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Characterization of Dietary Phosphorus-Dependent Duodenal Calcium Uptake in Vitamin D-Deficient Chicks

C. Tony Liang, Janice Barnes, Bertram Sacktor*, Richard A. Balakir
National Institute on Aging, National Institutes of Health, Gerontology Research Center, Baltimore, Maryland 21224

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Abstract. The effect of dietary phosphorus on intestinal calcium uptake was examined in duodenal cells isolated from vitamin D-deficient chicks. Cells from chicks on a high phosphorus diet accumulated calcium at a rate 38% higher than cells from animals on a normal phosphorus diet. Diet high in calcium did not affect calcium absorption in duodenal cells. The dietary phosphorus effect on calcium absorption was specific. Uptake of α-methyl glucoside was not altered. Increase in calcium absorption by a high phosphorus diet was not due to a change in cellular energy metabolism nor to the content of phosphorus in cells. Kinetically, a high phosphorus diet increased the V_{max} of calcium uptake; the affinity for calcium was unaffected. The effectiveness of dietary phosphorus to enhance the intestinal calcium uptake could also be demonstrated in brush border membrane vesicles. The increase in calcium uptake was not due to an alteration in membrane binding capacity nor to calcium efflux from vesicles. To test the hypothesis that a high phosphorus diet may affect membrane transport by altering phospholipid metabolism in duodenal cells, we examined the phospholipid content in isolated brush border membranes. The content of phosphatidylcholine, phosphatidylserine, phosphatidyinositol and phosphatidylethanolamine was not altered by the high phosphorus diet. These findings suggest that the vitamin D-independent and dietary phosphorus-dependent effect on intestinal calcium absorption was primarily due to a change in the calcium flux at the luminal side of the cells. However, the precise mechanism is still not clear.

Key words: Intestinal calcium uptake — Dietary phosphorus and calcium — Intestinal brush-border calcium uptake — Vitamin D-deficient chicks

Introduction

Studies in both humans and animals have shown that diets low in calcium (Ca) or phosphorus (P) could increase intestinal Ca absorption and this effect could be attributed to the enhanced production of $1,25(OH)_2D_3$ [1, 6, 7, 15–18]. However, some evidence also suggested a dietary P effect on intestinal Ca absorption independent of $1,25(OH)_2D_3$ metabolism. Analysis of Ca balance in vitamin D-deficient rats showed a net negative Ca absorption for animals on a low P diet, whereas a net positive absorption was observed for animals on a normal P diet [4]. Similar results on intestinal Ca^{2+} uptake by everted sacs were obtained in vitamin D-deficient rats fed a diet containing low or normal P [4]. Moreover, experiments with cultured intestinal explants prepared from vitamin D-deficient chicks showed that the concentration of phosphate in culture medium could affect Ca^{2+} uptake in a manner similar to the dietary P [3]. Since intact tissue was used in these studies, the site of this vitamin D-independent and dietary P-dependent effect at the cellular level was not vigorously examined.

We have established the isolated chick duodenal cell as a viable model to study the regulatory mechanism on intestinal Ca^{2+} absorption [12]. In this paper, we used this model to examine the effect of dietary P and Ca on Ca^{2+} uptake in duodenal cells isolated from vitamin D-deficient chicks. Using isolated brush border membrane from duodenum, we were able to identify the

* Deceased

possible site of action by dietary P at the membrane level.

Materials and Methods

Animals

One-day-old white leghorn cockerels were kept in a darkened room with yellow lights for 19–24 days and fed a vitamin D-deficient diet (Teklab diet TD 75007), containing 0.37% Ca and 0.37% P. The vitamin D-deficient animal was characterized by low serum Ca and P, high mortality rate, abnormal breast bone and undetectable intestinal CaBP level. The chicks were then randomly divided into two groups. One group was kept on the same diet (control diet). The other group was fed a vitamin D-deficient high P diet (0.37% Ca and 0.75% P). Some of the chicks were fed a vitamin D-deficient high Ca diet (1.2% Ca and 0.37% P). The P content in our control diet is similar to the normal P diet used in the study by Brautbar et al. [4]. This experimental dietary regimen was maintained for one week.

Isolation of Cells

Duodenal cells were isolated using a method modified from the published procedures [9]. The preparative procedure was carried out at room temperature. The isolation medium contained (mM) 240 mannitol, 3 K_2HPO_4, 1 $MgCl_2$, 5 NaCl, 0.5 β-hydroxybutyrate, 2.5 glutamine, 10 mannose, 1 mg/ml bovine serum albumin, and 20 HEPES, adjusted to pH 7.4 with KOH. Chick duodenum was collected and the luminal contents were flushed out with 5 ml of isolation medium. The intestine was slit lengthwise and washed twice with 20 volumes of isolation medium. The duodenum was then cut into small segments about 2 cm in length and transferred to a 50 ml polyethylene beaker containing 20 ml of medium. Hyaluronidase (1 mg/ml) was added and the beaker was placed in a water bath at 37°C for 30 min with vigorous shaking (130 oscillations/min, 2 cm path length). Duodenal cells were then detached by gentle agitation of the tissue segments with a glass rod. The cell suspension was filtered through 2 layers of cheesecloth and the cells in the filtrate collected by centrifugation at 120 × g for 2 min. The cells were washed three times by resuspension of the pellet in 15 ml of isolation medium with a plastic pipette followed by recentrifugation. The washed cells were finally resuspended at a concentration of 1 to 2 mg of cell protein/ml of isolation medium. Microscopic examination of the preparations showed a mixed population of single cells and some cell aggregates, a few of these containing as many as 20 cells.

Cellular Uptake of Calcium

Uptake of $^{45}Ca^{2+}$ was carried out by a centrifugation method. Briefly, a 100 μl aliquot of the cell suspension was equilibrated in a shaking water bath for 5 min at 37°C. Uptake was initiated with the addition of 100 μl of a solution containing (mM) 140 KCl, 10 HEPES, adjusted to pH 7.4 with KOH, and 2 $^{45}CaCl_2$(0.5 μCi). Except when noted, the uptake period was 5 min. The uptake was terminated by removing 180 μl of the reaction mixture and mixing it with 1 ml of ice-cold stopping solution consisting of (mM) 140 KCl, 10 HEPES, and 2 EGTA, (pH 7.5) in an Eppendorf tube. The cells were centrifuged at 4°C for 2 min, and the pellet washed with 1 ml of ice-cold stopping solution and recentrifuged. The washing step was repeated again. The loss of the intracellular ^{45}Ca in the centrifugation and washing was approximately 5%. The cell pellet was treated with 0.5 ml of 1 N NaOH for at least 30 min. The mostly solubilized cells were transferred to a scintillation vial and combined with 0.5 ml of water used to rinse the Eppendorf tube. Radioactivity was measured in a scintillation counter. Uptake of $^{45}Ca^{2+}$ was linear within the range of cell protein used (0.2 to 0.8 mg protein) for at least 5 min.

Cellular Uptake of α-Methyl Glucoside

Uptake of α-methyl glucoside was determined as described [10, 11]. Briefly, uptake was initiated by mixing 100 μl of cell suspension with 100 μl of medium comprised of (mM) 140 NaCl or 140 KCl, 10 HEPES-KOH buffer, pH 7.4 and 0.1 [^{14}C]-α-methyl glucoside (0.5 μCi). Uptake was terminated by removing 180 μl of the reaction mixture and adding the aliquot to an Eppendorf centrifuge tube containing 1 ml ice-cold stopping solution containing (mM) 124 NaCl, 10 Na_2HAsO_4, and 1 HEPES-KOH buffer, pH 7.2. The cells were pelleted, washed twice with stopping solution and solubilized with 1 N NaOH for radioactivity measurement.

Cellular Metabolism

The metabolism of the isolated cells was evaluated by measuring the production of $^{14}CO_2$ from [1-^{14}C] glucose. Production of $^{14}CO_2$ was determined as described previously [12].

Brush Border Membrane Vesicles

Duodenal brush border membrane vesicles were prepared by the procedure described by Shultz et al. [19]. Briefly, the crude brush borders were prepared by Ca^{2+} precipitation followed by dissociation of the microvillar membranes from the crude brush borders by homogenization in 0.5 M Tris/HCl, pH 7.2. The microvillar membranes were separated from the core fraction on a discontinuous 37–60% (wt/wt) glycerol gradient. The brush border membrane preparations, essentially all right-side out [19], were enriched 16- to 20-fold in alkaline phosphatase specific activity relative to the mucosal homogenate. No appreciable difference in the marker enzyme enrichment was observed for brush border membranes isolated from control chicks and animals on high P diet.

Uptakes in Brush Border Membrane Vesicles

To measure Ca^{2+} uptake by the brush border membrane vesicles, the vesicles were pre-equilibrated with a buffer solution containing 140 mM choline chloride and 10 mM HEPES, adjusted to pH 7.4. To initiate uptake, 25 μl of a reaction medium containing mM 140 KCl, 10 HEPES, pH 7.4, and 2 $^{45}CaCl_2$ (about 10 μCi/ml) was added to 25 μl of the membrane vesicle suspension, and the reaction incubated at 20°C in a shaking water bath. Uptake was

terminated by the addition of ice-cold stopping solution, consisting of (mM) 140 KCl, 2 EGTA, and 10 HEPES, adjusted to pH 7.4, and the suspension applied on a Millipore filter (DAWP 02500). The filter was rapidly washed twice with 3 ml of stopping solution. $^{45}Ca^{2+}$ activity retained on the filter was estimated by liquid scintillation spectrometry.

Uptake of [^{32}P]-phosphate and [^{3}H]-glucose was measured by a similar method except that 200 μM [^{32}P]-phosphate (0.1 μCi) and 100 μM [^{3}H]-glucose (0.2 μCi) were used individually to replace $^{45}CaCl_2$ in the reaction medium. For Na^+-dependent uptake, NaCl was used instead of KCl in the reaction medium. The stopping solution consisted of mM 124 NaCl, 10 Na_2HAsO_4 and 1 HEPES, pH 7.5.

Efflux of Vesicular $^{45}Ca^{2+}$

Brush border membrane vesicles were loaded with $^{45}Ca^{2+}$ in a medium containing (mM) 140 KCl, 10 HEPES-KOH pH 7.4 and 1 $^{45}CaCl_2$ for 20 min at 20°C. Efflux was initiated by diluting with 5 volumes of solution containing (mM) 140 KCl, 10 HEPES-KOH pH 7.4 and 2 EGTA at 20°C. Efflux of ^{45}Ca was terminated by rapid filtration on a Millipore filter (DAWP 02500) and washed twice with 3 ml ice-cold diluting solution. Amount of $^{45}Ca^{2+}$ retained was determined by liquid scintillation spectrometry.

Phospholipid Determination

To 0.5 ml of brush border membrane suspension, 4 ml of chloroform-methanol mixture (1 : 1) was added. The extraction was carried out for 30 min with intermittent vortexing. An aliquot of 1 ml of H_2O was then added and the tubes were vortexed 30 sec. To separate the phases, the tubes were centrifuged in a clinical centrifuge for 10 min. Aliquot of the chloroform layer was removed and dried under nitrogen. Phospholipids were separated by thin layer chromatography using Silica Gel-60 plate and a solvent system of chloroform-methanol-glacial acetic acid-water (42 : 38 : 4 : 3.8). Phospholipids were visualized with I_2 and identified with the known standard. Gel containing phospholipid was removed and treated with 9 N H_2SO_4 and 0.5 ml of isopropanol at 120° for 4 hr for complete hydrolysis. Phosphate content was measured by the method of Bartlett [2].

Analytic Methods

Serum and cellular Ca was determined by atomic absorption. Serum and cellular P was measured spectrophotometrically [5] after deproteination with 5% TCA. Protein was determined by the Lowry method [13] using bovine serum albumin as the reference protein.

Materials

Hyaluronidase (bovine testes, type 1S, 300 U/mg) was purchased from Sigma. Fatty acid free bovine serum albumin was obtained from Miles Laboratory. [^{3}H]-glucose, [^{32}P]-phosphate, [^{14}C]-α-methyl glucoside, and $^{45}CaCl_2$ were from New England Nuclear.

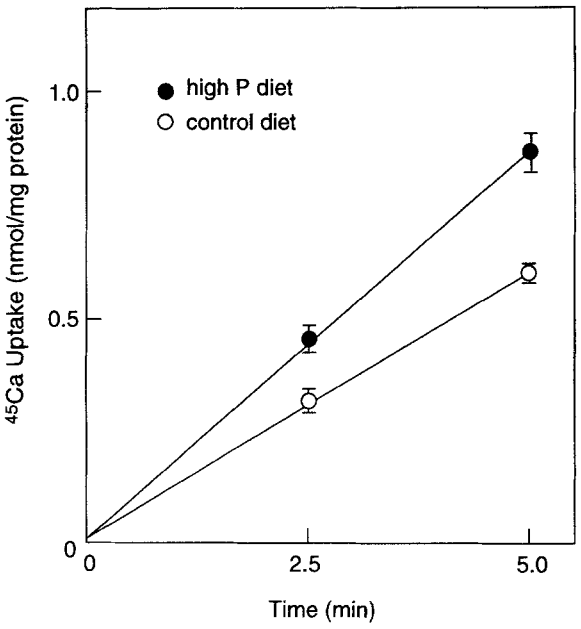

Fig. 1. Uptake of ^{45}Ca by duodenal cells isolated from animals on control and high P diet. Values represent means ± SE of four experiments.

Results

Effect of Dietary P and Ca on Cellular $^{45}Ca^{2+}$ Uptake

Cellular uptake of $^{45}Ca^{2+}$ was compared in cells isolated from chicks on control and high P diet. As shown in Fig. 1, $^{45}Ca^{2+}$ uptake at 2.5 and 5 min were consistently higher for chicks on high P diet. At 5 min, uptake of $^{45}Ca^{+}$ was 0.60 ± 0.002 and 0.83 ± 0.09 nmol/mg cell protein, respectively for cells isolated from chicks on control and high P diet ($P < 0.01$). Effect of high Ca diet on intestinal $^{45}Ca^{2+}$ uptake was also examined. Uptake activity at 5 min was 0.59 ± 0.05 and 0.55 ± 0.06 nmol/mg protein, respectively, for cells prepared from animals on control and high Ca diet.

Effect of Dietary P on Serum and Cellular Contents of Ca and P

Serum concentrations of Ca and P in chicks fed control and high P diets were shown in Table 1. Serum Ca decreased slightly in chicks fed with the high P diet. By comparison, serum Ca increases slightly in chicks fed the high Ca diet (6.6 ± 0.4 and 8.4 ± 0.4 mg/dl, respectively, for chicks on control and high P diet, $P < 0.01$), whereas serum P is not affected (3.6 ± 0.5 and 3.3 ± 0.4 mg/dl, respectively, for chicks

Table 1. Effect of dietary P on serum and cellular contents of Ca and P

Diet	Serum		Cellular content	
	Ca	P	Ca	P
	mg/dl		nmol/cell protein	
Control	7.0 ± 0.3	3.9 ± 0.4	9.5 ± 0.5	18.0 ± 0.6
High P	6.2 ± 0.3[a]	6.5 ± 0.4[b]	10.1 ± 0.6	17.6 ± 1.0

Values represent the mean ± SE of 19 experiments for serum Ca, P and 4 experiments for cellular Ca, P, respectively.
[a] $P < 0.05$ *vs.* control.
[b] $P < 0.001$ *vs.* control.

Table 2. Effect of high P diet on α-methyl glucoside uptake in duodenal cells

Diet	α-Methyl glucoside uptake nmol/mg protein · 2 min	
	Na^+	K^+
Control	0.078 ± 0.010	0.024 ± 0.002
High P	0.080 ± 0.013	0.027 ± 0.002

Values represent the mean ± SE of eight experiments. The uptake was initiated by mixing 100 μl of cell suspension with 100 μl of the reaction solution. After mixing, the extracellular medium contained either 70 mM Na^+ and 70 mM K^+ or 140 mM K^+. The final concentration of α-methyl glucoside was 50 μM.

on control and high Ca diet). Cellular contents of Ca and P in the isolated duodenal cells were also determined. No significant difference between chicks on control and high P diet was observed.

Effect of High P Diet on α-Methyl Glucoside Uptake

To test whether this dietary P effect on Ca^{2+} uptake was specific, uptake of α-methyl glucoside was also measured (Table 2). Uptake of this glucose analogue was stimulated threefold by a Na^+ gradient in chick duodenal cells. However, Na^+-dependent α-methyl glucoside uptake (Na^+) was not affected by the high P diet. No apparent difference was found in Na^+-independent (K^+) α-methyl glucoside uptake in control and experimental animals.

Effect of High P Diet on Metabolism

Since uptake of $^{45}Ca^{2+}$ was energy dependent [8], it is conceivable that a high P diet may change the energy production in cells and result in a change of $^{45}Ca^{2+}$ uptake. To monitor the energy metabolism, the oxidation of 1-[^{14}C]-glucose was measured. Production of [^{14}C]-CO_2 was 29.9 ± 2.7 and 29.7 ± 4.0 nmol/mg cell protein for cells from chicks on control and high P diet.

Kinetics of $^{45}Ca^{2+}$ Uptake

The kinetics of $^{45}Ca^{2+}$ uptake in duodenal cells from chicks on control and high P diet are shown in Fig. 2. Uptake of $^{45}Ca^{2+}$ increased as the concentration of Ca^{2+} increased. Rate of uptake followed the Michaelis-Menten equation. An apparent K_m of 0.32 mM Ca^{2+} was observed for cells isolated from animals on the control diet and was not significantly affected by the high P diet. However, the apparent V_{max} was increased from 0.67 to 0.87 nmol/mg cell protein · 5 min by high P diet.

Effect of Dietary P on $^{45}Ca^{2+}$ Uptake in Isolated Duodenal Brush Border Membrane Vesicles

Uptake of $^{45}Ca^{2+}$ into isolated duodenal brush border membrane vesicles was followed for 60 min. At 1 and 5 min, Ca uptakes into vesicles prepared from chicks on the high P diet were significantly higher than in the animals on the control diet (Fig. 3). Uptake of ^{45}Ca at 1 min was increased 44% by the high P diet (1.25 ± 0.09 and 0.87 ± 0.07 nmol/mg protein for vesicles prepared from chicks on high P and control diet, respectively). After 10 min, $^{45}Ca^{2+}$ uptake leveled off and, at 60 min, uptake activities were not different between the two dietary groups.

Effect of Dietary P on Uptake of Glucose and Phosphate in Duodenal Brush Border Membrane Vesicles

Uptakes of glucose and phosphate were measured at 15 sec and 60 min with an inward Na^+ or K^+ gradient and were shown in Table 3. At 15 sec, glucose uptake was stimulated by the Na^+ gradient approximately fivefold. However, Na^+-dependent (Na^+) and independent (K^+) glucose uptake activities were not affected by the high P diet. Phosphate uptake was also enhanced by Na^+ and this uptake activity was not altered by dietary P. At 60 min, glucose uptake activities with and without the Na^+ gradient were not different. Unlike phosphate uptake, Na^+-stimulated glucose uptake was higher at 15 sec as compared to uptake at 60 min. Furthermore, vesicle size was estimated from the glucose uptake value at 60 min and was not altered by the high P diet.

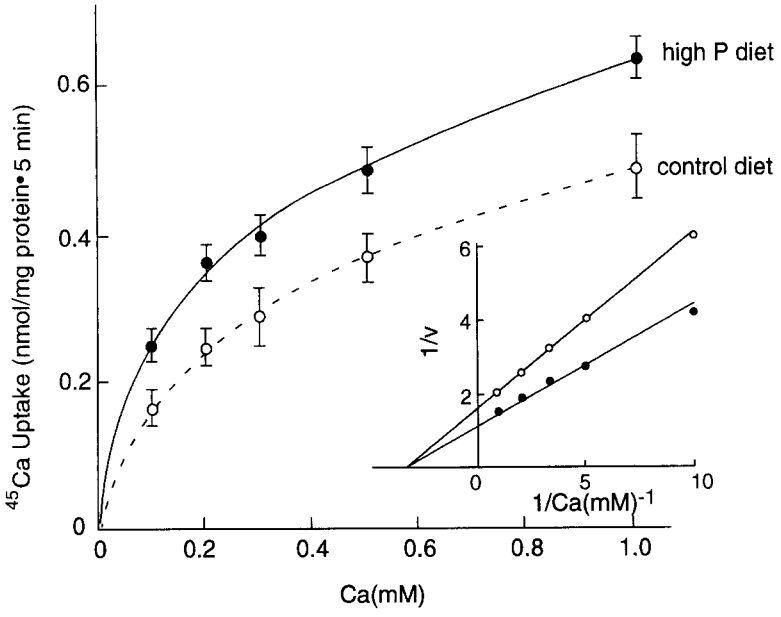

Fig. 2. Effect of high P diet on kinetics of ^{45}Ca uptake into duodenal cells. The indicated Ca^{2+} concentration, from 0.1 to 1.0 mM, was the final concentration in the uptake medium. Inset is a Lineweaver-Burk plot of the data. Values represent the mean ± SE of seven experiments.

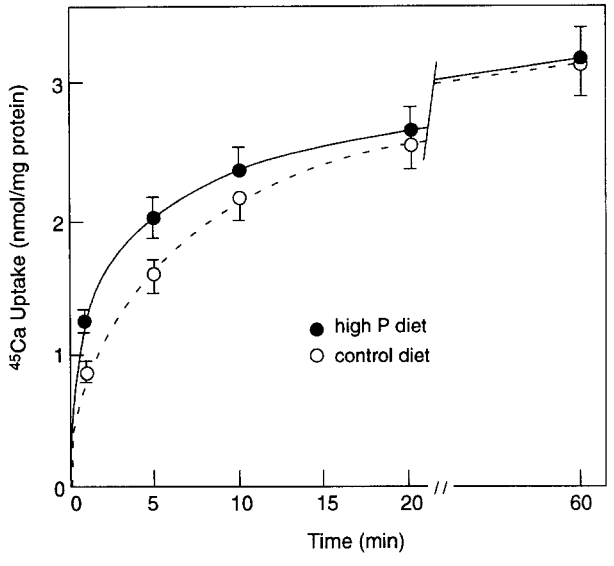

Fig. 3. Ca^{2+} uptake into duodenal brush border membrane vesicles from animals on control and high P diet. Values represent the mean ± SE of six experiments.

Efflux of $^{45}Ca^{2+}$ from Brush Border Membrane Vesicles

Efflux of ^{45}Ca was initiated by diluting the membrane vesicles with EGTA solution and the result was shown in Fig. 4. Initial rate of the efflux was rapid, followed by a slow rate after 1 min. After 5 min, intravesicular $^{45}Ca^{2+}$ was approximately half of the initial Ca content. However, the efflux rate was comparable for both groups.

Effect of Osmolarity on Uptake of $^{45}Ca^{2+}$ into Brush Border Membrane Vesicles

To resolve the question as to whether this dietary P effect on $^{45}Ca^{2+}$ uptake by brush border membrane vesicles was due to an increase of influx or to enhanced binding capacity to $^{45}Ca^{+}$, the effect of osmolarity in the incubation medium was studied. The intravesicular space was reduced by increasing the medium osmolarity from 0.3 to 0.8 mosm. As shown in Fig. 5, uptakes of $^{45}Ca^{2+}$ were found to be inversely proportional to the vesicle size which suggested $^{45}Ca^{2+}$ was indeed taken into the osmolarity-sensitive intravesicular space. When extrapolated to infinite osmolarity, an indicator of volume-independent binding, no difference was found between vesicles prepared from chicks on control and high P diet. Therefore, the high P diet induced an increase of Ca^{2+} influx across the luminal membrane of the duodenal cell, not a change in Ca^{2+} binding capacity.

Effect of Dietary P on Composition of Phospholipids in Brush Border Membrane

Change in phospholipid composition in brush border membrane by vitamin D was postulated as the possible mechanism that stimulated duodenal Ca^{2+} uptake [14]. Phospholipid composition of brush border membrane prepared from chicks on control and high P diet was determined. As shown in Table 4, no significant difference in content of phosphatidylcholine, phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine was found in brush bor-

Table 3. Uptake of glucose and phosphate in duodenum brush border membrane vesicles

Diet	Substrate	Uptake			
		nmol/mg protein			
		15 sec		60 min	
		Na^+	K^+	Na^+	K^+
Control	Glucose	0.061 ± 0.007	0.014 ± 0.002	0.038 ± 0.005	0.036 ± 0.006
$N = 6$	Phosphate	0.180 ± 0.020	0.066 ± 0.011	0.285 ± 0.023	0.229 ± 0.031
High P	Glucose	0.052 ± 0.009	0.015 ± 0.005	0.030 ± 0.004	0.033 ± 0.003
$N = 5$	Phosphate	0.179 ± 0.023	0.064 ± 0.013	0.273 ± 0.040	0.235 ± 0.040

Values represent the mean ± SE of 5–6 experiments. The uptake was initiated by mixing 25 μl of membrane vesicles with 25 μl of the reaction solution containing 200 μM and 100 μM of phosphate and glucose, respectively. After mixing, the extravesicular medium contained 70 mM NaCl and 70 mM KCl (Na^+) or 140 mM KCl (K^+). The final concentration of glucose and phosphate was 50 and 100 μM, respectively.

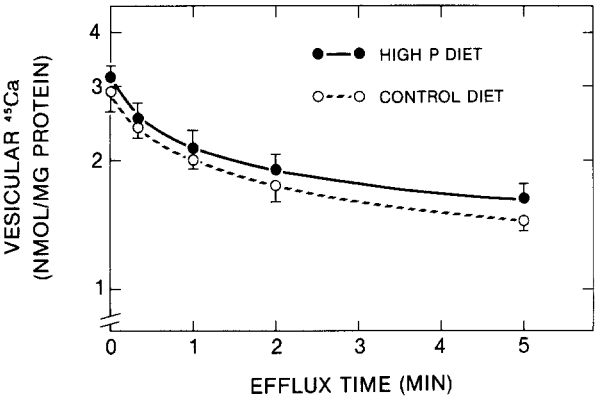

Fig. 4. Effect of dietary P on Ca^{2+} efflux from brush border membrane vesicles. Membrane vesicles were pre-loaded with $^{45}CaCl_2$ for 20 min. Efflux was initiated by dilution with EGTA. Values represent mean ± SE of four experiments.

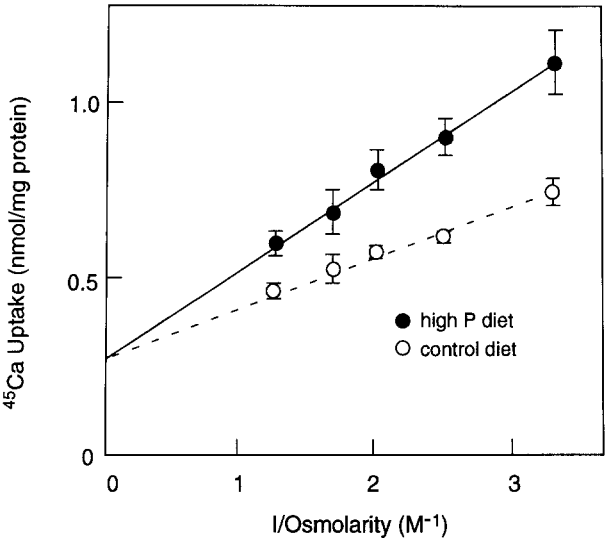

Fig. 5. Effect of osmolarity on Ca^+ uptake in duodenal brush border membrane vesicles from control chicks and animals on high P diet. Values represent mean ± SE of four experiments.

der membrane from chicks on control and high P diet.

Discussion

The present results demonstrate that Ca^{2+} uptake by duodenal cells could be regulated by dietary P independent of its effect on vitamin D metabolism. Cellular uptake of Ca^{2+} was increased approximately 35% in animals on high P diet as compared to the control diet. Our results are consistent with previous findings that high P content in diet [4] could elevate intestinal Ca^{2+} absorption in vitamin D-deficient animals. It is also apparent that this dietary effect was limited to the P content in diet. High Ca diet did not affect the intestinal Ca^{2+} uptake. Kinetically, the apparent K_m was not affected by dietary P. However, the apparent V_{max}, the maximum uptake capacity was elevated by high P diet. The effect of high P diet on intestinal Ca^{2+} absorption was specific. Uptake of α-methyl glucoside was not affected by this dietary manipulation. To examine the possible cause which may lead to this dietary effect, we carried out a series of experiments. Despite the increase in serum P when the animals were kept on a high P diet, the cellular contents of P which can affect the Ca^{2+} influx was not altered by the high P diet. It should be noted that intracellular Ca and P are compartmentalized in the cell. Cytosolic P and free Ca are probably better parameters for

Table 4. Effect of dietary P on phospholipid composition in isolated duodenal brush border membrane

Diet	Phospholipid content (μg phosphorus/mg protein)			
	Phosphatidylcholine	Phosphatidylserine	Phosphatidylinositol	Phosphatidylethanolamine
Control	1.58 ± 0.18	0.99 ± 0.06	0.37 ± 0.05	3.62 ± 0.23
High P	1.51 ± 0.14	0.93 ± 0.06	0.35 ± 0.08	3.57 ± 0.14

Values represent the mean ± SE of five experiments.

correlation. Since intestinal Ca^{2+} uptake is highly energy dependent [8] we examined whether energy metabolism was different in cells isolated from chicks on high P diet. Our findings that 1-[^{14}C]-glucose oxidation was not different between two groups suggested that alteration in metabolic pathway was not the cause of the dietary effect. Our results contradict the theory postulated by Brautbar et al. [4] that change in cellular P and subsequent effect on energy production was the cause of this dietary effect.

Experiments on uptake of Ca^{2+} in isolated brush border membrane vesicles showed a comparable effect of dietary P on Ca^{2+} uptake activity. One possible explanation for this increase in Ca^{2+} uptake in brush border membrane vesicles was that the efflux of Ca^{2+} was reduced. Analysis of ^{45}Ca efflux from vesicles preloaded with $^{45}Ca^{2+}$ showed that this was not the case. By examining the effect of osmolarity on Ca^{2+} uptake in membrane vesicles, we concluded that the increase in Ca^{2+} uptake was due to an increase in Ca^{2+} influx at the brush border. Binding capacity to Ca^{2+} was unaffected by the high P diet. The high P dietary effect on Ca^{2+} uptake in intestinal brush border membrane vesicles was also specific. It seems that the general transport activity is not affected by the dietary treatment.

Change in phospholipid metabolism was postulated as the possible mechanism for action of vitamin D on intestinal Ca^{2+} uptake [14]. Elevated serum P observed in chicks on a high P diet might alter phospholipid metabolism in duodenal cells. We have determined the content of phosphatidylcholine, phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine in duodenal brush border membrane prepared from chicks on control and high P diet and found no significant difference between the two groups.

The significance of this dietary P-dependent regulation on intestinal Ca absorption should not be overlooked. In a previous publication, we showed that repletion of chicks on D-deficient normal P diet with 1,25-$(OH)_2D_3$ increases Ca uptake into duodenal brush border membrane vesicles 73% [ref. 12, Fig. 11]. In the present study, change of the regimen from a D-deficient diet with normal P to a D-deficient diet with high P increases Ca uptake in duodenal brush border 44%. Thus, the influence of a high P diet on intestinal Ca absorption with respect to the entry of Ca at the brush border membrane is significant. However, it should be noted that vitamin D also regulates the efflux of Ca at the basolateral membrane, synthesis of calcium binding protein and Ca flux into intracellular organelles [20] which may affect intestinal Ca absorption in a broader manner.

References

1. Armbrecht, H.J., Gross, C.J., Zerser, T.V. 1981. Effect of dietary calcium and phosphorus restriction on calcium and phosphorus balance in young and old rats. *Arch. Biochem. Biophys.* **210:**179–185
2. Bartlett, G.R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:**466–468
3. Birge, S.J., Miller, R. 1977. The role of phosphate in the action of vitamin D on the intestine. *J. Clin. Invest.* **60:**980–988
4. Brautbar, N., Levine, B.S., Walling, M.W., Coburn, J.W. 1981. Intestinal absorption of calcium: role of dietary phosphate and vitamin D. *Am. J. Physiol.* **241:**649–653
5. Chen, P.S., Jr., Toribara, T.Y., Warner, H. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28:**1756–1758
6. Gray, R.W., Napoli, J.L. 1983. Dietary phosphate deprivation increase 1,25-dihydroxyvitamin D_3 synthesis in rat kidney *in vitro*. *J. Biol. Chem.* **258:**1152–1155
7. Hughes, M.R., Brumbaugh, P.F., Hausssler, M.R., Wergedal, J., Baylink, D.J. 1975. Regulation of serum 1α,25-dihydroxyvitamin D_3 by calcium and phosphate in the rat. *Science* **190:**578–580
8. Kendrick, N.C., Kabakoff, B., DeLuca, H.F. 1981. Oxygen-dependent 1,25-dihydroxycholecalciferal-induced calcium ion transport in rat intestine. *Biochem. J.* **194:**178–186
9. Kimmich, G.A. 1970. Preparation and properties of mucosal epithelial cells isolated from small intestine of the chicken. *Biochemistry* **9:**3659–3668
10. Liang, C.T., Barnes, J., Cheng, L., Balakir, R., Sacktor, B. 1982. Effect of 1,25-$(OH)_2D_3$ administered *in vivo* on phosphate uptake by isolated chick renal cells. *Am. J. Physiol.* **242:**C312–C318
11. Liang, C.T., Barnes, J., Balakir, R., Cheng, L., Sacktor, B. 1982. *In vitro* stimulation of phosphate uptake in isolated chick renal cells by 1,25-dihydroxycholecalciferol. *Proc. Natl. Acad. Sci. USA* **79:** 3532–3536

12. Liang, C.T., Barnes, J., Balakir, R.A., Sacktor, B. 1986. Characterization of 1,25-dihydroxyvitamin D_3-dependent calcium uptake in isolated chick duodenal cells. *J. Membrane Biol.* **90:**145–156
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265–275
14. Matsumoto, T., Fontaine, O., Rasmussen, H. 1981. Effect of 1,25-dihydroxyvitamin D_3 on phospholipid metabolism in chick duodenal mucosal cell. *J. Biol. Chem.* **256:**3354–3360
15. Morrissey, R.L., Wasserman, R.H. 1971. Calcium absorption and calcium-binding protein in chick on differing calcium and phosphorus intakes. *J. Biol. Chem.* **220:**1509–1515
16. Pansu, D., Bellaton, C., Bronner, F. 1981. Effect of Ca intake on saturable and nonsaturable components of duodenal Ca transport. *Am. J. Physiol.* **240:** 632–637
17. Rader, J.I., Baylink, D.J., Hughes, M.R., Safilian, E.F., Haussler, M.R. 1979. Calcium and phosphorus deficiency in rats: effects on PTH and 1,25-dihydroxyvitamin D_3. *Am. J. Physiol.* **236:**E118–E122
18. Riborich, M.L., DeLuca, H.F. 1978. Effect of dietary calcium and phosphate on intestinal calcium absorption and vitamin D. metabolism. *Arch. Biochem. Biophys.* **188:**145–156
19. Schultz, T.D., Bollman, S., Kumar, R. 1982. Decreased intestinal calcium absorption *in vivo* and normal brush border membrane vesicle calcium uptake in cortisol-treated chickens: evidence for dissociation of calcium absorption from brush border vesicle uptake. *Proc. Natl. Acad. Sci. USA* **79:**3542–3546
20. Wasserman, R.H., Fullmer, C.S. 1983. Calcium transport protein, calcium absorption, and vitamin D. *Annu. Rev. Physiol.* **45:**375–390