

Review: Regulation of gastrointestinal and renal transport of calcium and phosphorus in ruminants

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In comparison to monogastric animals, ruminants show some peculiarities in respect to the regulation of mineral homeostasis, which can be regarded as a concerted interplay between gastrointestinal absorption, renal excretion and bone mobilisation to maintain physiological Ca and phosphate (P_i) concentrations in serum. Intestinal absorption of Ca or P_i is mediated by two general mechanisms: paracellular, passive transport dominates when luminal Ca or P_i concentrations are high and transcellular. The contribution of active transport becomes more important when dietary Ca or P_i supply is restricted or the demand increased. Both pathways are modulated directly by dietary interventions, influenced by age and regulated by endocrine factors such as 1,25-dihydroxyvitamin D₃. Similar transport processes are observed in the kidney. After filtration, Ca and P_i are resorbed along the nephron. However, as urinary Ca and P_i excretion is very low in ruminants, the regulation of these renal pathways differs from that described for monogastric species, too. Furthermore, salivary secretion, as part of endogenous P_i recycling, and bone mobilisation participate in the maintenance of Ca and P_i homeostasis in ruminants. Saliva contains large amounts of P_i for buffering rumen pH and to ensure optimal conditions for the rumen microbiome. The skeleton is a major reservoir of Ca and P_i to compensate for discrepancies between demand and uptake. But alterations of the regulation of mineral homeostasis induced by other dietary factors such as a low protein diet were observed in growing ruminants. In addition, metabolic changes, for example, at the onset of lactation have pronounced effects on gastrointestinal mineral transport processes in some ruminant species. As disturbances of mineral homeostasis do not only increase the risk of the animals to develop other diseases, but are also associated with protein and energy metabolism, further research is needed to improve our knowledge of its complex regulation.

Keywords: 1,25-dihydroxyvitamin D₃, hypocalcaemia, hypophosphatemia, mineral homeostasis, parathyroid hormone

Implications

Disturbances of mineral homeostasis are of significant relevance not only in dairy cows but also in beef cattle and small ruminants. In addition, the contribution of excreted phosphorus to the pollution of surface waters necessitates a revision of our livestock feeding regimes. The present review gives an overview on our current knowledge of the regulation of mineral transport across gastrointestinal and renal epithelia derived from functional and structural studies in different ruminant species as affected by age, lactation, feeding regime, etc. It highlights the physiological differences between monogastric animals and ruminants as well as the importance of combining different scientific approaches to improve our understanding of the complex mechanisms crucial for the maintenance of mineral homeostasis.

Introduction

Depending on management strategies, milk fever occurs in dairy cows with an incidence of 0% to 1%, 1.4% to 4%, and 5.7% to 6% in the first, the second and the third lactation, while the prevalence of subclinical hypocalcaemia defined as serum Ca concentration <2 mM amounts to 5.7% to 25%, 29.0% to 41%, and 49% (Reinhardt *et al.*, 2011; Venjakob *et al.*, 2017). The physiological response to transient hypocalcaemia is an increase in bone mobilisation followed by enhanced gastrointestinal absorption (van't Klooster, 1976). If these mechanisms are compromised, either the extent or the duration of hypocalcaemia is exacerbated resulting in increased risks of developing different diseases in early lactation depending on the duration of hypocalcaemia (Neves *et al.*, 2018). Reliable data on the prevalence of peripartum hypocalcaemia in small ruminants are scarce. Like cows, goats develop

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hypocalcaemia usually at the onset of lactation, while Ca homeostasis of sheep is generally more severely challenged during late gestation (Oetzel, 1988; Brozos *et al.*, 2011).

Homeostatic control of phosphate (P_i) is also challenged at the onset of lactation. Subclinical hypophosphatemia around parturition is observed in >50% of dairy cows (Macrae *et al.*, 2006), and low serum P_i concentrations in cows suffering from milk fever are associated with an increased risk of developing downer cow syndrome (Menard and Thompson, 2007). However, high P percentage of pre-calving diets was identified as a risk factor for hypocalcaemia in a meta-analysis (Lean *et al.*, 2006), and a prepartum ration low in P seems to have beneficial effects on Ca homeostasis, probably because of an impact on bone mobilisation and vitamin D metabolism (Cohrs *et al.*, 2018).

Restriction of P and CP intake may occur for economic reasons or because animals are kept on deficient pastures (McGrath *et al.*, 2012; Elfers *et al.*, 2015). This might be especially relevant in growing or fattening animals. On the other hand, environmental pollution with P_i and N of animal origin is leading to legal incentives to reduce the P and CP content of ruminant rations to the lowest possible level that does not negatively affect health and productivity.

In contrast to monogastric species, including rats and horses, no significant changes in renal Ca and P_i excretion are observed in bovines kept on restricted alimentary Ca supply, and the adaptation of gastrointestinal absorption seems to be less pronounced (Martz *et al.*, 1999; van Doorn *et al.*, 2004; Zhang *et al.*, 2008; Taylor *et al.*, 2009). In Table 1 we present data from balance studies carried out in different ruminant species to illustrate that Ca and P absorption and secretion out of and into different gastrointestinal segments as well as urinary excretion are influenced by age, lactation and type of diet. There is inconsistency in the contribution of the forestomach of ruminants to overall Ca absorption (Table 1), which can partly be explained with differences in the composition of rations as mineral homeostasis interferes with other dietary factors such as dietary cation–anion difference, Mg and CP supply (Goff, 2008; Muscher and Huber, 2010; Elfers *et al.*, 2016a; Wilkens *et al.*, 2018). Therefore, studies using a more mechanistic approach are an important tool to enhance our knowledge.

Unfortunately, most research on the physiological mechanisms to maintain mineral homeostasis has used rodents as models for mammals in general. It is, therefore, the aim of this review to summarise the most important peculiarities of Ca and P_i transport across gastrointestinal and renal epithelia found in ruminants and highlight differences in comparison to monogastric animals. Throughout the following text, specific results obtained in ruminants will be indicated, while more general aspects often refer to studies done in rats and mice.

Methods

To evaluate renal and gastrointestinal Ca or P_i absorption and secretion *in vivo*, several different quantitative methods applied: balance studies using intact or cannulated animals, radioisotope tracer techniques, and the administration of stable strontium that can be used for this purpose, as its absorption shows a close correlation with that of Ca. As these experiments do not give explanations for sometimes inconsistent results, *ex vivo* methods are necessary to reveal the underlying mechanisms more precisely: isolated perfused organs, micropuncture experiments on renal transport, the everted sac technique that allows to control the composition of the luminal and serosal buffer solution and thus the chemical gradient, and the Ussing chamber is used to investigate transport mechanism by altering both the chemical and electrical gradients across the epithelium to differentiate between passive and active, paracellular and transcellular mechanisms. These functional studies are completed by *in vitro* experiments – for example, the quantification of RNA and protein expression of transporters and the functional characterisation applying electrophysiological techniques on cloned transporters. Although all these methods can greatly improve our understanding of physiological processes, the artificial conditions used or the fact that transporter abundance does not always represent *in vivo* activity may also provide challenges in interpretation. Taken together, our knowledge will probably increase if we combine the information derived from all these different approaches.

Endocrine control of calcium and phosphate transport

The concentrations of ionised Ca (Ca^{2+}) and P_i in blood are regulated in a narrow range by 1,25-dihydroxyvitamin D_3 (1,25-(OH) $_2D_3$), parathyroid hormone (PTH), calcitonin and fibroblast growth factor 23 (FGF23). Homeostasis is maintained by the interplay of gastrointestinal absorption, renal resorption and mobilisation of these inorganic ions from bone. Within minutes, a drop in blood Ca^{2+} induces the release of PTH from the parathyroid gland (Kumar and Thompson, 2011) that stimulates the mobilisation of Ca and P_i from the skeleton (Ben-awadh *et al.*, 2014). In monogastric animals, PTH also increases renal Ca resorption and P_i excretion by direct, rapid mechanisms (Besarab and Swanson, 1982). Furthermore, PTH enhances the expression and activity of 1 α -hydroxylase (CYP27B1), an enzyme that converts 25-hydroxyvitamin D_3 (25-OHD $_3$) to the biologically most active vitamin D metabolite 1,25-(OH) $_2D_3$ (Fraser and Kodicek, 1973). Furthermore, direct effects of plasma Ca and P_i on 1,25-(OH) $_2D_3$ concentrations were shown in rats (Bushinsky *et al.*, 1985; Bushinsky *et al.*, 1989).

In lactating animals, PTH-related peptide (PTHrP) is secreted by the mammary gland into both milk and blood. Although it can bind the PTH receptor, it is probably not

Table 1 Results from balance studies done with different ruminant species: intake, urinary excretion (UEX), pre-intestinal (PRE) and intestinal (INT) net absorption (ABS), faecal excretion (FEX) in grams per day, apparent digestibility (AD) in percentage

Ca						P						Treatment	Source and animals
Intake	UEX	ABS		FEX	AD	Intake	UEX	ABS		FEX	AD		
		PRE	INT					PRE	INT				
24.9	1.20	1.2	-0.2	23.9	4.2	16.6	5.26	-19.3	25.9	10.0	39.8	GS	Khorasani and Armstrong (1992)
25.1	1.00	1.9	2.6	20.7	17.7	16.8	4.50	-18.9	27.6	8.1	51.6	GS + F	
25.2	0.48	1.1	1.6	22.6	10.3	16.8	5.33	-18.5	26.1	9.3	44.9	GS + FF	
33.6	1.06	1.6	0.3	31.6	5.8	10.7	0.29	-17.9	21.4	7.2	32.4	Hay	
37.1	0.35	5.4	-1.3	33.0	11.1	14.8	2.53	-15.2	19.6	10.4	30.3	Hay + SM	
48.2	1.92	14.5	1.9	31.8	34.1	17.4	3.36	-18.1	26.3	9.1	47.4	GCS + FF	
51.4	0.49	20.9	-0.8	31.4	39.0	18.1	2.57	-16.7	23.4	11.3	37.2	GCS	Khorasani <i>et al.</i> (1997)
51.7	1.38	19.8	-4.3	36.2	29.9	21.5	5.85	-12.4	21.1	12.8	40.4	GCS + FF + SM	
												50% concentrate	
												50% silage	
115.0	n.d.	-9.0	36.8	78.6	31.7	82.0	n.d.	-60.5	85.0	65.5	20.1	Triticale	
118.0		6.5	28.6	83.6	29.2	90.0		-53.9	81.0	55.0	38.9	Oat	
150.0		19.1	28.0	107.4	28.4	97.0		-42.9	81.3	66.9	31.0	Barley	
231.0		49.8	24.6	156.5	32.3	105.0		-33.9	70.5	68.3	35.0	Alfalfa	
114.7	1.00	n.d.	n.d.	78.2	32.1	61.3	0.74	n.d.	n.d.	40.3	34.1	Second week of lactation,	Taylor <i>et al.</i> (2009)
129.5	0.96			84.6	36.2	47.9	2.95			28.3	46.6	increasing Ca intake	
205.7	1.10			148.3	26.0	58.0	1.04			36.8	39.0		
125.3	0.64			88.1	29.6	82.5	0.65			50.3	38.6	Eighth week of lactation,	
191.4	0.51			138.9	26.5	80.0	0.91			49.6	36.3	increasing Ca intake	
243.3	0.91			168.2	30.8	78.0	0.81			49.9	37.0		
71.8	0.42	11.1	16.3	44.4	38.2	41.3	3.56	-12.1	35.4	18.0	56.5	High DCAD	Oehlschlaeger <i>et al.</i> (2014)
72.4	6.10	-2.9	26.7	48.6	32.8	40.4	5.05	-26.8	50.0	17.2	57.5	Low DCAD	
71.9	10.15	3.3	34.7	33.9	52.9	42.4	7.80	-23.8	53.3	12.9	69.6	Low DCAD + 25-OHD	McGrath <i>et al.</i> (2012)
65.7	0.9	n.d.	n.d.	60.7	7.6	26.1	0.9	n.d.	n.d.	20.4	21.8	Control	
66.4	2.5	n.d.	n.d.	55.8	16.0	26.4	1.1	n.d.	n.d.	17.2	34.8	25-OHD	Brangus steers
6.4	n.d.	0.5	0.0	5.9	7.8	0.96	n.d.	-1.88	1.65	1.19	-24.0	P depletion	Breves <i>et al.</i> (1985)
6.2		0.0	1.3	4.9	21.0	4.19		-2.51	4.27	2.43	42.0	P repletion	Black headed mutton wethers
8.32	0.09	1.04	-0.03	7.31	12.1	5.83	2.16	-4.25	7.12	2.96	49.2	0.09% Na	Khorasani and Armstrong (1990)
8.32	0.01	1.12	0.05	7.15	14.1	5.76	1.92	-3.86	6.56	3.06	46.9	0.6% Na	
8.46	0.02	1.32	-0.14	7.28	13.9	5.85	2.26	-3.28	6.53	2.60	55.6	1.3% Na	
8.49	0.06	1.29	-0.03	7.50	11.7	5.81	2.17	-4.33	7.32	2.82	51.5	0.65% K	Suffolk halfbred wethers
8.28	0.01	1.07	-0.21	7.00	15.5	5.72	2.06	-3.36	6.21	2.87	49.8	3.0% K	
7.22	0.14	n.d.	n.d.	5.09	29.5	2.94	0.04	n.d.	n.d.	1.75	40.5	1.09% Ca, 0.46% P	Pfeffer <i>et al.</i> (1995)
5.76	0.20	n.d.	n.d.	5.35	7.1	1.08	0.01	n.d.	n.d.	1.11	-2.7	1.09% Ca, 0.20% P	
3.07	0.06	n.d.	n.d.	1.51	50.8	3.20	0.34	n.d.	n.d.	1.69	47.2	0.39% Ca, 0.46% P	Saanen-type, male goat kids
2.39	0.22	n.d.	n.d.	1.97	17.6	1.18	0.02	n.d.	n.d.	1.03	12.7	0.39% Ca, 0.21% P	
												Adequate P	Müschen <i>et al.</i> (1988)

Table 1 (Continued)

Intake	Ca					P					Source and animals	
	UEX	ABS			AD	Intake	UEX	ABS				AD
		PRE	INT	FEX				PRE	INT	FEX		
22.4	n.d.	n.d.	n.d.	15.7	30.0	8.12	n.d.	n.d.	3.70	54.5	First to sixth week	Saanen-type, female goats, lactating
20.6			15.2	26.3	7.18			3.29	54.1	Seventh to 11th week		
17.4			14.6	15.9	6.48			3.08	52.5	Twelfth to 16th week		
										Reduced P		
22.9			16.5	27.9	8.31			4.62	44.4	First to sixth week		
17.4			13.6	21.8	5.56			3.13	43.7	Seventh to 11th week		
13.1			13.1	22.3	3.23			1.86	42.6	Twelfth to 16th week		
										Deficient P		
24.0			17.7	26.3	8.58			4.62	46.2	First to sixth week		
10.1			8.7	13.8	1.46			0.95	34.6	Seventh to 11th week		
15.5			12.0	22.6	5.88			2.60	55.8	Twelfth to 16th week		

GS = ryegrass silage; F = formic acid; FF = formic acid and formaldehyde; SM = soybean meal; GCS = ryegrass and clover silage; DCAD = dietary cation-anion difference; 25-OHD = 25-hydroxyvitamin; n.d. = not determined.

involved in vitamin D metabolism but likely to act on bone mobilisation (Hernández-Castellano *et al.*, 2019). In addition, it was suggested to exert effects on renal Ca handling such as prolactin (Herm *et al.*, 2015).

Depending on the concentrations of plasma Ca and calcitonin, 1,25-(OH)₂D₃ either increases or inhibits bone mobilisation (Kurbel *et al.*, 2003). Via its genomic effects on Ca transporter expression that become present after a certain time lag, 1,25-(OH)₂D₃ stimulates renal resorption and intestinal absorption of Ca (Dusso *et al.*, 2005) and limits its own synthesis by inhibiting CYP27B1 and stimulating the expression of 24-hydroxylase, the enzyme that initiates the inactivation of both 25-OHD₃ and 1,25-(OH)₂D₃ (Chen and DeLuca, 1995; Beckman and DeLuca, 2002).

In addition, 1,25-(OH)₂D₃ induces the production of a bone-derived phosphatonin, FGF23 (Saji *et al.*, 2010), that interacts with PTH expression and vitamin D metabolism and thus decreases plasma concentrations of 1,25-(OH)₂D₃ (Schiavi and Kumar, 2004; Krajisnik *et al.*, 2007). Low dietary P intake decreased plasma concentrations of FGF23 and concomitantly increased 1,25-OH₂D₃ while plasma PTH was low (Antoniucci *et al.*, 2006).

Protein intake also interferes with vitamin D metabolism. Growth hormone acts mainly through insulin-like growth factor 1 (IGF1). Uncoupling of this somatotrophic axis indicated by low IGF1 plasma concentrations was observed during dietary protein restriction in growing goats and in peripartum dairy cows (Mischer *et al.*, 2011; Piechotta *et al.*, 2014). Reduced IGF1 was associated with decreased expression of CYP27B1 and affected bone mobilisation and intestinal Ca absorption probably via diminished plasma concentrations of 1,25-(OH)₂D₃ (Wilkens *et al.*, 2018).

As all these aspects might interfere with the strategies applied to stabilise mineral homeostasis in dairy cows and beef cattle – for example, dietary interventions, vitamin D supplementation, oral and parental administration of Ca, low IGF1 during negative energy balance, etc. (Reist *et al.*, 2003; Wilkens *et al.*, 2012a; Domino *et al.*, 2017) – a better understanding of the exact mechanisms is urgently needed.

Sites and mechanisms of gastrointestinal calcium absorption

Paracellular calcium absorption

Gastrointestinal Ca absorption can occur via the transcellular as well as paracellular pathways (Hoenderop *et al.*, 2005). Passive, paracellular absorption can take place when the chemical gradient is high enough (>6 mM on the luminal side) to overcome the electrical gradient and the barrier formed by tight junction proteins, both of which hinder the transport of cations (Bronner, 1987). As paracellular Ca transport is dependent on its luminal concentration, it is dominant when Ca intake is high (Bronner and Pansu, 1999) – for example, when Ca is provided as a bolus or via drenching. In addition, paracellular absorption can be driven by the so-called solvent drag effect. When water is absorbed due to hydrostatic and osmotic pressure, mineral

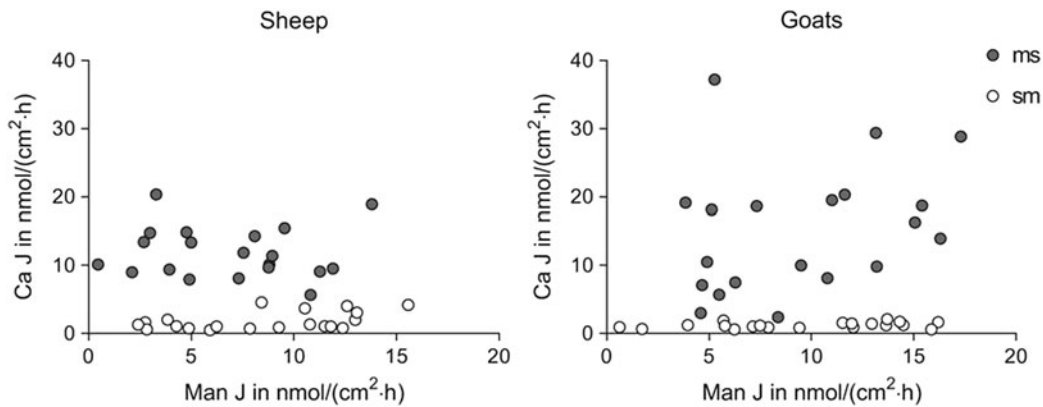


Figure 1 Unidirectional flux rates (J) from mucosal to serosal (ms) and from serosal to mucosal (sm) of Ca as a function of those of mannitol (Man) in the rumen tissues of sheep ($n=20$) and goats ($n=20$) determined using the Ussing chamber in the absence of any electrochemical gradient. As mannitol is used as a marker for paracellular transport of water, the lack of any relationship between Ca J ms and Man J ms indicates transcellular Ca absorption. Modified from Wilkens *et al.* (2011) and (2012b).

ions solubilised by water dipole-ion interactions can also pass through the paracellular pathway (Goff, 2018). The osmotic pressure contributing, to a large extent, to the solvent drag effect depends mainly on the transepithelial Na gradient generated by $\text{Na}^+\text{-K}^+\text{-ATPase}$ (Karbach, 1992).

Paracellular Ca transport in both directions can be found throughout the entire intestine, depending on the gradient. It is likely that the rumen multilayer epithelium is too dense to allow significant amounts of Ca to be absorbed via the interstitial fluid unless the luminal concentration of Ca is increased dramatically by additional supply. This hypothesis is also supported by the comparison of rumen Ca flux rates and mannitol flux rates that are used to estimate transepithelial movement of water (Figure 1) (Wilkens *et al.*, 2011 and 2012b).

Although it has been demonstrated that $1,25\text{-(OH)}_2\text{D}_3$ has an effect on the expression of several tight junction proteins (Chirayath *et al.*, 1998; Kutuzova and DeLuca, 2004), it is not clear to what extent it regulates paracellular Ca absorption. A stimulation of the expression of claudin-2 and claudin-12, tight junction proteins that increase the permeability for Ca, was found in response to long-term dietary Ca restriction in the small intestine of goats (Elfers *et al.*, 2016b); and in CaCo-2 cells treated with $1,25\text{-(OH)}_2\text{D}_3$, paracellular permeability was increased (Chirayath *et al.*, 1998).

Transcellular, pre-intestinal calcium absorption

In monogastric animals, transcellular Ca absorption mainly occurs in the duodenum and upper jejunum (Hoenderop *et al.*, 2005). The cellular mechanism consists of at least three steps: Ca enters the cell via the transient receptor potential vanilloid channel type 6 (TRPV6), is bound to the cytosolic protein calbindin- $\text{D}_{9\text{K}}$ (CaBP $_{\text{D}9\text{K}}$), translocated to the basolateral membrane and extruded mainly by the plasma membrane $\text{Ca}^{2+}\text{-ATPase}$ isoform 1b (PMCA1b) (Figure 2). A significant stimulation of expression by $1,25\text{-(OH)}_2\text{D}_3$ has been shown

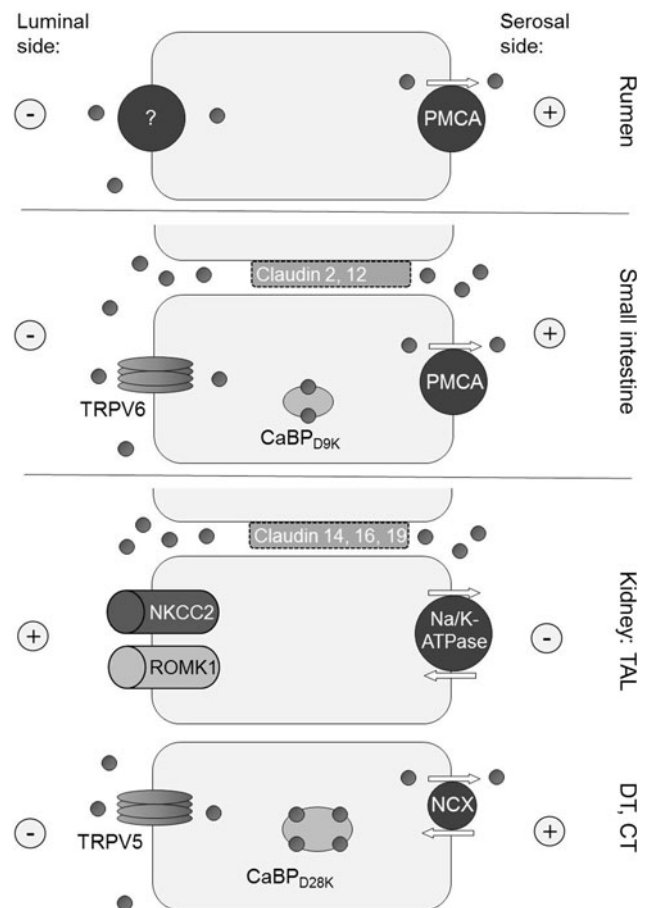


Figure 2 Ca transport mechanisms and transepithelial potential difference in the rumen, small intestine, the thick ascending limb of the loop of Henle (TAL), and the distal and connecting tubules (DT, CT) of the kidneys in ruminant species. PMCA, plasma membrane $\text{Ca}^{2+}\text{-ATPase}$ isoform 1b; TRPV6/5, transient receptor potential vanilloid channel type 6/5; CaBP $_{\text{D}9\text{K}}$ /CaBP $_{\text{D}28\text{K}}$, calbindin- $\text{D}_{9\text{K}}$ / $\text{D}_{28\text{K}}$; NKCC2, $\text{Na}^+\text{-K}^+\text{-Cl}^-$ co-transporter type 2; ROMK1, renal outer medullary K^+ channel type 1; NCX, $\text{Na}^+/\text{Ca}^{2+}$ exchanger type 1. Explanations of the mechanisms are given in the corresponding text.

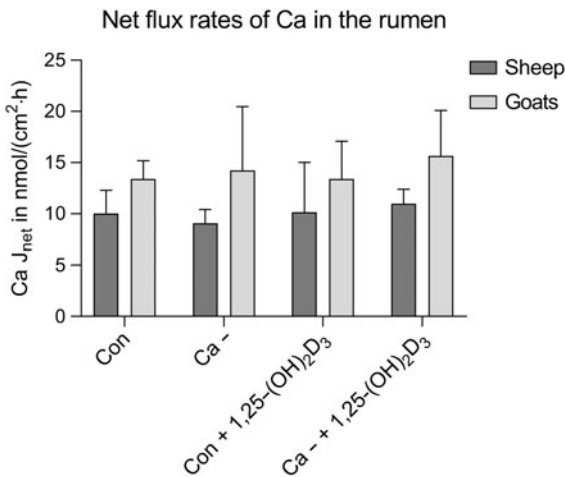


Figure 3 Rumen Ca net flux rates (J_{net}) of female sheep and goats aged 6 to 7 months kept on adequate (con, 0.92% and 1.10%, $n = 5$) or restricted Ca supply (Ca⁻, 0.26% and 0.22%, $n = 5$) treated with a placebo or fed the same diets and treated with 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃, $n = 5$) (0.5 µg/kg body weight) 12 h before sacrifice determined in Ussing chambers in the absence of any electrochemical gradient. Means ± SEM. Modified from Wilkens *et al.* (2011) and (2012b).

in all three of the abovementioned structures (Hoenderop *et al.*, 2005).

Marked differences to monogastric species have been described for ruminants, concerning the localisation and vitamin D sensitivity of Ca absorption along the gastrointestinal axis, particularly with respect to the forestomach compartment. While active Ca transport (3.0 ± 1.9 nmol/cm²·h) determined in Ussing chambers in the omasum has only been investigated and demonstrated in sheep (Höller *et al.*, 1988b), greater Ca net flux rates in the rumen were reported for sheep, goats (Figure 3) and cattle (13.8 ± 1.8 nmol/cm²·h). But as TRPV6 and CaBP_{D9K} are not expressed in ovine, caprine and bovine rumen epithelia, this pre-intestinal Ca absorption is probably not mediated by the classical mechanism described for the intestine of monogastric animals (Höller *et al.*, 1988a; Schröder *et al.*, 2001, 2015; Wilkens *et al.*, 2011, 2012b). Neither long-term dietary Ca restriction of sheep and goats nor administration of supraphysiological amounts of 1,25-(OH)₂D₃ resulted in increased Ca net flux rates across rumen epithelia measured in Ussing chambers (Figure 3) (Wilkens *et al.*, 2011 and 2012b). Hyde and Fraser estimated Ca transport *in vivo* by an administration of stable strontium. In contrast to the abovementioned studies, they observed that rumen Ca transport doubled after treatment of sheep with 1α-OHD₃ (Hyde and Fraser, 2014). However, no satisfying explanation for this inconsistency was found. It might be speculated that alterations regarding passage rate and rumen motility as a response to the hypercalcaemic effect of the treatment contribute to overall Ca transport *in vivo* (Daniel, 1983). *In vitro*, rumen Ca net flux rates of sheep determined in Ussing chambers seem to depend on the presence of short-chain fatty acids (SCFA; 0, 40 and 100 mmol/l in the mucosal buffer: 2.41 ± 0.55 , 9.59 ± 1.55 and 19.41 ± 3.37 nmol/cm²·h) and are increased by feeding 15 g of concentrate per kilogram body weight for 3 weeks in comparison to a

ration consisting of hay only (5.63 ± 0.54 , 17.43 ± 0.70 and 34.54 ± 2.67 nmol/cm²·h) (Uppal *et al.*, 2003a). Therefore, an apical transport mechanism based on a Ca²⁺/H⁺ exchange system was discussed (Lutz and Scharrer, 1991; Schröder *et al.*, 2015). Whether a higher Ca intake in the concentrate fed sheep might have altered rumen Ca transport mechanisms directly cannot be clarified. On the one hand, low luminal Ca concentrations before sacrifice did not influence the flux rates in sheep and goats (Figure 3). On the other hand, a greater contribution of pre-intestinal Ca to overall absorption was reported in a meta-analysis (Schröder and Breves, 2006).

As Na transport is also – although to a lesser extent – increased by higher luminal concentrations of SCFA (Uppal *et al.*, 2003b), rumen Ca transport could be mediated by a more complex ion exchanging mechanism. Another candidate for the apical uptake of Ca could be transient receptor potential vanilloid channel type 3 (TRPV3). In patch clamp measurements, agonists of this channel were shown to stimulate currents mediated by Ca²⁺, NH₄ and Na into HEK-293 cells expressing bovine TRPV3 (Schrapers *et al.*, 2018). An involvement of Na transport might also explain the finding that feeding a ration negative in dietary cation–anion difference (DCAD) to sheep affects the ratio of the electroneutral to the electrogenic component of rumen Ca transport from the mucosal to the serosal side (Figure 4) (Wilkens *et al.*, 2016). *In vivo* and *in vitro* studies have reported both a stimulating effect of essential oils, substances that are known to interfere with TRP channels, and also interactions between the absorption of Ca, NH₄ and Na. A conductance for NH₄ was blocked by divalent cations in bovine rumen epithelial cells. Addition of 10 µM

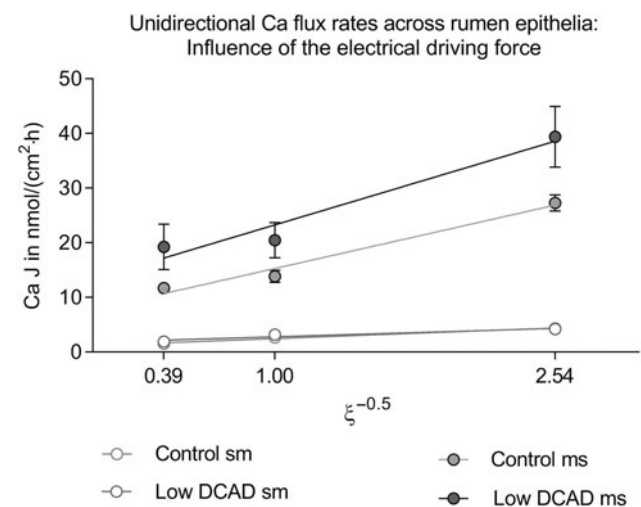


Figure 4 Correlation between electrical driving force and unidirectional Ca fluxes (J) from mucosal to serosal (ms) and from serosal to mucosal (sm) of castrated male sheep aged 8 months kept either on a ration positive in dietary cation–anion difference (DCAD) (control, $n = 4$) or negative in DCAD (low DCAD, $n = 5$). The electroneutral component of J_{ms} represented by the intercept of the linear function revealed by regression analysis is greater ($P < 0.01$) in sheep kept on a diet low in DCAD (control: $J_{ms} = 7.76 (\pm 1.23) + 7.53 (\pm 0.77) \cdot \xi^{-0.5}$; low DCAD: $J_{ms} = 13.32 (\pm 4.42) + 9.95 (\pm 2.77) \cdot \xi^{-0.5}$). Means ± SEM. Modified from Wilkens *et al.* (2016).

menthol enhanced Ca net flux rates determined for ovine rumen epithelia in Ussing chambers from 8.60 ± 1.43 to 13.24 ± 0.91 nmol/cm²·h and Na-mediated short-circuit currents (Rosendahl *et al.*, 2016). In dairy cows, oral administration of 1.2 g essential oils with menthol as the major compound increased plasma Ca from 2.46 to 2.53 mM and decreased plasma urea from 4.28 to 3.92 mM (Braun *et al.*, 2019).

As also shown in Table 1, these findings show that rumen Ca transport depends on luminal abundance of different factors and nutrients. Therefore, greater Ca flux rates determined for rumen tissue of lactating goats in comparison to dried-off animals (2.28 ± 0.35 v. 6.75 ± 1.16 nmol/cm²·h) could be either a direct effect of lactation or be caused by the different feeding regime and/or an enlargement of the luminal surface (Starke *et al.*, 2016). In cows, rumen Ca transport estimated by the administration of stable strontium is stimulated by lactation and decreased when forestomach motility is reduced (Hyde *et al.*, 2019). As impaired motility was observed with decreased plasma Ca concentrations (Daniel, 1983), inefficient ruminal Ca absorption following a disturbance of Ca mobilisation from the skeleton might aggravate hypocalcaemia in peripartum cows.

Transcellular, intestinal calcium absorption

Studies provide conflicting results on the intestinal absorption of Ca. 1,25-(OH)₂D₃-regulated proteins, essential for transcellular Ca absorption, have been identified in the small intestine of cattle (Yamagishi *et al.*, 2006; Schröder *et al.*, 2015), sheep (Schröder *et al.*, 2001; Wilkens *et al.*, 2009, 2011) and goats (Wilkens *et al.*, 2012b; Elfers *et al.*, 2015). However, Ca transport across ovine and caprine epithelia when determined *in vitro* in the absence of an electrochemical gradient appears to be very low compared to monogastric animals such as horses using the same methods (Figure 5) (Wilkens *et al.*, 2017). In the colon, Ca net flux rates are also very low. As in the rumen, significant active Ca transport (6.55 ± 2.01 nmol/cm²·h) across the colon of sheep is only detectable in the presence of SCFA. Unfortunately, no

published data are available on intestinal Ca transport determined for bovine epithelia.

In goats kept on a low Ca diet or treated with vitamin D, duodenal Ca flux rates measured in Ussing chambers were significantly increased in some (Wilkens *et al.*, 2012b) but not in all studies (Schröder *et al.*, 1997; Sidler-Lauff *et al.*, 2010). Higher flux rates and a more pronounced stimulation of transcellular Ca transport by dietary Ca restriction was accompanied by an increase in RNA, and protein expression of TRPV6 could be shown for the jejunum of goats indicating that this segment is more active for overall Ca absorption (Figure 6a) (Wilkens *et al.*, 2012b; Elfers *et al.*, 2015). Although the efficiency of net Ca absorption from the jejunum, measured by applying the Thiry-Vella loop technique, was increased in sheep with dietary Ca restriction (Abdel-Hafeez *et al.*, 1982), this could not be demonstrated in protein expression studies and Ussing chamber experiments (Figure 6b) (Wilkens *et al.*, 2011). In goats kept on a reduced protein diet, the intestinal absorption of Ca was diminished with a concomitant reduction of CaBP_{D9K} and PMCA1b, probably caused by decreased 1,25-(OH)₂D₃ concentrations (Figure 6c) (Elfers *et al.*, 2015).

Taken together with results from lactating and dried-off sheep and goats, it might be concluded that the responsiveness of intestinal Ca absorption to enhanced demand or restricted supply varies between different species and ages (Wilkens *et al.*, 2014; Klinger *et al.*, 2016; Starke *et al.*, 2016). In lactating and non-lactating cows, balance studies demonstrated that Ca digestibility is not increased with dietary Ca restriction, although lactation itself seems to enhance gastrointestinal absorption (Table 1). However, a full adaptation to increased Ca demand during lactation seems to take at least 2 days (van't Klooster, 1976).

Salivary secretion of phosphorus

As rumen P_i concentrations play a pivotal role for rumen buffering, fermentation and microbial protein synthesis, large amounts of P_i are secreted with saliva and resorbed in the lower digestive tract. Rumen P_i concentration thus depends on dietary P intake and the rate of salivary P_i secretion (Breves and Schröder, 1991). The role of salivary P_i is also reflected by the observation that salivary P_i concentrations and expression of NaPi IIb (SLC34A2) and P_i transporter PiT1 (SLC20A1), both Na-dependent P_i transporters, increase with age, that is, with the development of the gastrointestinal tract (Huber *et al.*, 2003). Interestingly, significant differences in both rumen P_i concentrations (see below) and salivary P_i were found when adult sheep (11.3 ± 1.2 mM) and goats (23.1 ± 3.2 mM) were kept on the same ration, indicating species differences in respect to salivary P_i secretion (Wilkens *et al.*, 2014).

Data on the regulation of salivary P_i secretion are inconsistent. Furthermore, data on potential molecular regulatory mechanisms of P_i transport in salivary glands are lacking. Intravenous loading with P_i resulted in an increase in P_i secretion via the parotid gland of sheep and cows, indicating

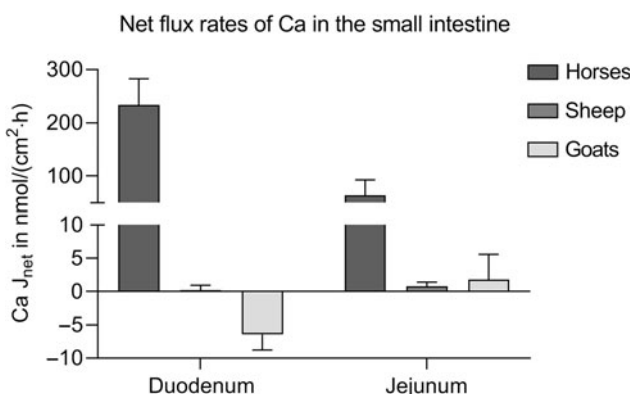


Figure 5 Intestinal Ca net flux rates (J_{net}) of horses of both sexes, aged 3 to 22 years ($n = 10$), female sheep ($n = 5$) and female goats ($n = 5$) aged 6 to 7 months kept on adequate Ca supply determined in Ussing chambers in the absence of any electrochemical gradient. Means \pm SEM. Modified from Wilkens *et al.* (2011), (2012b) and (2017).

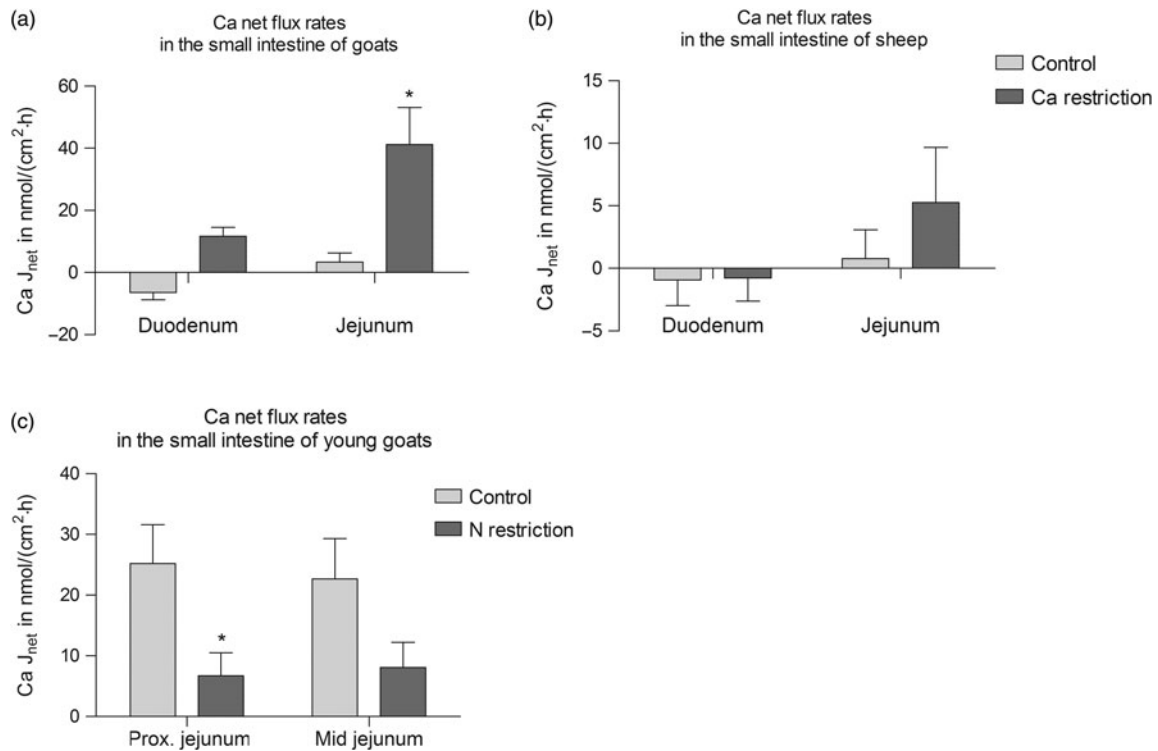


Figure 6 Intestinal Ca net flux rates (J_{net}) of female sheep and goats aged 6 to 7 months kept on adequate (control, 0.92% and 1.10%, $n = 5$) or restricted Ca supply (Ca restriction, 0.26% and 0.22%, $n = 5$) and male goats aged 3 to 4 months kept on adequate (control, 22% CP, $n = 7$) or restricted n supply (n restriction, 8% CP, $n = 6$) determined in Ussing chambers in the absence of any electrochemical gradient. Significant differences revealed by the Student's t test are marked with asterisks. Means \pm SEM; *, $P < 0.05$. Modified from Elfers *et al.* (2015), Wilkens *et al.* (2011) and (2012b).

plasma P_i concentration is the most important factor (Scott and Beastall, 1978; Riad *et al.*, 1987). In goats and sheep, the administration of PTH induced an increase in saliva P_i concentration in some studies (Wright *et al.*, 1982; Isac *et al.*, 1989), while others found a decreasing effect (Mañas-Almendros *et al.*, 1982). An injection of exogenous 1,25-(OH) $_2$ D $_3$ reduced salivary P_i concentrations in sheep and cows (Mañas-Almendros *et al.*, 1982; Riad *et al.*, 1987). A possible explanation for these contradictions could be the alteration of saliva flow rate, which is difficult to be addressed in the experimental design (Isac *et al.*, 1989). The salivary flow rate is mainly regulated by the physical nature of the diet fed (Wilson and Tribe, 1963). Pelleted diets induced lower daily saliva flow rates than chopped or long hay based on less chewing (Duric *et al.*, 1994).

However, rumen P_i concentrations were significantly increased in sheep (24.2 ± 1.0 v. 28.0 ± 0.9 mM) and goats (42.1 ± 2.9 v. 50.0 ± 3.8 mM) kept on a Ca-restricted ration for several weeks that led to an increase in an endogenous production of 1,25-(OH) $_2$ D $_3$, even though plasma (1.97 ± 0.13 v. 1.87 ± 0.21 mM) and salivary concentrations of P_i (37.3 ± 3.4 v. 37.0 ± 1.2 mM) were not affected by this feeding regime in goats (Wilkens *et al.*, 2012b, 2014).

Sites and mechanisms of gastrointestinal phosphorus absorption

The absorption of P_i takes place along the whole of the intestinal tract. In principle, P_i absorption can be divided into

a passive paracellular process and a saturable, active trans-cellular process. *In vivo* studies with the temporarily isolated reticulo-rumen from sheep demonstrated a positive linear relationship between rumen P_i concentrations and net P_i disappearance, indicating passive paracellular absorption of P_i . No indications of active P_i transport or saturation phenomena could be determined (Breves *et al.*, 1988; Beardsworth *et al.*, 1989). *In vitro* studies with rumen epithelium confirmed that no P_i net flux was found under short-circuit conditions, that is, in the absence of any electrochemical gradient, in Ussing chamber experiments (Breves *et al.*, 1988). A passive process of P_i absorption also occurs in the omasal epithelium of sheep (Höller *et al.*, 1988b). However, balance studies clearly indicate that there is no net absorption from but a substantial secretion of P_i into the forestomach *in vivo* (Table 1).

In ruminants as in monogastric species, the small intestine is the major site for P_i absorption (Pfeffer *et al.*, 1970). Dietary P concentration and 1,25-(OH) $_2$ D $_3$ are the main regulators of intestinal P_i transport in monogastric species. Paracellular P_i transport across the intercellular spaces of the small intestines has been postulated. However, no potential candidate genes which might mediate such mechanisms have been identified.

An H $^+$ -dependent P_i co-transport into duodenal brush border membrane vesicles (BBMV) from sheep and cattle was demonstrated, and this was stimulated by low dietary P (Shirazi-Beechey *et al.*, 1989, 1991). In the jejunum of sheep, the saturation of P_i absorption was demonstrated with

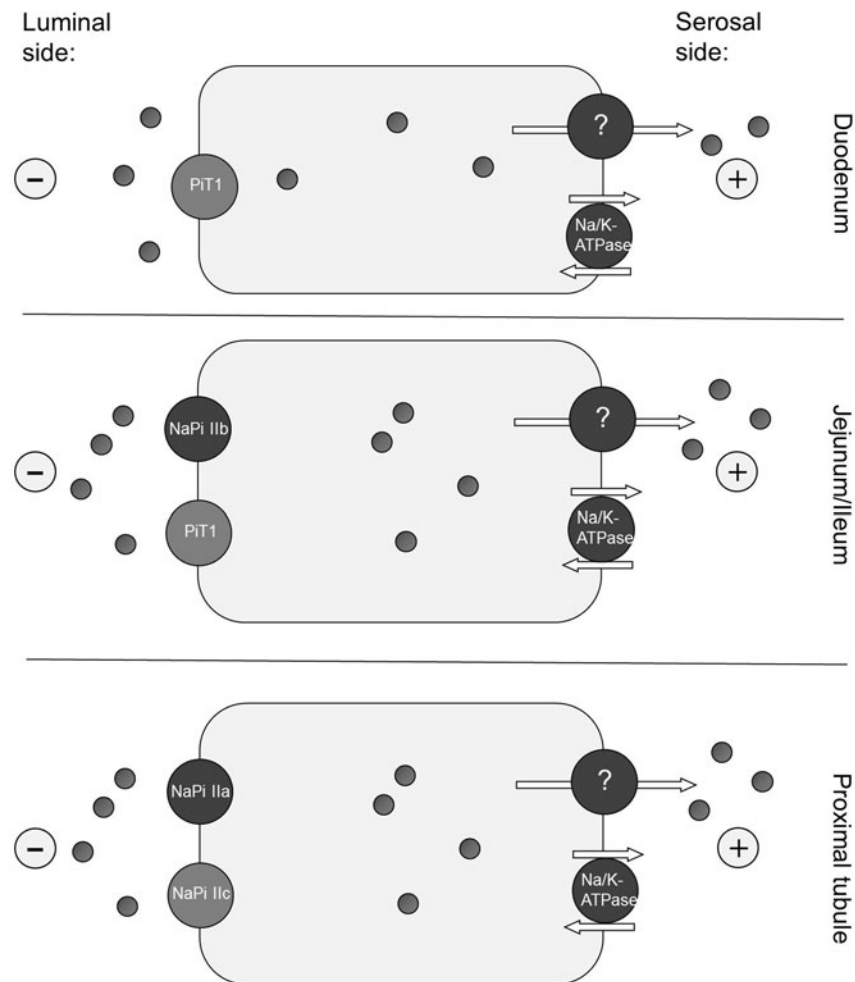


Figure 7 P_i transport mechanisms and transepithelial potential difference in the small intestine and proximal tubule of the kidneys in ruminant species. Apical entry occurs through Na-dependent P_i transporter family (NaPi) subtypes IIa and IIc or IIb and Na-dependent phosphate transporter 1 (PiT1). Basolateral extrusion mechanism of P_i is currently unknown. Further explanations of the mechanisms are given in the corresponding text.

the use of a Thiry-Vella loop when the infused solution was as high as 15 mM of P_i (Care *et al.*, 1980). Interestingly, studies on jejunal unidirectional P_i flux rates in Ussing chambers using intestinal tissue from sheep and goats demonstrated a substantial part of active P_i transport which was inhibited by arsenate by a reduction of luminal Na^+ concentrations and by serosal addition of ouabain. This Na^+ -dependent P_i co-transport could be stimulated by dietary P depletion, while changes in vitamin D metabolism were not involved (Schröder *et al.*, 1995). This provides evidence for an active P_i transport mechanism like that in non-ruminant species, with the highest absorption rates being found in the ileum of young goats (about 3 to 4 months) and adult sheep (Schröder *et al.*, 1995; Elfers *et al.*, 2015). To confirm that active P_i transport is Na^+ -dependent, P_i uptake studies into isolated BBMVs from goat jejunum were performed under different conditions of extravascular Na^+ and H^+ concentrations (Schröder and Breves, 1996). The results are similar to data from monogastric species and showed that a major proportion of jejunal P_i uptake is Na^+ -dependent, and can be stimulated by H^+ , in contrast to duodenal P_i transport which is H^+ -dependent and Na-sensitive.

After the molecular identification of an intestinal Na^+ -dependent P_i transporter in mice (NaPi IIb) (Hilfiker *et al.*, 1998), it could be shown that caprine NaPi IIb expression corresponded to murine NaPi IIb (Huber *et al.*, 2000). Both NaPi IIb mRNA and protein were absent in the duodenum of goats, while NaPi IIb was strongly expressed in the jejunum (Huber *et al.*, 2002). With jejunal BBMVs, it could be shown that a high linear correlation exists between transport capacity for P_i and NaPi IIb protein expression, indicating that the majority of Na-dependent P_i transport was mediated by NaPi IIb. Furthermore, the existence of an additional electrogenic Na-dependent P_i transporter, called PiT1, was shown in the small intestine of goats (Figure 7) (Elfers *et al.*, 2015). PiT1 belongs to the P_i transporter family that uses either Na or H^+ gradients to transport P_i (Saier, 2000). The mechanism for extrusion of P_i is still under investigation. In Holstein cows, the highest NaPi IIb RNA expression was found in the distal jejunum and ileum, while the expression in the upper intestinal segments was nearly absent (Foote *et al.*, 2011).

To characterise P_i transport in the duodenum in more detail, transepithelial P_i flux rates have been performed in Ussing chamber experiments, in the presence or absence

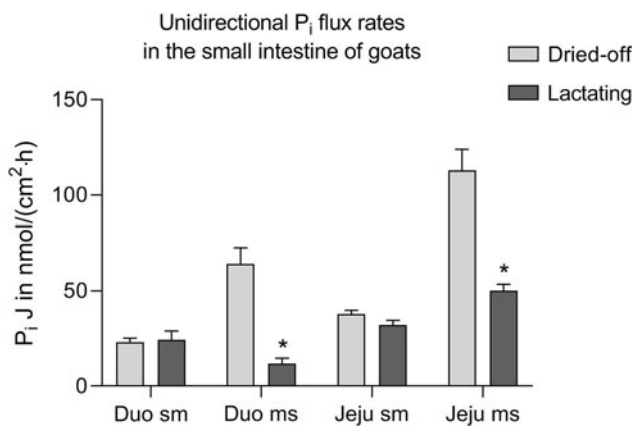


Figure 8 Unidirectional, duodenal (Duo) and jejunal (Jeju) P_i flux rates (J) from serosal to mucosal (sm) and from mucosal to serosal (ms) of dried-off ($n=6$) and lactating goats ($n=6$) determined in Ussing chambers in the absence of any electrochemical gradient. Significant differences revealed by the Student's t test are marked with asterisks. Means \pm SEM; *, $P < 0.05$. Modified from Starke *et al.* (2016).

of mucosal Na at different pH levels. From these studies, it could be concluded that at least two different P_i transport mechanisms exist in the goat intestinal tract which are regionally separate: in the duodenum, P_i uptake is mainly mediated by an H^+ -dependent Na-sensitive mechanism, while in the jejunum, P_i uptake is mainly Na-dependent and H^+ -sensitive (Schröder *et al.*, 1995; Huber *et al.*, 2002).

In lactating goats, P_i flux rates from the mucosal to the serosal side of the epithelium, determined in the duodenum and jejunum, were significantly smaller in comparison to dried-off animals, resulting in a reduced net absorption (Figure 8). This was accompanied by a downregulation of jejunal NaPi IIb, both on RNA and protein levels, probably as a consequence of either higher P intake or enhanced mobilisation of P_i from the skeleton (Starke *et al.*, 2016). In line with other studies, NaPi IIb was not detectable in the duodenum (Huber *et al.*, 2002). An ontogenetic study with goats found that P_i -binding properties changed during the development of the gastrointestinal tract of growing animals, indicating that alterations of NaPi IIb and/or PiT1 must be taking place (Huber *et al.*, 2003).

Interestingly, dietary P depletion modifies the intestinal absorption of P_i in young goats (4 to 5 months) but without the involvement of vitamin D metabolism (Schröder *et al.*, 1995). Therefore, an unknown P_i -sensing mechanism is hypothesised in the small intestine of ruminant species. Even when 1,25-(OH)₂D₃ concentrations were altered during dietary protein reduction, modulation of the expression of NaPi IIb and PiT1 in the small intestine was not found (Elfers *et al.*, 2015). These results contrast with data from monogastric species where a 1,25-(OH)₂D₃-dependent regulation of NaPi IIb was found (Murer *et al.*, 2004).

In young lambs (1 week old), the efficacy of P_i absorption from the colon was almost the same as in the upper and mid-jejunum, but the velocity of P_i absorption decreased during subsequent development (Scharer, 1985). In adult sheep fitted with re-entrant cannulae, the proximal colon

was perfused with an electrolyte solution free of P_i , and net P_i secretion was determined. Net absorption of P_i from the colon was shown when P_i concentrations of the electrolyte solution were between 2.5 and 6.5 mM (Höller *et al.*, 1988c).

Renal handling of calcium

In the kidneys of rats and hamsters, 70% of filtered Ca is resorbed paracellularly in the proximal tubules, while up to 20% is resorbed in the thick ascending limb of the loop of Henle (TAL) (Lassiter *et al.*, 1963). In the proximal tubules, where an osmotic gradient is built up due to the resorption of Na, glucose and amino acids, paracellular Ca transport is driven mainly by the solvent drag effect (Friedman and Gesek, 1995). In TAL, a lumen-positive transepithelial potential difference is generated by the electroneutral uptake of Na, K and Cl via the Na⁺-K⁺-2Cl⁻-co-transporter (NKCC) followed by the basolateral extrusion of Cl and the apical secretion of K. Tight junctions in this segment contain claudin-16 that increases cation permeability, claudin-19 that blocks anion permeability, and claudin-14 that decreases cation permeability mediated by claudin-16 (Negri, 2015). Interestingly, we observed a downregulation of claudin-19 with dietary Ca restriction in sheep and goats, which contrasts findings in rats (Frick *et al.*, 2013), and an upregulation of claudin-16 during lactation in goats (unpublished results).

Active, transcellular, 1,25-(OH)₂D₃-regulated Ca transport is found in the distal and connecting tubules. For active resorption of Ca, a transport mechanism similar to that generally accepted for the small intestine (TRPV5, calbindin-D_{28K} and basolateral extrusion by the Na⁺/Ca²⁺ exchanger NCX1) has been described (Figure 2) (Hoenderop *et al.*, 2002). In rodents fed a diet low in Ca, there was an increase in RNA expression of TRPV5 and CaBP_{D28K} (Hoenderop *et al.*, 2002; Ko *et al.*, 2009). Furthermore, it was demonstrated in mice that lactation stimulated renal RNA expression of TRPV5 and CaBP_{D28K} (van Cromphaut *et al.*, 2003). For adult sheep and goats, we found that ruminant kidney does not respond to a challenge of Ca homeostasis by altered expression of structures mediating Ca resorption. With respect to CaBP_{D28K}, we even observed a downregulation in dietary Ca-restricted or lactating goats, instead of the stimulation that has been reported for mice (Herm *et al.*, 2015). Interestingly, in lactating goats, urinary Ca excretion was not increased. We speculated that enhanced resorption in TAL mediated by prolactin and/or PTHrP might have compensated for the downregulation of TRPV5, CaBP_{D28K} and NCX1 RNA expressions in the distal parts of nephron (Herm *et al.*, 2015). Our findings on the structural level regarding animals kept on a low Ca diet could be explained by characteristically low renal Ca excretion in adult ruminants that cannot be further diminished when Ca homeostasis is challenged. As in cattle and lactating cows (Table 1), fractional excretion of Ca was not reduced by dietary Ca restriction in

small ruminants (sheep: 0.83 ± 0.22 v. $1.06 \pm 0.24\%$, goats: 0.71 ± 0.13 v. $1.03 \pm 0.21\%$).

However, in young goats (3 to 4 months) kept on a Ca-reduced diet, a stimulation of CaBP_{D28K} and NCX1 RNA expression occurred based on elevated 1,25-(OH)₂D₃ levels. This is in line with data from balance studies conducted with goat kids (Table 1). A concomitant decrease in dietary Ca and protein in these young animals caused a decrease in 1,25-(OH)₂D₃ concentrations, resulting in a downregulation of TRPV5, CaBP_{D28K} and NCX1 protein expressions (Firmenich *et al.*, 2018).

A way to increase renal Ca excretion in ruminants is to feed a ration negative in DCAD (Table 1). This feeding regime induces a compensated acidosis that results in increased tissue responsiveness to PTH (Goff *et al.*, 2014). In addition, DCAD treatment leads to significant changes in Ca balance before parturition. Several studies in dairy cows have demonstrated that urinary pH is decreased, while renal excretion of Ca is increased up to 10-fold (0.4 ± 0.2 v. 4.1 ± 0.9 g per day) (Grünberg *et al.*, 2011; Wilkens *et al.*, 2012a). As TRPV5 activity is pH-dependent, increased renal Ca excretion might be caused by a direct inhibitory effect of tubular acidosis on renal resorption of Ca as shown in the kidney of dogs and for rabbit TRPV5 (Sutton *et al.*, 1979; Yeh *et al.*, 2003). In preliminary experiments conducted with sheep, we observed that the expression of TRPV5, CaBP_{D28K} and NCX1 was not significantly altered under these conditions (unpublished results). Assuming that this occurs in cows kept on a low DCAD ration, too, this might indicate that renal resorption is immediately restored when the ration is changed postpartum. An adaptation on the functional level occurs faster than the stimulation of gene expression and could contribute to the beneficial effects of a low DCAD diet in peripartum cow.

Renal handling of phosphorus

During normophosphataemia, about 98% to 99% of filtered P_i is resorbed in the kidneys of ruminant species (Widiyono *et al.*, 1998). The mean plasma threshold for renal P_i excretion in goats lies around 4.3 mM (Widiyono *et al.*, 1998). Filtered P_i is reabsorbed mainly by the proximal tubule cells. The uptake of filtered P_i at the apical side is mediated by Na⁺-dependent P_i transporters: electrogenic NaPi Ila (SLC34A1) and electroneutral NaPi Iic (SLC34A3) in both ruminant and non-ruminant species (Figure 7) (Biber and Murer, 1994; Shirazi-Beechey *et al.*, 1996; Huber *et al.*, 2003; Starke *et al.*, 2013). Ovine and caprine amino acid sequence, kinetic and stoichiometric parameters of renal cortex Na⁺-dependent P_i transport are comparable to the type Ila Na⁺/P_i co-transport in monogastric species (Schröder *et al.*, 2000). Basolateral P_i extrusion mechanism is still unknown.

Changes in dietary P intake and consequent changes in extracellular P_i and PTH are the main regulators of renal P_i

transporters in monogastric species (Biber *et al.*, 1998). In mature goats and sheep on a P-reduced diet, no changes in renal transport capacities (Schröder *et al.*, 2000) or on NaPi Ila expression (Huber *et al.*, 2007) were determined. In contrast, dietary P restriction altered urinary P_i excretion in goat kids (Table 1). In young goats (3 to 4 months) on a high P diet, there was a decrease in renal P_i reabsorption capacity and an internalisation of NaPi Ila occurred (Huber *et al.*, 2007; Muscher *et al.*, 2008). Strong correlations between NaPi Ila mRNA and plasma P_i as well as plasma PTH concentrations indicated that elevated P_i and high PTH concentrations were able to modulate renal P_i excretion by reducing P_i reabsorption (Muschler *et al.*, 2008). This phenomenon is different to that in monogastric animals where NaPi Ila expression was decreased only at the protein level (Murer *et al.*, 1999).

Besides dietary P and PTH, a reduction in dietary protein also modulates mineral homeostasis in young goats (4 to 5 months) (Muschler *et al.*, 2011). A significant increase in NaPi Ila expression and a concomitant decrease in PTH receptor expression were observed in young goats (4 to 5 months) when dietary protein was diminished. The concentration of 1,25-(OH)₂D₃ was reduced while PTH levels were not affected (Starke *et al.*, 2013; Starke and Huber, 2014; Firmenich *et al.*, 2018). The stimulation of NaPi Ila expression during a protein-reduced diet is not obvious. It was postulated that a reduction in P_i concentrations in the ultrafiltrate stimulated the expression of NaPi Ila in apical membranes. The decline in P_i in the ultrafiltrate could be caused by a drop in the glomerular filtration rate (GFR) to conserve urea because a reduction in GFR by 60% was detected in goats fed a low protein diet (Eriksson and Valtonen, 1982; Valtonen *et al.*, 1982). Therefore, an unknown P_i-sensing mechanism(s) in the proximal tubules must exist. Interestingly, a stimulation of NaPi Ila expression was accomplished by 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 (Schröder *et al.*, 2000).

In pre-ruminant animals, the kidneys are the main excretory pathway for an excess of P_i. During the development of the rumen, changes occurred. When the threshold of plasma P_i exceeded, renal elimination of P_i is neither stimulated nor eliminated, but more P_i is secreted in the saliva to the rumen, where it is used by microorganisms. Therefore, PTH-mediated regulation of renal P_i excretion is less important in adult ruminants than in growing ruminants.


In adult ruminants, renal P_i excretion does not seem to be regulated. An intravenous infusion of PTH did not alter renal excretion of P_i in sheep, and a dietary Ca restriction for several weeks did not affect fractional excretion of P_i in small ruminants (sheep: $1.23 \pm 0.23\%$ v. $0.82 \pm 0.07\%$, goats: $2.26 \pm 0.80\%$ v. $2.79 \pm 0.80\%$) (Clark *et al.*, 1975; Herm *et al.*, 2015). This is in line with former results from sheep and cows (Braithwaite, 1975; Taylor *et al.*, 2009).


Conclusions and perspectives

The regulation of mineral homeostasis in ruminants differs not only from monogastric animals but also between and within ruminant species. Although the molecular structures that are involved in Ca and P_i transports in the intestinal tract and the kidneys have been characterised in several ruminant species, the modulation of these by different dietary interventions, by the supply of other minerals and nutrients, or as a consequence of hormonal changes in 1,25-(OH)₂D₃, FGF23, PTH or calcitonin are still under investigation. Ruminal Ca transport mechanisms are still not clarified. In addition, more information is required in respect to the contribution of salivary mineral secretion and bone turnover. Further research is also needed to better understand imbalances of mineral homeostasis, such as hypocalcaemia and the capacities of ruminants to adapt to marginal mineral supply when kept on P-deficient pasture. In this regard, the interplay between mineral homeostasis, availability and digestibility of nutrients and metabolic pathways regulating energy and protein metabolism should be elucidated as they are important for lactating cows as well as animals kept for meat production.

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Declaration of interest

The authors declare that there are no conflicts of interest.

Ethics statement

None.

Software and data repository resources

None of the data were deposited in an official repository.

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