Pflügers Archiv - European Journal of Physiology (2019) 471:185–191
https://doi.org/10.1007/s00424-018-2181-5

INVITED REVIEW

Mechanisms and regulation of epithelial phosphate transport in ruminants: approaches in comparative physiology

Alexandra S. Muscher-Banse[1] · Gerhard Breves[1]

Received: 7 June 2018 / Revised: 6 July 2018 / Accepted: 9 July 2018 / Published online: 16 July 2018

Abstract

Ruminants have a unique utilization of phosphate (P_i) based on the so-called endogenous P_i recycling to guarantee adequate P_i supply for ruminal microbial growth and for buffering short-chain fatty acids. Large amounts of P_i enter the gastrointestinal tract by salivary secretion. The high saliva P_i concentrations are generated by active secretion of P_i from blood into primary saliva via basolateral sodium (Na^+)-dependent P_i transporter type II. The following subsequent intestinal absorption of P_i is mainly carried out in the jejunum by the apical located secondary active Na^+-dependent P_i transporters NaPi IIb (SLC34A2) and PiT1 (SLC20A1). A reduction in dietary P_i intake stimulates the intestinal P_i absorption by increasing the expression of NaPi IIb despite unchanged plasma 1,25-dihydroxyvitamin D_3 concentrations, which modulate P_i homeostasis in monogastric species. Reabsorption of glomerular filtrated plasma P_i is mainly mediated by the P_i transporters NaPi IIa (SLC34A1) and NaPi IIc (SLC34A3) in proximal tubule apical cells. The expression of NaPi IIa and the corresponding renal Na^+-dependent P_i capacity were modulated by high dietary phosphorus (P) intake in a parathyroid-dependent manner. In response to reduced dietary P_i intake, the expression of NaPi IIa was not adapted indicating that renal P_i reabsorption in ruminants runs at a high level allowing no further increase when P intake is diminished. In bones and in the mammary glands, Na^+-dependent P_i transporters are able to contribute to maintaining P_i homeostasis. Overall, the regulation of P_i transporter activity and expression by hormonal modulators confirms substantial differences between ruminant and non-ruminant species.

Keywords 1,25-dihydroxyvitamin D_3 · Goat · NaPi IIa · NaPi IIb · PiT1 · Phosphate · PTH

Phosphate recycling in ruminants

In contrast to all other mammals, an endogenous phosphate (P_i) circulation has developed in ruminants. This species' specific trait has to be discussed concerning two major physiological functions of P_i in the forestomach region. Firstly, P_i is an essential component of microbial cell mass and is therefore needed for microbial protein synthesis as a dominating component of microbial growth since P_i is needed for the synthesis of new bacterial nucleic acids and other cell components. Secondly, besides bicarbonate P_i serves as a buffering system for short-chain fatty acids which are produced at high rates as end products of ruminal microbial fermentation. Thus, the endogenous P_i circulation maintains the chemical homeostasis in the rumen and P_i supply to ruminal microorganisms especially in situations of limited dietary phosphorus (P) supply.

In small and large ruminants, the salivary glands are the major site for endogenous P_i secretion into the gastrointestinal tract. This substantially exceeds dietary P intake under normal feeding conditions. The high P_i secretion rates are mediated by both high volume flow rates of saliva in ruminants and the ability of the salivary glands to enrich P_i compared with plasma P_i. The ability to enrich P_i in saliva has also been demonstrated for free-ranging ruminants such as roe deer [14]. Thus, daily secretion rates between 5 and 10 g P_i in sheep and goats and between 30 and 60 g P_i in cows [6] can be achieved. In order to inhibit substantial losses of the overall P pool, P_i has to be effectively absorbed. In studies in sheep which were equipped with duodenal and ileal cannula, it could be demonstrated that this mainly takes place in the small intestines.

This article is part of the special issue on Phosphate transport in Pflügers Archiv – European Journal of Physiology

✉ Alexandra S. Muscher-Banse
alexandra.muscher@tiho-hannover.de

[1] Department of Physiology, University of Veterinary Medicine Hannover, Bischofsholer Damm 15/102, 30173 Hannover, Germany

Phosphate transport in the ruminant intestine

Although the small intestines had been identified as the major site for P_i absorption in various studies carried out about 50 years ago [19, 41], the first data on the epithelial mechanisms in ruminants were published approximately 30 years ago. In P_i uptake, studies on brush border membrane vesicles (BBMV) which had been prepared from the upper small intestine of young sheep, a P_i uptake was described which depended on the pH gradient between the buffer solutions outside and inside the vesicles [53]. By replacing of mannitol or gluconate in the incubation medium by more permeable anions such as SCN^- or Cl^-, no effects on the P_i transport rate were determined which was interpreted as evidence for an electroneutral P_i transport [54]. Therefore, it was concluded that in contrast to monogastric animals, P_i uptake across the enterocyte apical membrane is mediated by a proton (H^+)-driven electroneutral mechanism. The transport capacity of this system increased in response to P depletion [54]. The molecular basis of a potential duodenal H^+-dependent P_i transport system has not yet been identified.

This concept, however, could not be confirmed by direct P_i flux measurements across intact epithelial tissues from different intestinal segments in sheep and young goats [47]. In these studies, unidirectional P_i flux rates were measured in Ussing chambers in the absence of an electrochemical gradient, and in both species, high P_i net flux rates could be determined in the mid-jejunum. This clearly indicated the existence of active transport mechanism. In young goats, the highest P_i absorption along the intestinal axis was measured in the ileum [12]; this was also demonstrated in adult sheep [47]. An explanation for this could be a pH of 8.0 in the ileum which could cause a shift in the equilibrium of constant P_i to a more divalent P_i (HPO^{2-}_4), which is favorably transported by the electrogenic Na^+-dependent P_i transporter NaPi IIb (SLC34A2). For further characterization of active P_i transport, it could be demonstrated that around 60% of active P_i transport in the mid-jejunum could be inhibited when either the mucosal sodium (Na^+) concentration was reduced from 148.2 to 1.8 mmol/l, ouabain was adjusted to a concentration in the serosal compartment of 0.1 mmol/l for complete inhibition of basolateral Na^+/K^+-ATPase [5] or arsenate was added to the mucosal buffer solution at a concentration of 5 mmol/l. Arsenate has been established as a competitive inhibitor of renal and intestinal Na^+-coupled P_i cotransport into BBMV vesicles of monogastric species [1, 10, 21, 42, 52].

Despite the fact that Na^+-dependency of a substantial proportion of active P_i transport could clearly be demonstrated in these experiments, it could not be concluded that this transport was identical to the secondary active Na^+/P_i cotransport as suggested for the sheep ileum [43, 44]. The manipulations on mucosal or serosal Na^+ might have also affected the apical Na^+/H^+ antiporter. Thus, Na^+-driven H^+ extrusion could have been limited by these manipulations, therefore inhibiting P_i uptake by an H^+/P_i cotransporter.

In order to further clarify how Na^+ and H^+ are involved in P_i transport P_i uptake, studies into BBMV from goat jejunum were carried out under different conditions with regard to the extravesicular Na^+ and H^+ concentrations [45]. The Na^+-dependent P_i uptake as a function of extravesicular P_i concentration was saturable, following a simple Michaelis-Menten kinetic and resulted in a V_{max} of 0.423 ± 0.080 nmol mg^{-1}protein· $15\ s^{-1}$ and a K_m of 0.029 ± 0.007 mmol/l. These kinetic data are in accordance with respective data obtained from monogastric species. At an extravesicular Na^+ concentration of 100 mmol/l, a decrease in extravesicular pH from 7.4 to 5.4 led to a significant increase in P_i uptake by about 60%. This effect could not be observed when extravesicular Na^+ was completely replaced by K^+. The results do not agree with flux data obtained for ileal tissues. This could be due to the fact that by using intact epithelial tissues for flux measurements still include the microclimate which cannot be assumed for BBMV. These data suggested that a major proportion of jejunal P_i uptake in goat jejunum is Na^+-dependent and can be stimulated by H^+.

This assumption could be confirmed after the murine type II Na^+/P_i cotransporter had been identified [20]. In a first approach, it could be demonstrated by applying Northern blot analysis of mRNA of mouse and goat jejunum that the hybridization signal of goat intestinal mRNA was located in the same range as the mouse-specific NaPiIIb band. At protein level, a strong Na^+/P_i cotransporter type IIb specific immunoreaction was shown when antibodies raised against N terminal-specific oligopeptide of mouse Na^+/P_i cotransporter type IIb were used [23]. This could be confirmed by further comparative studies in goat duodenum and jejunum. Northern blot analysis of duodenal and jejunal poly(A) + RNA was performed, and hybridization with a goat-specific NaPi IIb probe revealed strong bands in the jejunum but not in the duodenum. The lack of NaPi IIb expression in goat duodenum was confirmed by Western blot analysis when NaPi IIb protein could only be detected in the jejunum with a mouse-specific NaPi IIb antibody. Immunohistochemically, the NaPi IIb protein localization could be shown in goat jejunum but not in goat duodenum. In addition, the relative amounts of NaPi IIb protein in BBMV of goat jejunum as a function of V_{max} of jejunal Na^+-dependent P_i transport could be described by a positive correlation indicating that a higher capacity of NaPi IIb transport was correlated with an increased abundance of NaPi IIb protein which also underlined that the major extent of Na^+/P_i transport was mediated by NaPi IIb [24]. The expression of another electrogenic Na^+-dependent P_i transporter named PiT1 (SLC20A1) was shown in caprine intestinal epithelia [12].

In order to clarify the role of the goat duodenum for P_i transport, transepithelial P_i flux rates were measured in Ussing chambers in the presence or absence of mucosal Na^+

at pH 7.4 or 5.4, respectively. At a mucosal pH of 7.4 and in the presence of Na^+, small net flux rates (17.1 ± 3.3 nmol cm^{-2} h^{-1}) were measured which were in the same range as previously determined by Schroder et al. [47]. Reducing the mucosal pH to 5.4 resulted in a significant increase in net flux above 200 nmol cm^{-2} h^{-1} with Na^+ and around 50 nmol cm^{-2} h^{-1} without Na^+ in the mucosal buffer. The K_m values were about tenfold higher (0.4 mmol/l) compared with the K_m values of the jejunal NaPi IIb transporter. From these studies, it was concluded that at least two different mechanisms are involved in goat intestinal P_i absorption. In the duodenum, it is mediated by an H^+-dependent and Na^+-sensitive system which is not upregulated in response to dietary P_i depletion. In the jejunum, P_i transport is mediated by a Na^+-dependent and H^+-sensitive mechanism which is mainly represented by NaPi IIb and regulated by P intake [24].

The lack of mRNA and protein expression of NaPi IIb in the duodenum and their presence in the jejunum has also been found in lactating and dried-off goats. Interestingly, both, the jejunal mRNA and protein expression of NaPi IIb were significantly downregulated in lactating goats in comparison with dried-off goats [60]. The expression of NaPi IIb mRNA was also studied in Holstein cows, and it could be shown that expression of NaPi IIb was highest in the distal jejunum and in the ileum and virtually absent in the duodenum and in the proximal jejunum [17].

The morphological and functional ontogenesis of the forestomach system during the first months of life has to be regarded as the major developmental process in young ruminants. With regard to the specific functions of P_i for microbial processes in the rumen, it was therefore of interest to measure the expression of intestinal NaPi IIb as a function of time during early development. These experiments were carried out in goats' tissues obtained during the first week of life, within week 4–5, 8–11 and up until the fifth month. The kinetic parameters were measured by uptake studies into BBMV, and relative expression of NaPi IIb was recorded by molecular methods [25]. From these studies, it could be concluded from the different K_m values that in the first week of life, covalent modifications of NaPi IIb and/or PiT1 might have been present in the jejunum which affected binding properties because the K_m value in this group was significantly higher than in all other groups.

From many studies on monogastric species, it has been shown that the active intestinal P_i absorption is upregulated in response to either dietary P or calcium (Ca) depletion and that this effect is mediated by 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) [18, 28, 40]. However, data from monogastric species is not consistent because in mice deficient for the vitamin D receptor (VDR) or the 1alpha hydroxylase and fed a low P_i diet, the expressions of intestinal NaPi IIb and renal NaPi IIa were regulated like in wild type animals although 1,25-$(OH)_2D_3$-VDR axis was not involved [8]. A P depletion in sheep and goats neither affected plasma 1,25-$(OH)_2D_3$ concentrations [7, 45] nor the metabolic clearance rate or the production rate of 1,25-$(OH)_2D_3$ [30]. In order to detect whether in ruminants the regulation of the vitamin D hormone systems is mediated by the VDR level of enterocytes rather than by the hormone production rate, studies on the kinetic parameters of the VDR were performed in lactating and in young male goats. For lactating goats, increased binding affinities of the VDR could be demonstrated [46] which, however, could not be confirmed for young goats [48]. Despite the lack of response from the vitamin D hormone system, jejunal P_i net flux rates in young goats were significantly increased in response to P depletion. Minor increases were also shown for duodenal P_i net flux rates [47]; this, however, could not be confirmed by Huber et al. [24]. In contrast to data from chicks and rabbits [11, 42] and from rats [9], no effects of P depletion on V_{max} of the Na^+/P_i cotransport system could be detected for young goats by measuring P_i uptake into BBMV [45].

Based on efficient rumino-hepatic circulation of urea, ruminants cope easily with a reduction in dietary protein to lower the excretion of nitrogen (N) into the environment. However, changes in mineral homeostasis like reduced blood Ca concentrations and decreased serum 1,25-$(OH)_2D_3$ levels were detected in young goats kept on a reduced protein diet [12, 35, 38]. The decrease in 1,25-$(OH)_2D_3$ did not modulate the expression of NaPi IIb in the small intestine of goats [12] whereas in monogastric species, the expression of intestinal NaPi IIb is regulated in a 1,25-$(OH)_2D_3$-dependent manner [34].

Phosphate transport in the ruminant parotid gland

The parotid glands of ruminants are able to secrete large amounts of P_i from blood into saliva. The acinar cells of sheep parotid glands secrete 5 to 10 l per day of iso-osmotic saliva which contains 10 to 40 mmol/l P_i. These high concentrations of P_i in the saliva require a high flux through the acinar cells. In ovine acinar cells, it was shown that this flux was mediated by an Na^+-dependent uptake of P_i and that it was inhibited by phosphonoformate in parotid basolateral membrane vesicles [62]. Therefore, it was concluded that this Na^+-dependent P_i transport was mediated by an Na^+-dependent P_i transporter type IIb located on the basolateral membrane of acinar cells [25, 27]. The activity of this transport system was not regulated by a dietary P or Ca depletion in parotid glands [27, 62] whereas the intestinal absorption of P_i was stimulated by such a dietary intervention [54]. The mechanism of apical P_i extrusion into the primary saliva is unknown to date.

Phosphate transport in the ruminant kidney

In the kidneys, most of the filtered P_i is reabsorbed across the proximal tubule cells. This process is mediated predominantly by the apically located Na^+-dependent P_i transport proteins named NaPi IIa (SLC34A1) and NaPi IIc (SLC34A3) in both ruminant and non-ruminant species [2, 25, 55, 59]. About 90% of amino acid sequence, homology exists between ruminant (goat, sheep, or bovine) and rat renal NaPi IIa [23, 49, 64]. However, the bovine NaPi IIa and NaPi IIc sequences from native ruminant renal tissue has only a 59 and 56% sequence identity, respectively, with the cloned Na^+-dependent P_i transporter of bovine renal epithelial cell line NBL-1 while the homology with NaPi IIb was 97% [64].

Under normophosphatemic conditions in ruminants, the excretion of P_i is very low based on efficient tubular P_i reabsorption rates to prevent this urinary P_i loss [63]. However, the functional and modulatory background for this event has not been identified so far because the kinetic and stoichiometric parameters of renal cortex Na^+-dependent P_i transport are comparable to the type IIa Na^+/P_i cotransport in monogastric species [49].

In monogastric species, NaPi IIa is mainly regulated by fluctuating P levels in the extracellular fluid [3]. A low P_i diet increased the expression of NaPi IIa in the kidney of rats [33] whereas in goats and sheep neither a P nor a Ca depletion caused significant effects on renal P_i transport capacities [49] or on NaPi IIa expression [27], thus assuming that the P supply was still adequate in ruminants. On feeding a high P diet to young ruminants, a decrease in renal P_i reabsorption capacity based on internalized NaPi IIa protein occurred [27, 36]. Strong correlations between NaPi IIa mRNA and plasma P_i as well as plasma parathyroid hormone (PTH) levels indicated that elevated P_i and PTH concentrations were able to modulate the renal P_i excretion by reducing P_i reabsorption [36]. This phenomenon is different to monogastric animals where the NaPi IIa expression was decreased only at protein level [32].

In young goats, a modulation of mineral homeostasis caused by a reduction in dietary protein under isoenergetic conditions was shown [38]. During a dietary protein reduction, a significant increase in NaPi IIa protein expression and a concomitant decrease in PTH receptor protein expression were observed in young goats, whereas serum 1,25-$(OH)_2D_3$ concentrations were diminished and PTH levels were unaffected [15, 58, 59]. Reason for this stimulated NaPi IIa expression could be a decrease in P_i concentrations in the ultrafiltrate caused by a drop in the glomerular filtration rate (GFR) to conserve urea. A reduction in the GFR by 60% was detected in goats fed a low-protein diet [13, 61]. Such a chronic tubular P_i depletion could cause an increase in NaPi IIa protein expression by unknown P_i sensing mechanism(s) in the proximal tubules, whereas the corresponding RNA was not affected like in monogastric species [4, 29]. Interestingly, a stimulation of NaPi IIa expression could be achieved by a dietary protein reduction and thereby presumably a reduction in P_i in the ultrafiltrate. A direct dietary P_i depletion without manipulation of GFR did not show the same effects in the ruminant kidney [49].

Overall, the role of the kidneys in the modulation of P homeostasis in ruminants is not clarified completely because in preruminant animals, the kidneys are the main excretory pathway for an excess of P_i like in monogastric species. However, during the development of the ruminant, a transition occurred, and an excess of P_i is not excreted by the kidneys anymore but is secreted in the saliva and transferred to the rumen where it is taken care of by microorganisms. Therefore, the PTH-mediated regulation of renal P_i excretion is less important in adult ruminants than in young ruminants and monogastric species.

Phosphate transport in the ruminant mammary gland

Similarly as for Ca ruminant, milk also contains high concentrations of P which can be allocated to different chemical fractions. According to studies, in normal goat milk, approximately 30% of the total P concentration around 20 mmol/l were present as inorganic soluble P, and the remainder were either non-covalently bound to protein or covalently bound to casein [39]. Thus, for P_i, a concentrating ability of plasma P_i between 4 and 5 mmol/l can be assumed for the mammary gland, suggesting similarity to the parotid gland. The expression of NaPi IIb in the apical membrane of mice mammary gland has been demonstrated for the first time by Miyoshi et al. [31]. In their study, however, NaPi IIb could only be detected when the alveolar epithelium had developed its full secretory function. It could not be shown in virgin or early pregnancy mice. They have suggested the physiological function of NaPi IIb as a potential marker of secretory functions in the mammary gland. In order to characterize the potential role of NaPi IIb in the mammary gland of ruminants, experiments were performed in lactating goats [26]. In these experiments, NaPi IIb protein could be detected in fractions of the apical membrane which could also be confirmed by immunohistochemistry. For functional characterization, apical membrane vesicles from alveolar epithelial cells were prepared from fresh goat milk in accordance with the approach introduced by Shennan [50]. These membranes were then subjected to Na^+-dependent P_i uptake as a function of time, P_i concentration in the extravesicular buffer, and in the absence or presence of phosphonoformic acid (PFA). PFA competitively inhibited Na^+/P_i transport [22]. In NaPi IIb-transfected PS120 cells and in *Xenopus laevis* oocytes, 5 mmol/l PFA inhibited nearly the entire P_i uptake [65]. These approaches showed the overshoot profile as a function of time, V_{max} of 0.9 nmol mg^{-1}protein·

Table 1 Location, identity, and main features of intestinal P_i transport in ruminants

Location	Identity	Main features of P_i transport
Duodenum	No NaPi IIb PiT1	H^+-dependent and Na^+-sensitive P_i transport No modulation by dietary P intake
Jejunum	NaPi IIb PiT1	Na^+-dependent and H^+-sensitive P_i transport Modulation of NaPi IIb by dietary P intake
Ileum	NaPi IIb PiT1	No correlation between transepithelial flux rates and NaPi IIb protein expression

$10s^{-1}$ and a K_m of 0.2 mmol/l, indicating a system with higher transport capacity and lower affinity in comparison with jejunal NaPi IIb. PFA led to a significant decrease in P_i uptake. Although these data clearly indicate the presence of NaPi IIb in apical membranes of goat alveolar epithelial cells, with regard to the transmembrane Na^+ gradient in alveolar epithelial cells, it is quite unlikely that the substantial secretion of inorganic P is mediated by this mechanism. Thus, there might be a further basolateral mechanism for P_i secretion. Therefore, it can be assumed that the modulation of apical NaPi IIb in mammary glands is necessary to guarantee adequate intracellular P_i supply for the cells during different stages of lactation [37] Reason for this is because mammary blood flow is diminished during involution [16], and the activity of the basolateral-located Na^+-dependent P_i transporter is reduced by milk stasis [51]. However, this is not yet fully understood and further studies are needed.

Phosphate transport in the ruminant bone

The majority of P_i is present in the skeleton primarily complexed with Ca in the form of hydroxyapatite crystals. In bovine articular chondrocytes, two P_i transport mechanisms, a Na^+-dependent and a Na^+-independent one, were characterized [56, 57]. The Na^+-dependent component had a K_m value for P_i of 0.17 mmol/l whereas the Na^+-independent part was not fully saturable, indicating both carrier-mediated P_i uptake and diffusive pathway in chondrocytes [57]. Both, the Na^+-dependent P_i transport mechanism and the Na^+-independent one were blocked by phosphonoacetate and arsenate, even though parts of the Na^+-independent component were resistant. On a molecular basis, the mRNA expression of PiT1 and PiT2 (SLC20A2) could be shown in bovine articular chondrocytes [57].

Conclusion and outlook

In ruminants, a number of specific features in P_i homeostasis have been documented in recent years. Firstly, the endogenous P_i cycle ensures a high availability of P_i in the forestomach region for microbial and buffer features. Secondly, intestinal P_i absorption is mediated by at least two different mechanisms: an H^+-dependent and Na^+-sensitive P_i transport in the duodenum which is not modulated by dietary P intake whereas the NaPi IIb and PiT1 could only be detected in jejunal and ileal tissues. This system is H^+-sensitive and is significantly upregulated in response to dietary P depletion without any changes in the vitamin D hormone system. Thirdly, the role of the kidneys for regulating P_i homeostasis is by far less important as compared with monogastric species which is due to the fact that under physiological P_i conditions, the reabsorption of P_i already runs at a very high level in the kidneys. Therefore, additional adaptation processes cannot occur (Table 1).

Further experimental studies should focus on a more detailed characterization of duodenal P_i transport and on those mechanisms which are involved to mediate adaptational jejunal P_i transport. In addition, the potential interaction between P_i homeostasis and other nutrient systems need further clarification.

Acknowledgements The authors wish to thank Frances Sherwood-Brock for proofreading the manuscript.

Funding information The research was partly supported by the Deutsche Forschungsgemeinschaft (SFB 280, Br 780/4-2, Br 780/11-1, Br 780/11-2, Mu 3585/1-1).

References

1. Berner W, Kinne R, Murer H (1976) Phosphate transport into brush-border membrane vesicles isolated from rat small intestine. Biochem J 160:467–474
2. Biber J, Murer H (1994) A molecular view of renal Na-dependent phosphate transport. Ren Physiol Biochem 17:212–215
3. Biber J, Murer H, Forster I (1998) The renal type II Na+/phosphate cotransporter. J Bioenerg Biomembr 30:187–194
4. Biber J, Hernando N, Forster I, Murer H (2009) Regulation of phosphate transport in proximal tubules. Pflugers Arch 458:39–52. https://doi.org/10.1007/s00424-008-0580-8
5. Blaustein MP (1993) Physiological effects of endogenous ouabain: control of intracellular Ca2+ stores and cell responsiveness. Am J Phys 264:C1367–C1387. https://doi.org/10.1152/ajpcell.1993.264.6.C1367
6. Breves G, Schroder B (1991) Comparative aspects of gastrointestinal phosphorus metabolism. Nutr Res Rev 4:125–140. https://doi.org/10.1079/NRR19910011

7. Breves G, Beyerbach M, Holler H, Lessmann HW (1985) Fluid exchange in the rumen of sheep in low and adequate phosphorus administration. Dtsch Tierarztl Wochenschr 92:47–49
8. Capuano P, Radanovic T, Wagner CA, Bacic D, Kato S, Uchiyama Y, St-Arnoud R, Murer H, Biber J (2005) Intestinal and renal adaptation to a low-Pi diet of type II NaPi cotransporters in vitamin D receptor- and 1alphaOHase-deficient mice. Am J Physiol Cell Physiol 288:C429–C434. https://doi.org/10.1152/ajpcell.00331.2004
9. Caverzasio J, Danisi G, Straub RW, Murer H, Bonjour JP (1987) Adaptation of phosphate transport to low phosphate diet in renal and intestinal brush border membrane vesicles: influence of sodium and pH. Pflugers Arch 409:333–336
10. Danisi G, van Os CH, Straub RW (1984) Phosphate transport across brush border and basolateral membrane vesicles of small intestine. Prog Clin Biol Res 168:229–234
11. Danisi G, Caverzasio J, Trechsel U, Bonjour JP, Straub RW (1990) Phosphate transport adaptation in rat jejunum and plasma level of 1, 25-dihydroxyvitamin D3. Scand J Gastroenterol 25:210–215
12. Elfers K, Wilkens MR, Breves G, Muscher-Banse AS (2015) Modulation of intestinal calcium and phosphate transport in young goats fed a nitrogen- and/or calcium-reduced diet. Br J Nutr 114: 1949–1964. https://doi.org/10.1017/S000711451500375X
13. Eriksson L, Valtonen M (1982) Renal urea handling in goats fed high and low protein diets. J Dairy Sci 65:385–389. https://doi.org/10.3168/jds.S0022-0302(82)82202-9
14. Fickel J, Goritz F, Joest BA, Hildebrandt T, Hofmann RR, Breves G (1998) Analysis of parotid and mixed saliva in roe deer (Capreolus capreolus L.). J Comp Physiol B 168:257–264
15. Firmenich CS, Elfers K, Wilkens MR, Breves G, Muscher-Banse AS (2018) Modulation of renal Ca and Pi transporting proteins by dietary nitrogen and/or calcium in young goats. J Anim Sci. https://doi.org/10.1093/jas/sky185
16. Fleet IR, Peaker M (1978) Mammary function and its control at the cessation of lactation in the goat. J Physiol 279:491–507
17. Foote AP, Lambert BD, Brady JA, Muir JP (2011) Phosphate transporter expression in Holstein cows. J Dairy Sci 94:1913–1916. https://doi.org/10.3168/jds.2010-3691
18. Fox J, Care AD, Blahos J (1978) Effects of low calcium and low phosphorus diets on the duodenal absorption of calcium in betamethasone-treated chicks. J Endocrinol 78:255–260
19. Grace ND, Ulyatt MJ, Macrae JC (1974) Quantitative digestion of fresh herbage by sheep .3. Movement of Mg, Ca, P, K and Na in digestive-tract. J Agric Sci 82:321–330
20. Hilfiker H, Hattenhauer O, Traebert M, Forster I, Murer H, Biber J (1998) Characterization of a murine type II sodium-phosphate cotransporter expressed in mammalian small intestine. Proc Natl Acad Sci U S A 95:14564–14569
21. Hoffmann N, Thees M, Kinne R (1976) Phosphate transport by isolated renal brush border vesicles. Pflugers Arch 362:147–156
22. Hoppe A, Lin JT, Onsgard M, Knox FG, Dousa TP (1991) Quantitation of the Na(+)-Pi cotransporter in renal cortical brush border membranes. [14C]phosphonoformic acid as a useful probe to determine the density and its change in response to parathyroid hormone. J Biol Chem 266:11528–11536
23. Huber K, Walter C, Schroder B, Biber J, Murer H, Breves G (2000) Epithelial phosphate transporters in small ruminants. Ann N Y Acad Sci 915:95–97
24. Huber K, Walter C, Schroder B, Breves G (2002) Phosphate transport in the duodenum and jejunum of goats and its adaptation by dietary phosphate and calcium. Am J Physiol Regul Integr Comp Physiol 283:R296–R302. https://doi.org/10.1152/ajpregu.00760.2001
25. Huber K, Roesler U, Muscher A, Hansen K, Widiyono I, Pfeffer E, Breves G (2003) Ontogenesis of epithelial phosphate transport systems in goats. Am J Physiol Regul Integr Comp Physiol 284: R413–R421
26. Huber K, Muscher A, Breves G (2007) Sodium-dependent phosphate transport across the apical membrane of alveolar epithelium in caprine mammary gland. Comp Biochem Physiol A Mol Integr Physiol 146:215–222. https://doi.org/10.1016/j.cbpa.2006.10.024
27. Huber K, Roesler U, Holthausen A, Pfeffer E, Breves G (2007) Influence of dietary calcium and phosphorus supply on epithelial phosphate transport in preruminant goats. J Comp Physiol B 177: 193–203. https://doi.org/10.1007/s00360-006-0121-8
28. Jungbluth H, Binswanger U (1989) Unidirectional duodenal and jejunal calcium and phosphorus transport in the rat: effects of dietary phosphorus depletion, ethane-1-hydroxy-1,1-diphosphonate and 1,25 dihydroxycholecalciferol. Res Exp Med (Berl) 189:439–449
29. Madjdpour C, Bacic D, Kaissling B, Murer H, Biber J (2004) Segment-specific expression of sodium-phosphate cotransporters NaPi-IIa and -IIc and interacting proteins in mouse renal proximal tubules. Pflugers Arch 448:402–410. https://doi.org/10.1007/s00424-004-1253-x
30. Maunder EM, Pillay AV, Care AD (1986) Hypophosphataemia and vitamin D metabolism in sheep. Q J Exp Physiol 71:391–399
31. Miyoshi K, Shillingford JM, Smith GH, Grimm SL, Wagner KU, Oka T, Rosen JM, Robinson GW, Hennighausen L (2001) Signal transducer and activator of transcription (Stat) 5 controls the proliferation and differentiation of mammary alveolar epithelium. J Cell Biol 155:531–542. https://doi.org/10.1083/jcb.200107065
32. Murer H, Forster I, Hernando N, Lambert G, Traebert M, Biber J (1999) Posttranscriptional regulation of the proximal tubule NaPi-II transporter in response to PTH and dietary P(i). Am J Physiol 277: F676–F684
33. Murer H, Hernando N, Forster I, Biber J (2000) Proximal tubular phosphate reabsorption: molecular mechanisms. Physiol Rev 80: 1373–1409. https://doi.org/10.1152/physrev.2000.80.4.1373
34. Murer H, Forster I, Biber J (2004) The sodium phosphate cotransporter family SLC34. Pflugers Arch 447:763–767. https://doi.org/10.1007/s00424-003-1072-5
35. Muscher A, Huber K (2010) Effects of a reduced nitrogen diet on calcitriol levels and calcium metabolism in growing goats. J Steroid Biochem Mol Biol 121:304–307. https://doi.org/10.1016/j.jsbmb.2010.03.084
36. Muscher A, Hattendorf J, Pfeffer E, Breves G, Huber K (2008) Hormonal regulation of phosphate homeostasis in goats during transition to rumination. J Comp Physiol B 178:585–596. https://doi.org/10.1007/s00360-007-0248-2
37. Muscher A, Breves G, Huber K (2009) Modulation of apical Na+/Pi cotransporter type IIb expression in epithelial cells of goat mammary glands. J Anim Physiol Anim Nutr 93:477–485. https://doi.org/10.1111/j.1439-0396.2008.00831.x
38. Muscher AS, Piechotta M, Breves G, Huber K (2011) Modulation of electrolyte homeostasis by dietary nitrogen intake in growing goats. Br J Nutr 105:1619–1626. https://doi.org/10.1017/S0007114510005350
39. Neville MC, Peaker M (1979) The secretion of calcium and phosphorus into milk. J Physiol 290:59–67
40. Peterlik M, Wasserman RH (1980) Regulation by vitamin D of intestinal phosphate absorption. Horm Metab Res 12:216–219. https://doi.org/10.1055/s-2007-996246
41. Pfeffer E, Thompson A, Armstrong DG (1970) Studies on intestinal digestion in the sheep. 3. Net movement of certain inorganic elements in the digestive tract on rations containing different proportions of hay and rolled barley. Br J Nutr 24:197–204
42. Quamme GA (1985) Phosphate transport in intestinal brush-border membrane vesicles: effect of pH and dietary phosphate. Am J Phys 249:G168–G176. https://doi.org/10.1152/ajpgi.1985.249.2.G168

43. Schneider KM, Straub RW, Danisi G (1985) Phosphate-transport in the gut of the ruminant. Experientia 41:834–835
44. Schneider KM, Straub RW, Danisi G (1985) Sodium phosphate cotransport in ruminant intestine. Miner Electrol Metab 11:339–339
45. Schroder B, Breves G (1996) Mechanisms of phosphate uptake into brush-border membrane vesicles from goat jejunum. J Comp Physiol B 166:230–240
46. Schroder B, Breves G, Pfeffer E (1990) Binding properties of duodenal 1,25-dihydroxyvitamin D3 receptors as affected by phosphorus depletion in lactating goats. Comp Biochem Physiol A Comp Physiol 96:495–498
47. Schroder B, Kappner H, Failing K, Pfeffer E, Breves G (1995) Mechanisms of intestinal phosphate transport in small ruminants. Br J Nutr 74:635–648
48. Schroder B, Pfeffer E, Failing K, Breves G (1995) Binding properties of goat intestinal vitamin D receptors as affected by dietary calcium and/or phosphorus depletion. Zentralbl Veterinarmed A 42:411–417
49. Schroder B, Walter C, Breves G, Huber K (2000) Comparative studies on Na-dependent Pi transport in ovine, caprine and porcine renal cortex. J Comp Physiol 170:387–393
50. Shennan DB (1992) K+ and Cl- transport by mammary secretory-cell apical membrane-vesicles isolated from milk. J Dairy Res 59: 339–348
51. Shillingford JM, Shennan DB, Beechey RB (1995) The regulation of anion transport in lactating rat mammary tissue. Biochem Soc Trans 23:26S
52. Shirazi-Beechey SP, Gorvel JP, Beechey RB (1988) Phosphate transport in intestinal brush-border membrane. J Bioenerg Biomembr 20:273–288
53. Shirazi-Beechey SP, Kemp RB, Dyer J, Beechey RB (1989) Changes in the functions of the intestinal brush border membrane during the development of the ruminant habit in lambs. Comp Biochem Physiol B 94:801–806
54. Shirazi-Beechey SP, Beechey RB, Penny J, Vayro S, Buchan W, Scott D (1991) Mechanisms of phosphate transport in sheep intestine and parotid gland: response to variation in dietary phosphate supply. Exp Physiol 76:231–241
55. Shirazi-Beechey SP, Penny JI, Dyer J, Wood IS, Tarpey PS, Scott D, Buchan W (1996) Epithelial phosphate transport in ruminants, mechanisms and regulation. Kidney Int 49:992–996
56. Solomon DH, Browning JA, Wilkins RJ (2007) Inorganic phosphate transport in matrix vesicles from bovine articular cartilage. Acta Physiol (Oxf) 190:119–125. https://doi.org/10.1111/j.1748-1716.2007.01670.x
57. Solomon DH, Wilkins RJ, Meredith D, Browning JA (2007) Characterisation of inorganic phosphate transport in bovine articular chondrocytes. Cell Physiol Biochem 20:99–108. https://doi.org/10.1159/000104158
58. Starke S, Huber K (2014) Adaptive responses of calcium and phosphate homeostasis in goats to low nitrogen intake: renal aspects. J Anim Physiol Anim Nutr 98:853–859. https://doi.org/10.1111/jpn.12144
59. Starke S, Cox C, Sudekum KH, Huber K (2013) Adaptation of electrolyte handling to low crude protein intake in growing goats and consequences for in vivo electrolyte excretion. Small Ruminant Res 114:90–96. https://doi.org/10.1016/j.smallrumres.2013.06.008
60. Starke S, Reimers J, Muscher-Banse AS, Schroder B, Breves G, Wilkens MR (2016) Gastrointestinal transport of calcium and phosphate in lactating goats. Livest Sci 189:23–31
61. Valtonen MH, Uusi-Rauva A, Eriksson L (1982) The effect of protein deprivation on the validity of creatinine and urea in evaluation of renal function. An experimental study in the goat. Scand J Clin Lab Invest 42:507–512
62. Vayro S, Kemp R, Beechey RB, Shirazi-Beechey S (1991) Preparation and characterization of basolateral plasma-membrane vesicles from sheep parotid glands. Mechanisms of phosphate and D-glucose transport. Biochem J 279(Pt 3):843–848
63. Widiyono I, Huber K, Failing K, Breves G (1998) Renal phosphate excretion in goats. Zentralbl Veterinarmed A 45:145–153
64. Wood IS, Ford LT, Scott D, Rees WD, Shirazi-Beechey SP (1998) Sequence comparisons of ruminant renal Na+/phosphate co-transporters. Biochem Soc Trans 26:S121
65. Xu H, Inouye M, Missey T, Collins JF, Ghishan FK (2002) Functional characterization of the human intestinal NaPi-IIb cotransporter in hamster fibroblasts and Xenopus oocytes. Biochim Biophys Acta 1567:97–105