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ORIGINAL PAPER

Influence of dietary calcium and phosphorus supply on epithelial phosphate transport in preruminant goats

Korinna Huber · Uta Roesler · Antje Holthausen · Ernst Pfeffer · Gerhard Breves

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Abstract P homeostasis affected by high or low Ca and/or P supply in preruminant goats was characterized by balance studies in vivo. The main excretion pathway was the renal P_i excretion whose extent was modulated by variations in dietary P and/or Ca supply. Faecal P excretion remained low irrespective of dietary regimen. The balance data were combined with respective in vitro data on P_i transport properties and their adaptation in response to changes in dietary Ca and/or P intake. Therefore, P_i transport capacities were determined by P_i uptake into brush border membrane vesicles of jejunum and kidney. Epithelial P_i transporters were determined semiquantitatively by northern and western blot analyses in jejunum, kidney and salivary gland. Renal P_i transport was downregulated by doubling dietary P supply while doubling both, Ca and P as well as restrictive Ca at unchanged P led to slight, but not significant reductions in renal P_i transport. Jejunal P_i transport was reduced by P excess (doubling P and doubling both, Ca and P), but only NaPi IIb protein expression was significantly diminished. In conclusion, the significance of epithelial adaptation to dietary Ca and P supply for P homeostasis is discussed in preruminant goats.

Keywords Preruminant goats · Renal and jejunal P_i transport · NaPi II · Ca and P supply

Abbreviations

BBMV	Brush border membrane vesicles
ELISA	Enzyme-linked immunosorbent assay
K_m	Half-maximal saturation value
MR	Milk replacer
NaPi II	Sodium phosphate cotransporter type II
PTH	Parathyroid hormone
SDS	Sodium dodecyl sufate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SLC34	Solute carrier family 34
SSC	Sodium chloride sodium citrate
V_{max}	Maximal transport rate

Introduction

In contrast to monogastric animals, phosphorus (P) homeostasis in ruminant goats is characterized by almost complete phosphate (P_i) reabsorption in the kidney at physiological plasma P_i concentrations. When renal P_i threshold is exceeded by increased plasma P_i concentrations, renal P_i excretion also increases. Homeostatic responses to variations in dietary P supply are mainly mediated by modulation of intestinal P_i absorption and with it, faecal P_i excretion. Additionally, recycling of P_i via saliva enables a high regulatory faecal P_i excretion. Thereby, plasma P_i level is maintained below the renal P_i threshold preventing renal P_i wasting

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K. Huber (✉) · U. Roesler · G. Breves
Department of Physiology, School of Veterinary Medicine Hannover, Bischofsholer Damm 15/102, 30173 Hannover, Germany
e-mail: korinna.huber@tiho-hannover.de

E. Pfeffer
Institute of Animal Nutrition, Rheinische Friedrich-Wilhelms University Bonn, 53115 Bonn, Germany

A. Holthausen
Fa. Lohmann, Animal Health GmbH, 27472 Cuxhaven, Germany

under physiological conditions. Homeostatic responses to dietary variations of P and Ca supply are based on epithelial P_i transport capacities. Secondary active, Na^+-dependent P_i transport processes were identified across the apical membranes of epithelial cells in jejunum and renal proximal tubuli (Schröder and Breves 1996; Schröder et al. 2000). Structurally, in goat jejunum and kidney cortex P_i transporters were characterized which belong to the NaPi type II transporter family as described by Murer et al. (2000), caprine renal NaPi to subtype IIa (Schröder et al. 2000) and caprine intestinal NaPi to subtype IIb (Huber et al. 2002). These transporters have recently been renamed as members of the solute carrier family SLC34, NaPi IIa as SLC34A1 and NaPi IIb as SLC34A2 (Murer et al. 2003). P_i transport in ovine and caprine salivary glands is also based on a Na^+-dependent mechanism (Shirazy-Beechey et al. 1996) and structurally, a NaPi type II transporter was identified (Huber et al. 2003).

While P homeostasis in ruminant goats is well characterized, data on P homeostasis in preruminant goats are only scarce. "Preruminant" means that the metabolizable energy for the animal is not provided by digestion of nutrients in the rumen but only by digestion in the intestines. Since the forestomachs are not yet developed, preruminant goats are also denominated as "functionally monogastric" animals. In this situation the kidneys are regarded as the major regulatory organs, while faecal P_i excretion is low. Salivary P_i secretion is also low due to the lack of stimulation of saliva flow rate by roughage intake. However, the ability to concentrate P_i in saliva is existent from the third week of life (Huber et al. 2003).

With regard to P homeostasis, efficiency of intestinal P_i absorption and capacity of renal P_i reabsorption are also important for the capability of preruminant kids to adapt their P homeostasis similarly as in ruminant goats. Therefore, it was the aim of the present study to use milk-fed kids for investigating the molecular mechanisms of epithelial P_i transport of organs, which are actively involved later on in the endogenous P_i recycling in weaned ruminants. NaPi cotransporter type II expression was examined in jejunum, kidney and salivary gland; additionally, P_i transport capacities in jejunum and kidney were studied in preruminant goats.

Materials and methods

Animals and feeding

Thirty-six intact male kids of the Saanen type breed "Weiße Deutsche Edelziege" were used for the experiment. Kids were kept individually in boxes with straw bedding. During the experiment balance trials were performed in the same animals (Boeser 2004). General care, handling and maintenance of kids followed the procedure approved by the Animal Welfare Commissioner of the University of Bonn in accordance with the German Animal Welfare Law.

The liquid diets differed in their respective concentrations of Ca and P which were those found in original goat milk (o) or doubled (d) in the first experiment and (o) or reduced (r) in the second experiment. In the first experiment (high P/Ca exp.) dietary concentrations of Ca and/or P were doubled by addition of calcium lactate and/or sodium dihydrogenphosphate, which was fed during the experiment. Four to five kids were allotted to each of the treatments: control group (oCa/oP-1), Ca supplemented group (dCa/oP), P supplemented group (oCa/dP) and Ca and P supplemented group (dCa/dP). In the second experiment (low P/Ca exp.) with 17 animals, each group of 3–5 kids, concentrations of P and/or Ca were reduced to about two-third of the respective concentration in goat milk by diluting goat milk with 30% of a self-made, low P_i and Ca milk replacer (MR). MR was composed of butter, lactose, albumen, lecithin (10%) and water (in g/kg: 40, 40, 40, 20, 860). To elevate the levels of P and Ca the mixture of milk/MR of the control group (oCa/oP-2), the Ca restrictive group (rCa/oP) and the P restrictive group (oCa/rP) were supplemented with calcium lactate and/or sodium dihydrogenphosphate, respectively. Calculated contents of dry matter (DM), crude protein (XP), crude fat (XL), Ca and P in goat milk and MR and composition of the diets used in the high and low P/Ca experiments are given in Table 1.

Milk was fed to satiation twice daily and consumption was registered. Milk and MR supplemented with salts were easily accepted by the kids during the whole experimental period. The experiment was finished as soon as the kids had reached 15 kg body weight by slaughtering the animals; experimental period took 35–58 days in high P/Ca experiment and 40–66 days in low P/Ca experiment. Kids were observed to ingest some straw of the bedding which could not be quantified.

Slaughtering and tissue sampling

Kids were stunned using a captive bolt pistol and bled by severing the carotid arteries. Segments of jejunum, kidney and Gl. parotis were obtained immediately after slaughtering. After rinsing with ice-cold saline (0.9% NaCl) isolated jejunal mucosa

Table 1 Calculated contents of dry matter (DM), crude protein (XP), crude fat (XL), Ca and P in goat milk and milk replacer and composition of the diets used in the high and low P/Ca experiments

			Goat milk (g/kg)				Milk replacer (g/kg)	
DM			115				111	
XP			31				29	
XL			32				31	
Ca[a]			1.2				0.9	
P[a]			0.9				0.6	
Diets	dCa/dP	oCa/dP	dCa/oP	oCa/oP1	oCa/oP2	rCa/oP	oCa/rP	rCa/rP
Milk	1,000				700			
Milk replacer	–				300			
Ca-lactate	×	–	×	–	×	–	×	–
Na-phosphate	×	×	–	–	×	×	–	–
Ca[b]	2.58	1.19	2.49	1.19	1.19	0.89	1.18	0.8
P[b]	1.80	1.54	0.84	0.83	0.89	0.90	0.64	0.64

[a] Calculated P and Ca contents in goat milk and milk replacer

[b] Measured P and Ca contents in daily freshly prepared diets

as well as sliced jejunal pieces of 1–2 m length were frozen in liquid nitrogen and stored at −80°C for structural and functional analyses. Kidney cortex was prepared for both, structural and functional analyses. The procedure of slaughtering and tissue sampling was done as described by Schröder and Breves (1996) in order to ensure the integrity and the viability of the tissues.

Structural and functional analyses of P_i transporters

Northern and Western blot analyses were performed as described in detail by Huber et al. (2002, 2003).

Northern blot analyses

Briefly, total RNA (kidney) or poly(A)$^+$RNA (jejunum, salivary gland) was fractionated in 1.0% formamide/agarose gels and was transferred by capillary blotting onto nitrocellulose membranes. Prehybridisation/hybridisation was performed in a solution containing 5× SSC, 5× Denhardt´s solution, 0.1% SDS, and 40% formamide at 42°C. For blocking unspecific bindings sites of membranes denatured herring sperm DNA (20 μg/cm^2) was added. Radioactive labelled NaPi IIb (small intestines), IIa (kidneys), II (salivary glands) and β-actin specific probes were created by using a random prime labelling system (AmershamPharmaciaBiotech, rediprime™II, Buckinghamshire, UK). Hybridisation was performed at 42°C overnight. The membranes were washed 3× each time for 10 min with 2× SSC/0.1% SDS at room temperature, with 0.5× SSC/0.1% SDS at 37°C and finally, with 0.2× SSC/0.1% SDS at 42°C. The membranes were analysed after exposure to a phosphor imager screen for 2–4 h with a phosphor imager system (Fa. BioRad, Germany). Relative amounts of specific mRNA were quantified by reference to β-actin as internal standard using the quantitation software Quantity One (BioRad, Munich, Germany). To compare the relative differences between the feeding groups RNA or poly(A)$^+$RNA of three animals of each group were evaluated on each blot.

Western blot analyses

Intestinal brush border membranes isolated for uptake studies (preparation of BBMV see below) were fractionated by SDS-PAGE in an 8.4% discontinuous polyacrylamide gel according to Laemmli (1970). Separated proteins were transferred onto nitrocellulose membranes by tank blotting (blotting time 2 h). After blocking with 2% fat free milk solution overnight membranes were incubated for 2 h with a goat-specific anti NaPi type IIb antibody (1:2,000). The anti-NaPi IIb antibody was kindly provided by Prof. Dr. J. Biber and Prof. Dr. H. Murer, Institute of Physiology, University of Zürich-Irchel, Zürich, Switzerland. This antibody was directed against the murine NaPi IIb but expressed a high cross-homology to the caprine intestinal NaPi IIb (Huber et al. 2002).

Immunolocalisation of the primary antibody was performed using an anti rabbit secondary antibody (1:1,500) coupled with HRP. Bands were detected by enhanced chemiluminiscence system according to manufacturer´s protocol (Pierce, Rockford, USA). To quantify protein expression levels β-actin specific

immunodetection was performed on the same blots. Bands were analysed quantitatively using Quantity One software.

Preparation of brush border membrane vesicles (BBMV) and P_i transport measurements

BBMV were prepared from jejunal and renal proximal tubule epithelia by a modified Mg^{2+}-EGTA precipitation method. P_i uptake into BBMV was quantified by using the rapid filtration technique as described by Huber et al. (2002, 2003). Glucose uptakes were performed to test the quality of the vesicles and to determine the functional identity of basal membrane properties in all feeding groups.

Determination of P_i, Ca and calcitriol concentrations in plasma

Plasma P_i was determined colorimetrically using the vanadate–molybdate method (Kruse-Jarres 1979) and plasma Ca by the standard *o*-cresolphthaleine complex method (Sarker and Chaunan 1967). Plasma calcitriol concentrations were measured by a commercial ELISA (Serotec, Bensheim, Germany).

Statistics

Values are given as mean ± SEM; *N* numbers of animals are given in the respective figures or tables. Blotting results were depicted as relative amounts (specific band value/β-actin value). Details are given in the respective legend. All immunodetection and hybridisation experiments were performed at least twice resulting in consistent expression patterns. Significance of differences between the recorded parameters of the feeding groups was tested by one-way ANOVA and by Tukey's *t* test as a posttest (software Graphpad prism 3.0, San Diego, USA; http://www.graphpad.com). *P* values of <0.05 were set to be significant.

Results

Plasma Ca and P_i concentrations at the end of the experimental time

Plasma Ca and P_i concentrations are given in Table 2. In the high Ca/P experiment, only doubling P supply (oCa/dP) resulted in significant increases in plasma P_i concentrations without affecting plasma Ca concentrations compared to oCa/oP-1 group. Restriction of P supply resulted in significant decreases of plasma P_i concentrations in both, oCa/rP and rCa/rP groups, without any effect on plasma Ca with the exception of rCa/rP group which expressed significantly higher plasma Ca concentrations.

Plasma calcitriol concentrations during the experimental time in the high Ca/P experiment

Calcitriol levels were measured at the tenth day and at the end of the experiment from three animals/group. At the beginning of the high Ca/P supply only high Ca supply induced significant decreases of calcitriol concentrations irrespective of P supply; these differences, however, could not be confirmed at the end of the experiment (Table 3).

Table 3 Plasma calcitriol concentrations in kids at the beginning (tenth day after start of experimental feeding) and at the end of the high Ca/P experiment

Group	Beginning (pmol/ml)	End (pmol/ml)
oCa/oP-1	159.7 ± 23.9^{a}	116.8 ± 29.4
oCa/dP	204.0 ± 27.5^{a}	119.7 ± 17.2
dCa/oP	14.1 ± 5.5^{b}	66.8 ± 19.6
dCa/dP	48.7 ± 8.0^{b}	42.1 ± 13.8

Values are given as mean ± SEM, $N = 3$ animals/group. One-Way ANOVA resulted in $P < 0.0001$ for calcitriol. Means not sharing the same letter are significantly different within the column according to Tukey's test; a,b = at least $P < 0.01$, significance of difference between oCa/oP-1 and dCa/dP $P < 0.05$

Table 2 Phosphate (P_i) and calcium (Ca) concentrations (mmol/l) in plasma of kids at the end of the experiment reflecting the phosphorus and calcium status of the animals

Group	dCa/dP	oCa/dP	dCa/oP	oCa/oP-1	oCa/oP-2	rCa/oP	oCa/rP	rCa/rP
P_i	3.2 ± 0.1^{a}	4.6 ± 0.4^{b}	3.0 ± 0.3^{a}	3.0 ± 0.1^{a}	3.2 ± 0.05^{c}	$3.0 \pm 0.1^{c,z}$	2.6 ± 0.1^{d}	$2.7 \pm 0.1^{d,z}$
Ca	2.7 ± 0.1^{a}	$2.4 \pm 0.1^{a,x}$	$2.9 \pm 0.1^{a,y}$	2.6 ± 0.1^{a}	3.0 ± 0.04^{c}	2.9 ± 0.1^{c}	3.0 ± 0.1^{c}	3.5 ± 0.1^{d}

Values are given as mean ± SEM, $N = 4$–5/group. One-Way ANOVA resulted in $P < 0.01$ for P and Ca. Both experiments were analysed independently from each other. Means not sharing the same letter are significantly different according to Tukey's test: high P/Ca experiment (left side) a,b = $P < 0.01$ within the row in relation the oCa/oP-1, statistical difference between oCa/dP and dCa/oP x,y = $P < 0.01$; low P/Ca experiment (right side) c,d = at least $P < 0.05$ within the row in relation to oCa/oP-2, rCa/oP and rCa/rP not significantly different (z,z)

Structural and functional expression of P_i transport

Glucose uptake into renal and jejunal BBMV was demonstrated in all preparations indicating intact membrane vesicles. The uniform level of glucose uptake in all feeding groups (data not shown) suggested that basal membrane properties were not affected by the Ca and/or P supply. The influence of Ca and/or P supply in excess or in restriction on parameters of epithelial P_i transport is shown in Figs. 1 and 2 and Table 4, respectively.

Intestinal P_i transport

Maximal jejunal P_i transport rates (V_{max}) tended to be lower in the groups with an absolute P excess (oCa/dP; dCa/dP; Fig. 1a, Table 4). While expression of NaPi IIb mRNA was not influenced significantly by doubling P supply (Fig. 1b), expression of NaPi IIb protein tended to be reduced in these groups irrespective of the dietary Ca supply (Fig. 1c). This could not be confirmed statistically. In restrictive fed groups no differences occurred in the intestinal P_i transport properties (Fig. 2a–c, Table 4). The transporter P_i affinity given as K_m value was about 0.05 mmol/l with the exception of the control group and the oCa/rP group of the experiment with restrictive P supply. In these groups, K_m values were higher at about 0.3 mmol/l (Table 4).

Renal P_i transport

Renal P_i transport was significantly affected by doubling P (oCa/dP) supply (Fig. 1d). V_{max} of Na^+-dependent P_i

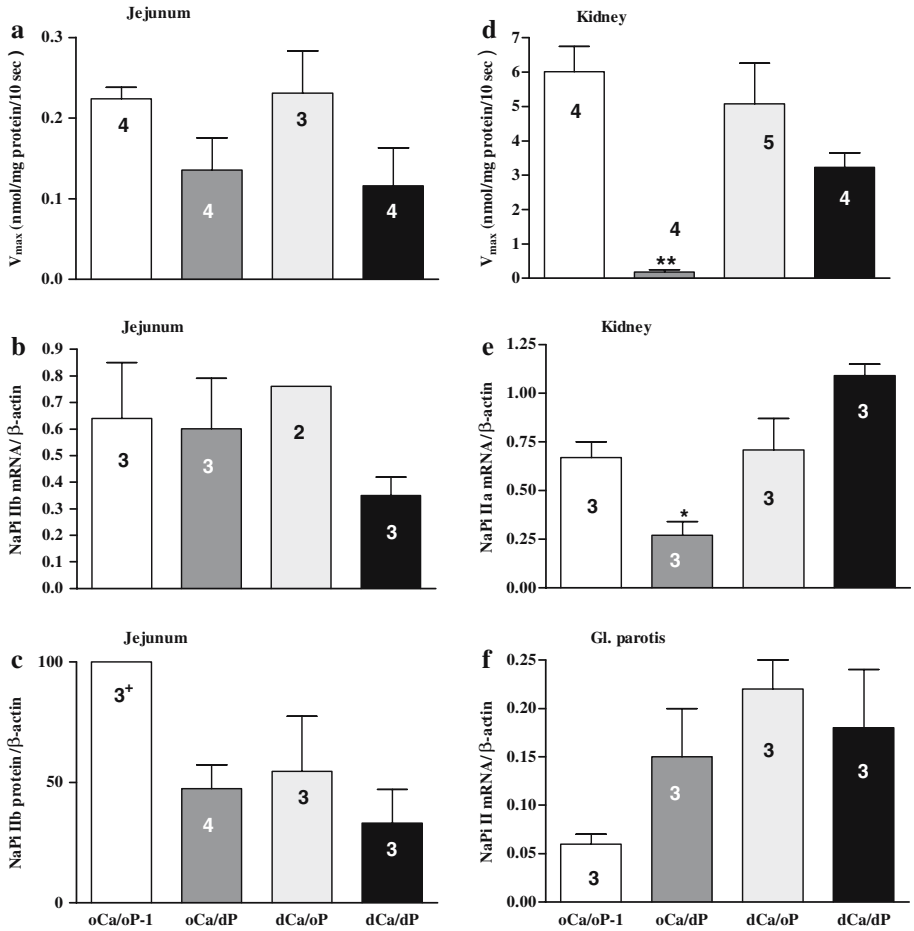

Fig. 1 Epithelial P_i transport in preruminant goats determined by in vitro studies: Effects of doubling dietary Ca and/or P supply on Na^+/P_i transport capacity and NaPi II expression in jejunum, kidneys and salivary glands in preruminant goats. Transport capacities were given in nmol/mg protein/10 s (detailed V_{max} and respective K_m values are summarized in Table 4); specific protein and mRNA levels are given as the ratio between NaPi II and β-actin. Values are given as mean ± SEM, $N = 4$–5/group for functional studies, $N = 2$–4/group for gene expression studies (respective N were given in the *bars*). Blotting experiments were done at least twice. Level of significance is $^*P < 0.05$, $^{**}P < 0.01$. *Left side* P_i transport capacity (**a**), NaPi IIb mRNA (**b**) and protein (**c**) expression in the jejunum. NaPi IIb protein expression was related to the protein amounts of oCa/oP-1 group (+) as 100% to compare different blots. *Right side* P_i transport capacity (**d**) and NaPi IIa mRNA (**e**) expression in the kidney and NaPi II mRNA expression in the Gl. parotis (**f**)

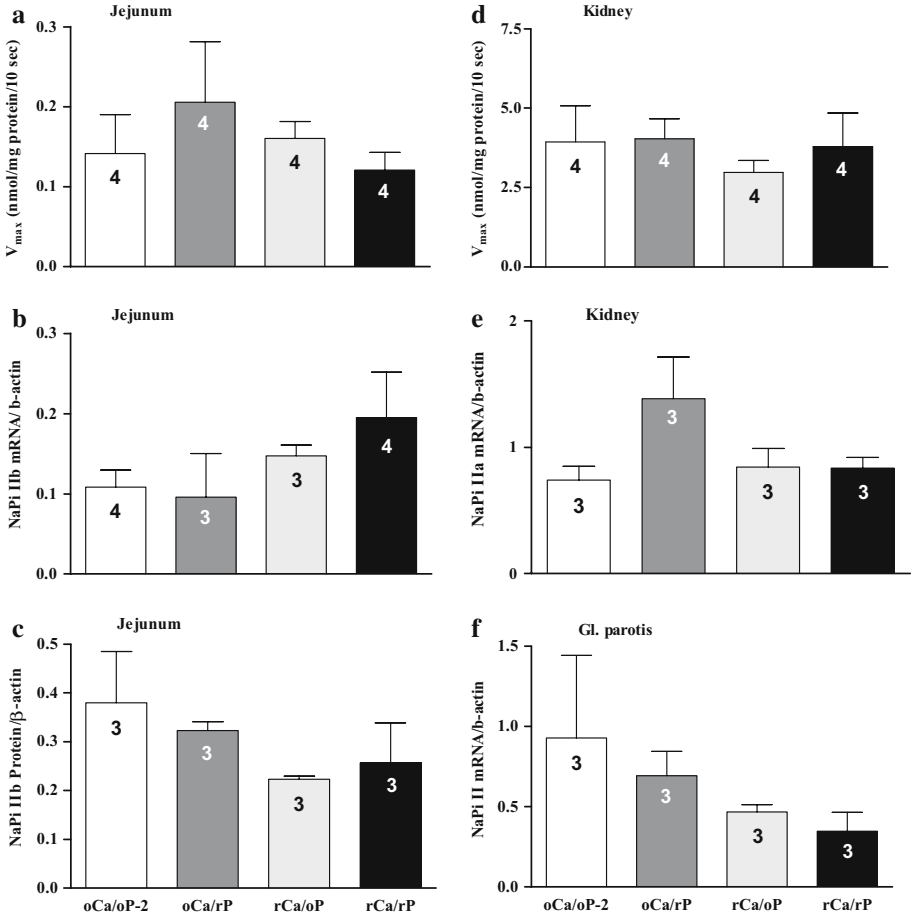

Fig. 2 Epithelial P_i transport in preruminant goats determined by in vitro studies: Effects of restriction of dietary Ca and/or P supply on Na^+/P_i transport capacity and NaPi II expression in jejunum, kidneys and salivary glands in preruminant goats. Transport capacities were given in nmol/mg protein/10 s (detailed V_{max} and respective K_m values are summarized in Table 4); specific protein and mRNA levels are given as the ratio between NaPi II and β-actin. Values are given as mean ± SEM, $N = 4$/group for functional studies, $N = 3$–4/group for gene expression studies (respective N were given in the bars). Blotting experiments were done at least twice. *Left side* P_i transport capacity (**a**), NaPi IIb mRNA (**b**) and protein (**c**) expression in the jejunum. *Right side* P_i transport capacity (**d**) and NaPi IIa mRNA (**e**) expression in the kidney and NaPi II mRNA expression in the Gl. parotis (**f**)

Table 4 Functional parameters of jejunal and renal Na^+-dependent P_i transport influenced by doubling or restriction of calcium and/or phosphorus supply in preruminant goats

	Jejunal Na^+/P_i transport		Renal Na^+/P_i transport	
	V_{max} (nmol/mg protein/10 s)	K_m (mmol/l)	V_{max} (nmol/mg protein/10 s)	K_m (mmol/l)
oCa/oP-1	0.22 ± 0.01^a	0.03 ± 0.008^b	6.02 ± 0.73^c	0.53 ± 0.09^e
oCa/dP	0.13 ± 0.06^a	0.03 ± 0.012^b	0.18 ± 0.07^d	0.08 ± 0.02^f
dCa/oP	0.23 ± 0.05^a	0.03 ± 0.010^b	5.08 ± 1.17^c	0.51 ± 0.07^e
dCa/dP	0.12 ± 0.05^a	0.04 ± 0.007^b	3.85 ± 0.38^c	0.67 ± 0.13^e
oCa/oP-2	0.14 ± 0.05^g	0.30 ± 0.210^h	3.94 ± 1.15^i	0.42 ± 0.08^k
OCa/rP	0.21 ± 0.08^g	0.33 ± 0.170^h	4.04 ± 0.62^i	0.42 ± 0.09^k
rCa/oP	0.16 ± 0.02^g	0.07 ± 0.010^h	2.97 ± 0.38^i	0.37 ± 0.05^k
rCa/rP	0.12 ± 0.02^g	0.06 ± 0.013^h	3.80 ± 1.05^i	0.49 ± 0.09^k

Values are given as mean ± SEM, $N = 3$–5/group. One-Way ANOVA resulted in $P < 0.01$ for V_{max} and K_m of renal Na^+ /P_i transport. Both experiments were analysed independently from each other. Means of columns sharing the same letter are not significantly different within the columns of high P/Ca experiment (upper part) and low P/Ca experiment (lower part) according to Tukey's test. c,d = $P < 0.01$, significance of difference between oCa/dP and dCa/dP $P < 0.05$; e,f = $P < 0.05$, significance of difference between oCa/dP and dCa/dP $P < 0.01$

transport was significantly reduced to 0.18 nmol P_i/mg protein/10 s versus 6.02 nmol P_i/mg protein/10 s (oCa/oP). K_m was also decreased from about 0.55 to 0.08 mmol/l indicating an increased P_i affinity. Expression of NaPi IIa mRNA in oCa/dP group was also significantly reduced (Fig. 1e). Doubling Ca or Ca and P supply did not affect the renal P_i transport parameters except for the increase of NaPi IIa mRNA in dCa/dP. Likewise, Ca and/or P restriction did not result in changes in P_i transport (Fig. 2d, e, Table 4).

P_i transport in the Gl. Parotis

Capacity to concentrate salivary P_i was existent, however, it was not influenced by Ca and/or P supplies (Huber, unpublished observations). NaPi II mRNA levels, however, tended to be higher in oCa/dP, dCa/oP and dCa/dP compared to oCa/oP-1 (Fig. 1f). Under restrictive Ca and P supply, NaPi II mRNA tended to decrease from oCa/oP-2 to oCa/rP, rCa/oP and rCa/rP (Fig. 2f).

Discussion

It was the aim of this study to describe adaptive changes in P homeostasis more precisely in the preruminant kid by combining in vitro and in vivo data of the same animals. For this experimental approach, however, it has to be considered that in vitro data can only reflect the status of the animals at the end of the experiments, while in vivo data describe changes during the whole experimental period.

Milk feeding was elongated until the kids reached 15 kg body mass to maintain the preruminant status and to adapt the animals to different Ca and/or P supply. The level of P_i uptake, accretion and excretion was obtained from balance trials (Table 5; Boeser 2004). Reducing the P supply (oCa/rP) or increasing the Ca supply (dCa/oP) resulted in an absolute and a relative P restriction, respectively. Likewise, increased P supply (oCa/dP) or decreased Ca supply (rCa/oP) produced an absolute and a relative P excess, respectively. Ca:P ratios of rCa/oP and oCa/dP were equally low (0.9, 0.8), while ratios of dCa/oP and oCa/rP are both higher (3.0, 1.9) than in the control groups (1.4, 1.3). Increasing or decreasing both Ca and P resulted in an absolute excess or restriction of P supply without changes in the Ca:P ratio. The proportions of changes in renal P_i excretion between the feeding regimes acted simultaneously irrespective of an absolute or relative P restriction or P excess and were independent of Ca:P ratio (Table 5). Changes in dietary Ca:P ratio might have an influence on the solubility of phosphate in the intestinal lumen. Calcium phosphate complexes could be generated by increased levels of either one or both of these minerals. Therefore, intestinal absorption should decrease and faecal excretion increase, while renal P_i excretion is diminished. Since excess of ingested P is completely excreted by the kidneys in these preruminant goats, solubility of Ca and P_i in the gut lumen was not influenced by changes in dietary Ca:P ratio.

To summarize the results determined in vivo, it was shown that dietary calcium (Ca) supply is the limiting factor for the phosphorus accretion in preruminant goats. Excessive P is exclusively excreted by the kidneys within this developmental stage in contrast to adult ruminants. These homeostatic differences between ruminant and preruminant goats are demonstrated schematically in Fig. 3.

Molecular and functional properties of epithelial P_i transport were characterized by molecular and cell biological methods in vitro:

Table 5 P intake, P accretion, P_i excretion with the urine and Ca:P ratio of Ca and P intake determined over the complete feeding period (Boeser 2004) and plasma P_i concentrations of preruminant goats

Group	P intake (g)	Plasma P_i (mmol/l)	P accretion (g)	P_i excretion with urine (g)	Ca:P ratio (Ca intake/P intake)
Groups with absolute Ca/P excess or Ca/P restriction					
dCa/dP	158	3.2	81	77	1.4
oCa/oP[a]	79	3.0	54	25	1.4
oCa/oP[b]	97	3.2	57	40	1.3
rCa/rP	76	2.7	55	22	1.3
Groups with relative P excess or P restriction					
rCa/oP	98	2.9	48	50	0.9
oCa/oP[b]	97	3.2	57	40	1.3
oCa/oP[a]	79	3.0	54	25	1.4
dCa/oP	75	2.1/3.0[c]	64	11	3.0
Groups with absolute P excess or P restriction					
oCa/dP	135	4.6	55	80	0.8
oCa/oP[a]	79	3.0	54	25	1.4
oCa/oP[b]	97	3.2	57	40	1.3
oCa/rP	65	2.6	53	12	1.9

[a] Values of control group of high Ca/P experiment

[b] Values of control group of restrictive Ca/P experiment

[c] Plasma P_i concentrations in dCa/oP show an time-dependent effect during feeding period; the initial one-third phase was characterized by a low plasma Pi concentration which increased to control levels at the last two-third of experimental period

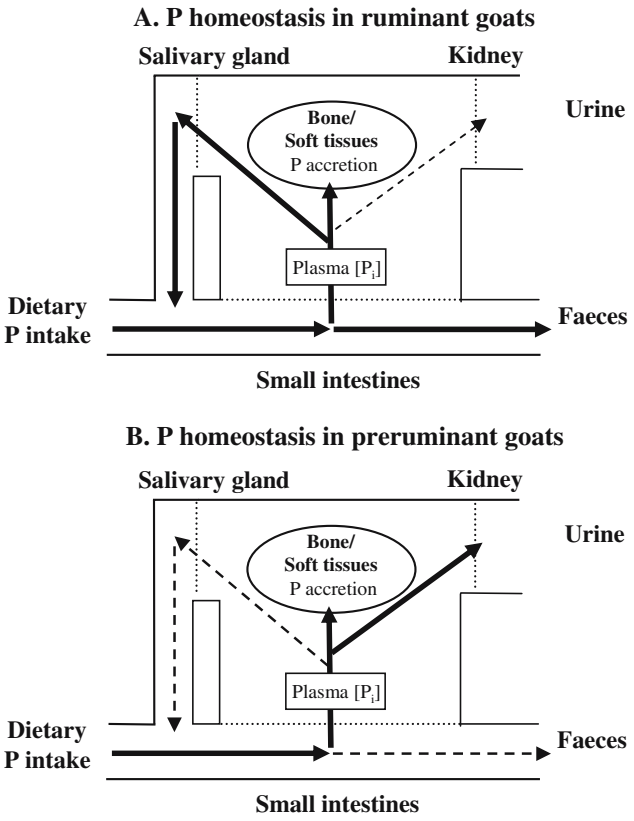

Fig. 3 P homeostasis is shown schematically in ruminant and preruminant goats. **a** Ruminant goats: P is excreted mainly by the faeces, while renal P_i excretion is physiologically low. By endogenous P_i recycling via concentrating it in the saliva and secreting it with the saliva into the rumen, high faecal excretion is supported. Renal P_i excretion increases only if plasma P_i threshold is exceeded due to reduced salivary secretion. Regulation of P homeostasis influenced by variations in dietary P supply occurs mainly by adaptation of intestinal P_i transport capacity, renal P_i transport capacity is constantly high within physiological plasma P_i levels. **b** Preruminant goats: P is excreted mainly be the urine, while faecal P excretion is low in milk-fed goats. Endogenous recycling of P_i is only slightly developed; the salivary glands concentrate P_i, but saliva secretion rate is low due to the lack of dietary mechanical stimuli. Increasing plasma P_i concentrations due to high dietary P intake and high intestinal absorption of P_i result in an increase in urinary P_i excretion. So, regulation of P homeostasis is based on renal P_i transport capacity at this developmental stage

Intestinal P_i transport

In ruminating goats faecal P excretion is the main excretory pathway (Fig. 3). Jejunal P_i absorption can modulate the extent of P_i excretion closely matched to the homeostatic P_i needs of the body. Jejunal Na^+/P_i transport is stimulated by a restrictive dietary P supply on protein and functional level (Huber et al. 2002). In preruminant goats stimulation by restrictive P and/or Ca supply failed whereas doubling P alone or doubling P and Ca tended to result in a decrease of Na^+/P_i transport capacity studied in vitro. Pooling results of all dP fed groups resulted in a significant decrease of Na^+/P_i transport capacity in dP (oP 0.23 ± 0.05 vs. dP 0.12 ± 0.10, $n = 7–8$, mean ± SEM, $P < 0.05$). Since jejunal P_i transport is mainly mediated by NaPi IIb in goats (Huber et al. 2002), NaPi IIb protein amounts tended to be lower in dP groups as a consequence.

Dietary Ca supply seemed to be irrelevant for adaptive molecular response of mid jejunum, but influenced calcitriol plasma levels (Table 3). Calcitriol is an important regulatory hormone for enhancement of Na^+/P_i transport (Cross and Peterlik 1982) and of NaPi IIb mRNA and protein expression regarding Ca and P homeostasis in monogastric animals (Xu et al. 2002; Hattenhauer et al. 1999). A vitamin D_3-responsive element on NaPi IIb gene was discussed (Xu et al. 2002). In mice, enhancement of Na^+/P_i transport and potentially, NaPi IIb expression in response to low dietary P supply was assumed to be based on increased plasma calcitriol levels due to low P intake. But recently, studies in VDR and renal 25-hydroxyvitamin D_3-1α-hydroxylase knock-out mice have shown that adaptive enhancement of Na^+/P_i transport and NaPi IIb expression due to low-P feeding did not depend on the vitamin D_3-VDR-axis (Capuano et al. 2005). Similarly, the findings regarding the adaptation to high P supply in jejunal P_i transport of goats indicated a vitamin D_3-independent effect on P_i absorption due to the different calcitriol levels found in oCa/dP and dCa/dP.

In contrast to these in vitro data from structural and functional studies, in vivo intestinal P_i absorption was not affected by the feeding regime as was shown in balance trials in the same animals (Boeser 2004). P_i excretion occurred via urine in all feeding groups and was adapted to P and Ca supply, respectively (Table 5). Therefore, it can be assumed that in preruminant goats P_i was completely absorbed in the intestine even though a small reduction occurred in the respective jejunal Na^+/P_i transport process in dP groups. Since balance studies show the overall P_i absorption along the intestinal tract, P_i transport mechanisms located in other regions of intestine than mid jejunum or time-dependent changes in intestinal P_i absorption capacity which occurred during the feeding period and were not recorded continuously might have been responsible for the complete absorption of ingested P_i in all feeding groups.

Importance of plasma Ca and P_i levels for P homeostasis

Ca was shown to be the limiting factor for P_i accretion (Boeser 2004). Therefore, plasma P_i levels were not affected in the dCa/dP group due to the high accretion

of P_i while in the oCa/dP group it was enhanced (Table 2). Salivary P_i secretion was still not established in these animals so that elimination of P_i via saliva from the plasma in all groups did not preponderate. In contrast, a 30 % reduction of P supply reduced plasma P_i level significantly, independently of Ca supply. But the extent of P_i reduction was not sufficient to stimulate either intestinal P_i absorption or renal P_i reabsorption as discussed in the following chapter. P accretion was unaffected in these animals.

Renal P_i transport

According to the results shown in Table 5 the kidneys were the main P_i excretory organ in milk-fed kids. A relative or an absolute excess of P resulted in an increase, a relative or an absolute P restriction in a decrease of renal P_i excretion. However, different physiological mechanisms were responsible for these changes in renal P_i excretion.

After a long-term high P supply (oCa/dP) renal P_i excretion was high due to the strong decrease in Na^+/P_i transport capacity. Since tubular P_i reabsorption is mediated by NaPi IIa the reduction in NaPi IIa mRNA was possibly based on a transcriptional regulation of renal P_i transport capacity. The characteristic K_m value for NaPi IIa was about 0.3 mmol P_i/l. The strong increase in transporter P_i affinity (expressed as K_m; from about 0.57 to 0.08 mmol P_i/l) indicated that the remaining P_i transport could no longer be mediated by NaPi IIa. A renal NaPi type II transporter with a comparable high P_i affinity (K_m 0.07 mmol/l) was described in rat kidneys characterized as a growth-related Na^+/P_i transporter only expressed at weaning (Segawa et al. 2002). Since goats approached the age of weaning at the end of the experiments and were also consuming straw from the litter an increased expression of this NaPi IIc (SLC34A3) might possibly have occurred. But NaPi IIa downregulation was the main adaptive change in the kidney due to a high P load.

The molecular signal of this downregulation is still unclear. However, it could be assumed that due to the small reduction in plasma Ca concentration PTH levels were elevated and could participate in the deactivation of NaPi IIa and renal Na^+/P_i transport. Confirming this assumption an increase in plasma PTH level was also observed in ruminating goats fed a high P diet (unpublished results). Plasma P_i concentrations were highest in the oCa/dP group (about 4.6 mmol/l) and P accretion was equal to oCa/oP-1 due to the relative lack of Ca. In all other groups plasma P_i concentrations did not exceed control levels. Since significant downregulation of renal Na^+/P_i transport appeared in this group only, the high plasma P_i level might have been the initial signal for the strong decrease in the renal P_i reabsorption capacity. In ruminating goats plasma P_i threshold for renal P_i excretion was about 4.3 mmol/l (Widiyono et al. 1998). V_{max} values of renal Na^+/P_i transport related to plasma P_i concentrations in a range from about 0.5 to 5.3 mmol/l of individual young goats resulted in equal high transport capacities up to plasma P_i values of about 3.5 mmol/l (Fig. 4). Beyond it, V_{max} was strongly low indicating that plasma threshold was reached in preruminant goats at lower plasma P_i concentrations than in ruminant goats.

Doubling both Ca and P resulted in a small, but not significant reduction of renal P_i transport despite the lack of a hyperphosphatemic stimulus, and P_i excretion was enhanced in comparison with the control group due to that. The high P accretion due to the higher availability of the limiting factor Ca might be responsible for maintaining control plasma P_i levels. NaPi IIa mRNA was enhanced, but this can not be explained so far.

A relative P excess and the lack of Ca in the rCa/oP group resulted likewise in a lower P accretion and a higher P_i excretion due to a slight, but not significant reduction of Na^+/P_i transport capacity.

An absolute restriction of P (oCa/rP) led to significantly decreased plasma P_i concentrations, whereas P

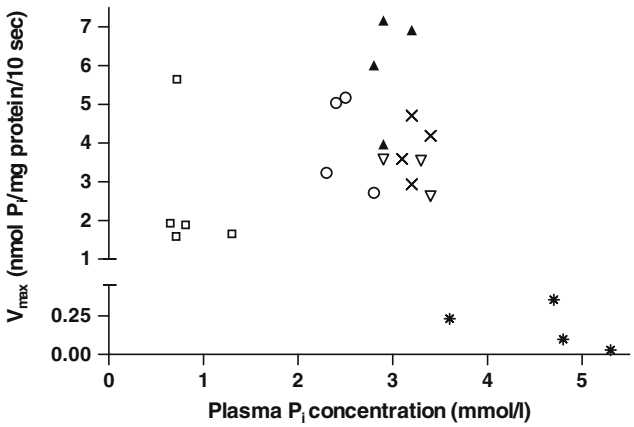

Fig. 4 V_{max} values of renal Na^+/P_i transport related to plasma P_i concentrations of individual goats. At physiological and low plasma P_i concentrations renal P_i transport remained constantly high indicating that P_i reabsorption capacity of kidneys is maximal within this range of plasma P_i concentrations. A strong decrease in V_{max} was observed in animals exhibiting plasma P_i levels above 3.5 mmol/l indicating that the plasma threshold for renal P_i excretion was exceeded in preruminant goats. *Asterisk* oCa/dP; *inverted triangle* dCa/oP; *multi symbol* dCa/dP; *triangle* oCa/oP; *circle* oCa/rP; *square* P depleted (ruminating). Data of animals were selectively chosen to cover a wide range of plasma P_i concentrations; therefore, also older P-depleted goats were included despite their ruminating status (Huber, unpublished data). Slightly lower V_{max} values of these animals were characteristic for ruminating goat at 4–5 month of age

accretion was as high as in oCa/oP-2. Renal P_i excretion was diminished without an effect on renal Na^+/P_i transport properties. Reduced plasma P_i level did not stimulate P_i reabsorption in the kidney like in rats in which low P supply increased NaPi IIa expression (Murer et al. 2000). Therefore, renal P_i excretion was reduced due to the lower plasma P_i levels which will have resulted in lower P_i concentrations in the ultrafiltrate. Analogously, in rCa/rP plasma P_i level and renal P_i excretion were reduced at unchanged P_i transport properties. In spite of a restriction of the limiting factor Ca, P accretion was almost as high as in oCa/oP-2 indicating that a restriction of about 30% of Ca as well as of P did not result in a Ca and/or P deficiency in preruminant goats.

In a special manner P homeostasis was influenced by a relative P restriction due to doubling Ca supply. A time dependent initial reduction of plasma P_i concentrations based on reduced feed intake was observed during the first 2 weeks of experimental feeding (Boeser 2004; Table 4). This should be responsible for the decreased renal P_i excretion due to the lower P_i content of the ultrafiltrate during this time. Na^+/P_i transport properties at the end of the experimental period were not affected similarly compared with absolute P restriction. Therefore, it could be assumed that the P supply in preruminant goats was still adequate despite a one-third reduction since no adaptive changes were seen in the properties of renal Na^+/P_i transport.

Salivary P_i transport

The extent of saliva flow was small in preruminant goats whereas the ability to concentrate P_i was increasingly adjusted (Huber et al. 2003; Boeser 2004). Mechanically stimulated salivary flow rate seemed to increase NaPi II expression (Huber et al. 2003). In milk-fed preruminant goats the saliva secretion was hardly stimulated mechanically, but it could be that the supplementation of Ca and P salts acted as a chemical stimulus for saliva secretion. Therefore, NaPi II expression was increased in the groups with salt supplementation, whereby this increase was highest in the group with supplementing Ca and P (oCa/oP-2, dCa/dP). But it was assumed that the overall volume of saliva flow was small and endogenous P_i cycling was still hardly developed.

In summary, aim of the study was to relate data from in vivo P balance studies in preruminant goats to in vitro determined functional and structural data on epithelial P_i transport and expression levels of Na^+/P_i transporters. In vitro studies were performed in tissues from the same animals which were used in balance trials.

Adaptation to dietary Ca and P supply is focused on renal P_i excretion in preruminants. In vivo observed high P_i excretion provoked by an absolute or relative excess of dietary P was based on different molecular mechanisms. Therefore, even though gene expression was only determined at the end point of the experimental period, long-time adaptive changes in the P_i absorbing epithelia could be detected and could be used for interpreting balance data. Thus, significance of adaptation of epithelial P_i transport could be defined allowing a more complex view of P homeostasis in preruminant goats.

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