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Gastrointestinal transport of calcium and phosphate in lactating goats

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ABSTRACT

Previous studies gave evidence for the assumption that in contrast to sheep, dairy goats can adapt to lactation-induced challenges of calcium (Ca) homeostasis by effective adaptation of gastrointestinal Ca absorption. Ca and phosphate (P_i) homeostasis are known to be closely related challenged during lactation. However, for both, Ca and P_i homeostasis, the underlying molecular mechanisms and their regulation during lactation in goats have only marginally been investigated so far. Therefore, the present study aimed to investigate the impact of lactation on gastrointestinal Ca and P_i absorption in dairy goats on a functional level using the Ussing chamber technique to determine macromineral fluxes across gastrointestinal epithelia, and on a structural level by analyzing gastrointestinal RNA and protein expression of key genes involved in transcellular Ca and P_i transport. Besides slightly increased functional Ca absorption and elevated expression of *transient receptor potential vanilloid channel type 6*, *calbindin* D_{9K} and *plasma membrane* Ca^{2+}*-ATPase type 1* in jejunum, an upregulation of Ca absorption in lactating goats also occurred in the rumen in terms of increased Ca fluxes. However, the exact molecular and regulatory mechanisms have to be examined in more detail. Interestingly, intestinal P_i absorption in duodenum and jejunum was downregulated by lactation on functional level, and in accordance, *sodium-dependent* P_i *cotransporter type IIb* RNA and protein expression were decreased in jejunum. Whether this downregulation of intestinal P_i absorption can be attributed to sufficient P_i supply from endogenous sources or to lactation-induced increased dietary P_i intake has still to be investigated.

1. Introduction

The understanding of peripartal challenges of calcium (Ca) homeostasis is a crucial point in maintaining animal welfare and health in dairy farming. Recently, we could demonstrate that in goats, the physiological increase in bone turnover providing Ca from the skeleton around parturition was normalized faster than in sheep and cows (Wilkens et al., 2014). Furthermore, goats were able to compensate for low dietary Ca intake by increasing intestinal Ca absorption, which could not be observed in sheep (Wilkens et al., 2012), and the incidence of peripartal hypocalcemia was much lower than in sheep or cows despite a milk yield comparable to that of cows (Oetzel, 1988). Thus, it is hypothesized that the greater capacity to adapt to lactation-induced challenges of Ca homeostasis in goats might be caused by efficient stimulation of gastrointestinal Ca absorption.

Balance studies focusing on the particular effect of lactation on gastrointestinal Ca absorption have been carried out in sheep (Braithwaite et al., 1969, 1970) and in cows (Ramberg et al., 1970; van't Klooster, 1976). Though, although investigations on intestinal Ca excretion in lactating goats have been made with respect to variations in dietary phosphorus (P) supply (Müschen et al., 1988), there is still a lack of data emphasizing the special impact of lactation on intestinal Ca absorption. Moreover, the mechanisms underlying lactation-induced changes in gastrointestinal Ca transport have exclusively been investigated in rodents so far (Ajibade et al., 2010; Charoenphandhu et al., 2009; Fudge and Kovacs, 2010).

Around parturition, not only Ca balance, but also phosphate (P_i) homeostasis is challenged and it has been shown that low plasma P_i concentrations in cows suffering from milk fever were associated with an increased risk to develop a downer cow syndrome (Menard and Thompson, 2007). The regulation of plasma P_i concentrations in ruminants is mostly accomplished by P_i absorption from the intestine. Gastrointestinal P_i transport in small ruminants has already been investigated in several studies (Beardsworth et al., 1989; Braithwaite, 1978; Huber et al., 2000, 2002; Muscher et al., 2012; Schröder et al., 1995; Schröder and Breves, 1996; Wadhwa and Care, 2002). Two transport systems for the apical uptake of P_i in the intestine could be detected, one of which in the duodenum was shown to be proton-dependent, whereas the other one was identified as the *sodium-dependent* P_i *cotransporter type*

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IIb (NaPiIIb) (Huber et al., 2000, 2002; Schröder et al., 1995; Schröder and Breves, 1996; Shirazi-Beechey et al., 1996). However, although changes of P homeostasis including an increased P absorption during lactation have already been demonstrated in small ruminants (Braithwaite, 1983), the exact regulatory molecular mechanisms underlying these changes are still unknown.

The current study aimed to examine the effect of lactation on gastrointestinal Ca and P_i transport in dairy goats on a functional and structural level. The functional level of gastrointestinal mineral transport was investigated by Ussing chamber technique, whereas investigations of the structures known to be involved in transcellular, active Ca absorption, TRPV6 (*transient receptor potential vanilloid channel type 6*, an apically located Ca channel), CaBP-D_{9K} (*calbindin D_{9K}*, a cytosolic protein mediating the transport of Ca through the cell), PMCA1 (*plasma membrane Ca^{2+}-ATPase type 1*, active basolateral extrusion of Ca) and the NaPiIIb were performed by analyzing the mRNA and protein expression.

2. Animals, materials and methods

2.1. Animals and feeding

The protocol of animal treatment was approved by the Animal Welfare Commissioner of the University of Veterinary Medicine Hannover, Foundation, Germany, and the experimental procedure was supervised according to the German Animal Welfare Law.

The study was performed with 12 multiparous Saanen type goats housed in a free-stall barn at the Department of Physiology of the University of Veterinary Medicine Hannover, Foundation, Germany. Six of the animals were lactating and were slaughtered on day 21 after parturition. The other six animals had been in milk for three months before drying-off. Six weeks after lactation had ceased the animals were sacrificed. Average body weights were 49.1 ± 1.7 kg (lactating goats) and 44.6 ± 2.4 kg (dried-off goats).

All animals had access to hay, minerals and water *ad libitum*. Lactating goats were milked and fed concentrate three times daily at 8.00, 14.00 and 20.00. The detailed composition of the diet is described elsewhere (Wilkens et al., 2014). The amount of concentrate was calculated individually according to the milk yield of the previous milking (800 g concentrate containing 0.90% Ca and 0.55% P per kg milk) to meet the recommendations for dairy goats as given by the German Society of Nutrition Physiology (GfE, 2003). Dried-off goats received 75 g concentrate per 10 kg body weight per day.

2.2. Sampling

All goats were killed by exsanguination from the carotid arteries following a standard abattoir stunning procedure. Samples of the ventral ruminal sac and rumen fluid were collected immediately after slaughtering. Furthermore, 30 cm long pieces of intestinal tissue were carefully removed directly distally from the pylorus and, respectively, 150 cm distally from the pylorus to collect duodenum and jejunum samples.

For Ussing chamber experiments, the ruminal mucosa was stripped off the underlying muscle layers and kept in a buffer solution (Table 1, Rumen serosal) aerated with carbogen at 38 °C until mounting into the Ussing chambers. Intestinal segments were opened along the mesenteric line, rinsed with ice-cold 0.9% (w/v) saline and kept in ice-cold buffer solution (Table 1, Jejunum mucosal) aerated with carbogen. Serosal and muscle layers were separated carefully and the epithelia were mounted into the Ussing chambers.

Samples for RNA isolation and for protein detection were rinsed with ice-cold saline. The epithelia were separated from the underlying submucosal and muscle layers. All samples were shock-frozen in liquid nitrogen and stored at −80 °C.

Table 1
Composition of buffer solutions used in Ussing chamber experiments. Concentrations given in mM. Determination of pH at 38 °C, buffers aerated with carbogen. Since only ionised Ca is transported, its concentration was adjusted in the buffer solutions and determined using an electrolyte analyzer (Rapidlab 248, Chiron Diagnostrics GmbH, Fernwald, Germany). All chemicals were of analytical grade (Merck, Darmstadt, Germany or Sigma-Aldrich Chemicals, St.Louis, USA).

	Jejunum		Rumen	
Substance	**Serosal**	**Mucosal**	**Serosal**	**Mucosal**
NaCl	113.6	113.6	55.5	57.0
KCl	5.4	5.4	5.0	5.0
$CaCl_2 \cdot 2H_2O$	1.2	1.2	2.2	1.45
(Ca^{2+})	0.9	0.9	0.8	0.8
$MgCl_2 \cdot 6H_2O$	1.2	1.2	1.2	1.2
$NaHCO_3$	21.0	21.0	21.0	2.0
$Na_2HPO_4 \cdot 2H_2O$	1.2	1.2	1.4	0.5
$NaH_2PO_4 \cdot H_2O$	–	–	1.4	2.3
Glucose	10.0	–	10.0	5.0
Na acetate · $3H_2O$	–	–	–	36.0
Na propionate	–	–	–	15.0
Na butyrate	–	–	–	9.0
Na gluconate	6.0	–	60.0	20.9
Mannitol	1.2	1.2	0.8	0.8
HEPES	7.0	20.0	–	–
1N HCl	0.2	0.2	0.4	0.8
1N NaOH	–	6.0	–	–
pH	7.42	7.42	7.52	6.50

2.3. Analysis of rumen fluid

Concentrations of Ca and P_i in rumen fluid were analysed after centrifugation at $18{,}000 \times g$ for 20 min at room temperature by standard spectrometric techniques (Kruse-Jarres, 1979; Sarkar and Chauhan, 1967).

2.4. Ussing chamber experiments

From each gastrointestinal segment, rumen, duodenum and jejunum, six pieces of tissue per animal were mounted into Ussing chambers with an exposed area of 2.00 cm^2 (rumen tissue), or 1.13 cm^2 for the intestinal segments. Both Ussing chamber compartments were connected to circulation reservoirs filled with respective buffer solutions (Table 1) maintained at 38 °C and aerated with carbogen.

A computer-controlled voltage clamp device (Mussler Scientific Instruments, Aachen, Germany) was used to determine the electrophysiological parameters including the transepithelial potential difference (PD_t), the tissue conductances (G_t) and the short circuit currents (I_{sc}). All experiments were carried out under I_{sc} conditions. The secretory response of the intestinal segments to forskolin (10 μM, added to the serosal side) and the collapse of the ruminal PDt induced by ouabain (100 μM, added to the serosal side) were applied to control the viability of the epithelium at the end of the experiments.

2.5. Determination of Ca, P_i and mannitol flux rates

Unidirectional Ca, P_i and mannitol flux rates were determined in duodenum and jejunum, whereas in rumen tissues unidirectional flux rates of Ca and mannitol were determined. ^{45}Ca, ^{32}P and ^{3}H-mannitol were used as tracers. Of each tracer, 5 μCi for intestinal tissue and 6 μCi for ruminal tissue were added to the chambers as $^{45}CaCl_2$, $H_3{}^{32}PO_4$ and ^{3}H-mannitol (PerkinElmer Life Sciences, Rodgau-Jürgensheim, Germany), respectively, either to