). Our findings support the work of Sweeny, *et al* (2), and Landsman, *et al* (3, 4), who showed that Pi depletion can elicit a rapid behavioral response in young animals. The present study provides a potentially intriguing aspect to this mineral appetite. We do not know whether the phosphate-seeking behavior observed in dialysis patients with Pi restriction is linked to Pi appetite. Further studies are needed to clarify the molecular targets involved in the induction of Pi appetite.

REFERENCES

1. Logman-Adham M : Adaptation to changes in dietary phosphorus intake in health and in renal failure. J Lab Clin Med 129 : 176-188, 1997
2. Sweeny J, Seibert HE, Woda C, Schulkin J, Haramati, Mulroney SE : Evidence for induction of a phosphate appetite in juvenile rats. Am J Physiol 275 : R1358-R1365, 1998
3. Landsman A, Lichtstein D, Bacaner M, Ilani A : Dietary phosphate-dependent growth is not mediated by changes in plasma phosphate concentration. Br J Nutr 86 : 217-223, 2001
4. Landsman A, Lichtstein D, Ilani A : Distinctive features of dietary phosphate supply : J Appl Physiol 99 : 1214-1219, 2005
5. Mulroney SE, Woda CB, Halaihel N, Louie B, McDonnell K, Schulkin J, Haramati A, Levi M : Central control of renal sodium-phosphate (NaPi-2) transporters. Am J Physiol Renal Physiol 286 : F647-F652, 2004