

# Mechanisms and regulation of calcium absorption from the gastrointestinal tract in pigs and ruminants: comparative aspects with special emphasis on hypocalcemia in dairy cows

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## Abstract

Adequate blood calcium (Ca) concentrations are a prerequisite to maintain several physiological functions of mammals such as pig and ruminants. Thus, blood Ca levels have to be regulated within very close limits. This is basically ensured through the coordinated effects of the calcitropic hormones parathyroid hormone (PTH) and calcitriol (1,25-dihydroxyvitamin D<sub>3</sub>). If plasma Ca decreases, one main effect of subsequently secreted PTH is the stimulation of renal reabsorption of Ca from the glomerular filtrate to reduce urinary Ca loss. Another important action of PTH is the induction of the renal enzyme 1 $\alpha$ -hydroxylase, which is responsible for the production of calcitriol. In most monogastric species, so far investigated, one of the most important effects of calcitriol is to stimulate active absorption of Ca from the gastrointestinal tract, particularly the upper small intestines, via a vitamin D-receptor-mediated genomic action. Whether this concept can be transferred without any constrictions to ruminants is still under discussion. Marked interspecies differences have to be recognized with respect to ruminant or non-ruminant animals, particularly with respect to individual segments along the gastrointestinal axis and with respect to vitamin D sensitivity of Ca transport mechanisms. This review will elucidate some of the current concepts related to the mechanisms and sites of Ca absorption in pigs and ruminants with special emphasis on dairy cows where Ca homeostasis is occasionally compromised at the time of parturition.

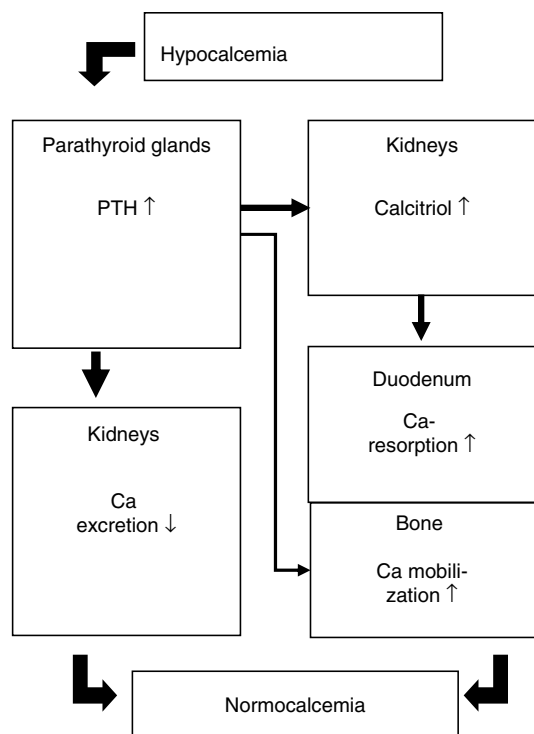
**Keywords:** pig, sheep, goat, cattle, gastrointestinal tract, calcium homeostasis, calcium absorption, calcitriol, vitamin D receptor, TRPV5, TRPV6, calbindin-D<sub>9k</sub>, PMCA

## Introduction

The role of calcium (Ca) as an essential constituent of many physiological processes is well documented. Whereas, at least in adult animals, almost 99% of total body Ca is localized within the bone (Flynn, 2003), the remaining Ca within intra- and extracellular fluids is a critical cation in numerous fundamental physiological functions. For example, Ca plays an important role as a second messenger in skeletal- and smooth-muscle

contraction (Wray *et al.*, 2005; Rossi and Dirksen, 2006), in blood clotting (Furie and Furie, 2005), in motor neuron acetylcholine release into the neuromuscular junction (Santafe *et al.*, 2005) as well as in secretory tissues like gastric, pancreatic and salivary glands (Melvin, 1999; Puscas *et al.*, 2001; Thevenod, 2002) or intestinal mucosa cells (Schröder *et al.*, 1996). Against this background it appears self-explanatory that extra- and intracellular Ca concentrations have to be regulated within very close limits. In most mammals, plasma Ca concentration is maintained between 2.2 and 2.5 mmol l<sup>-1</sup> through the coordinated effects of the calcitropic hormones parathyroid hormone (PTH) and calcitriol (1,25-dihydroxyvitamin

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**Fig. 1.** Mechanisms of the Ca homeostatic system during hypocalcemia (for details see text; ↑, increase; ↓, decrease; degrading line width of arrows symbolizes short-, middle- and long-term events, respectively).

D<sub>3</sub>) (Horst *et al.*, 2005). In case of decreased plasma Ca, one main effect of secreted PTH is to stimulate renal reabsorption of Ca from the glomerular filtrate to reduce urinary Ca loss. Another important action of PTH is the induction of the renal enzyme 1 $\alpha$ -hydroxylase, which results in increased calcitriol production (Fig. 1). In most monogastric species, so far investigated, one of the most important effects of calcitriol is to stimulate active absorption of Ca from the intestinal tract via a vitamin D receptor (VDR)-mediated genomic action (Anderson *et al.*, 2003). How far this concept can also be transferred to ruminants will be discussed later.

The mechanisms and regulation of gastrointestinal Ca absorption in pigs as a model for monogastric animals and different ruminant species have been investigated for many years and they have been more or less effectively characterized (for reviews see Horst *et al.*, 1994; Breves *et al.*, 1995, 1999; Schröder *et al.*, 2002). In principle, in both pigs and ruminants, Ca absorption from the gastrointestinal tract may be active, via the transcellular route, or passive, across paracellular pathways. Unfortunately, there is only little information on respective proportions of active and passive transport routes under different conditions, i.e. amount of dietary Ca supply, source of Ca, species, age, sex, and reproduction as well as production state. A number of studies have demonstrated that passive absorption of Ca at least in adult

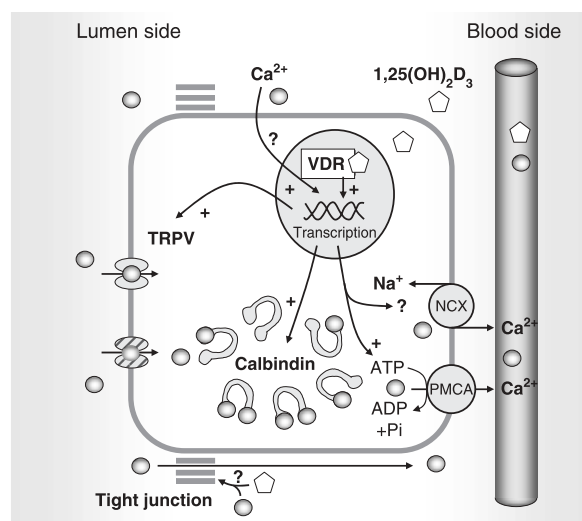
individuals dominates when Ca intake is adequate or high (Ireland and Fordtran, 1973; Bronner and Pansu, 1999), whereas others have questioned this (McCormick, 2002). Nevertheless, in growing piglets with adequate dietary Ca supply, it was shown that the active Ca transport component in the upper small intestines is necessary for maintenance of Ca homeostasis (Schröder *et al.*, 1993). On the other hand, plasma Ca could be normalized in VDR- or 1 $\alpha$ -hydroxylase knockout mice (Li *et al.*, 1998; Dardenne *et al.*, 2003) by feeding a so-called 'rescue diet' (2% Ca and 20% lactose). With normal Ca supplementation, these animals would become hypocalcemic. In cattle, oral administration of concentrated Ca supplements was effective to increase serum Ca in normo- and hypocalcemic animals within 30 min which was discussed with respect to passive Ca absorption (Horst *et al.*, 2005). However, from these studies, no conclusion can be drawn concerning the gastrointestinal site of Ca absorption. Thus, marked interspecies differences have to be recognized with respect to ruminant or non-ruminant animals, with respect to individual segments along the gastrointestinal axis and with respect to vitamin D sensitivity of Ca transport mechanisms.

## Sites and mechanisms of gastrointestinal Ca absorption

### Pigs

From a number of studies with pigs which were fitted with cannulae in the proximal duodenum and in the terminal ileum (or proximal cecum), it is well known that more than 90% of the daily Ca net absorption takes place in the small intestines, particularly in the proximal part (Partridge, 1978; Schröder, 1996). Other segments of the gastrointestinal tract, such as the stomach, cecum or colon, appear to be of minor relevance for Ca absorption at least under normal feeding conditions.

In principle, Ca absorption *in vivo* occurs in its soluble form via para- and/or transcellular pathways. Whereas, the individual proportions of distinct pathways in overall Ca net absorption in different intestinal segments are not clear, it is obvious that at least in weaning piglets as well adult pigs an intact vitamin D-hormone system is obligatory (Schröder *et al.*, 1993). This indicates that only passive Ca net absorption would not be sufficient to keep the animals in health (Schlumbohm and Harmeyer, 2004). Vice versa, significant passive contribution may be important as well. This implies potential roles of such mechanisms which are involved in modulating the paracellular permeability in Ca net absorption. The principal possibility for such a process has recently been demonstrated in human familial hypomagnesemia with hypercalciuria and nephrocalcinosis (Kausalya *et al.*, 2006). Affected patients are lacking of functioning claudin-16 as a tight junction protein of renal epithelial



**Fig. 2.** Proposed model of transcellular active Ca absorption in pig proximal small intestine. Effects of dietary Ca on gene expression (van de Graaf *et al.*, 2004) and of Ca and calcitriol on tight junction permeability (Brown and Davis, 2002; Wassermann, 2004) are under discussion (for more details see text; TRPV: epithelial Ca channels of the TRP superfamily;  $1,25(\text{OH})_2\text{D}_3$ , calcitriol; NCX,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger; PMCA, plasma membrane Ca-ATPase; Calbindin, calbindin- $\text{D}_{9k}$ ).

cells of the thick ascending limb of Henle's loop, where it plays a central role in the reabsorption of divalent cations and this contributes to impaired Ca homeostasis. Kaune (1996) reported that the addition of lactose to the diet increased the apparent digestibility of Ca in pig hindgut in the order of 25%. Although the underlying mechanisms in pig are so far unknown, theories that have been proposed on the basis of similar results from other animals include, among other effects such as Ca bioavailability, the possibility of an increased permeability of the intercellular junctions (Allen, 1982; Brommage *et al.*, 1993).

Transcellular Ca transport in the proximal small intestine in most vertebrate species so far investigated, including the pig, represents an active, well-regulated process which is stimulated by calcitriol, the biologically active form of vitamin  $\text{D}_3$  (Favus, 1985; Bronner, 1992; Kaune *et al.*, 1992; Brommage *et al.*, 1995; Bronner and Pansu, 1999; Schröder *et al.*, 2002). According to the present concept, transcellular Ca transport is generally envisaged as a three-step process, consisting of passive entry of Ca along its electrochemical gradient across the enterocyte's apical brush-border membrane via two closely related epithelial Ca channels, TRPV5 and TRPV6, subsequent calbindin- $\text{D}_{9k}$  facilitated transcytosis of Ca across the cytosol, and finally Ca pump-driven active Ca extrusion across the opposite basolateral membrane (Fig. 2, Table 1).

The apical epithelial Ca channels are two members of the super-family of transient receptor potential (TRP) channels which have recently been reviewed elsewhere

(Ramsey *et al.*, 2006). It is believed that these channels, which are constitutively active, convey an important rate-limiting step in active Ca transport and play, therefore, a pivotal role in Ca homeostasis (Van Abel *et al.*, 2005). Thus, the findings in different animal models and cell lines that the expression of TRPV5 and TRPV6 is controlled by calcitriol appears to be logical. In addition, in mice lacking a functional VDR, it was found that dietary Ca levels can exert a regulatory effect on TRPV5 and TRPV6 expression independent of calcitriol (Van Abel *et al.*, 2005). In pigs, low expression of TRPV5 and high expression of TRPV6 in intestinal mucosa was demonstrated by Northern analyses (Hinterding *et al.*, 2002). In detail, mRNA levels of both Ca channels were higher in the duodenum than in the jejunum and could not be detected in the ileum. Small but significant expression levels were observed in the proximal and distal colon. Respective data on the protein level are still missing and the findings concerning calcitriol effects on apical Ca channels are somehow contradictory. In a pig model with inherited calcitriol deficiency, hypocalcemia and clinical signs of rickets, apical Ca uptake into isolated duodenal brush-border membrane vesicles was significantly reduced indicating restricted function of apical Ca transport components. This assumption was supported by the finding that the dysfunction could be overcome by treating the animals with either calcitriol or vitamin  $\text{D}_3$  (Kaune *et al.*, 1992). In contrast, in normocalcemic piglets which were treated with vitamin  $\text{D}_3$  neither Ca uptake across the duodenal brush-border membrane nor TRPV5 or TRPV6 mRNA levels were affected (Brandenburger *et al.*, 2004). A reasonable explanation could be the assumption of a somehow compensatory mechanism due to the hypercalcemia which was found in response to increased plasma calcitriol levels in vitamin  $\text{D}_3$ -treated animals.

It has been predicted from mathematical modeling that, in addition to the apical Ca uptake process, cytosolic Ca diffusion (Ca transcytosis) may present a second rate-limiting step of epithelial Ca transport (Slepchenko and Bronner, 2001; Bronner, 2003). Ca transcytosis implies the presence of calbindin- $\text{D}_{9k}$ , a 9 kDa and vitamin D-dependent cytosolic Ca-binding protein. This protein has been proposed to facilitate the movement of Ca ions across the cytosol from the apical to the basolateral side of the enterocyte (Pansu *et al.*, 1989; Bronner, 1992, 2003; Feher *et al.*, 1992; Kumar, 1995; Christakos *et al.*, 1996). Evidence for the contribution of similar cytosolic processes has also been described for pigs (Hofmann *et al.*, 1979; Fox *et al.*, 1985; Schröder *et al.*, 1993, 1998). In chick intestine, transcytotic vesicular Ca transport has been proposed, which depends on adequate function of the cytoskeleton (Nemere and Norman, 1988; Nemere, 1992). However, it has to be kept in mind that, although discussed over nearly two decades, the exact mechanisms that are involved in so-called Ca transcytosis have not yet been completely clarified.

**Table 1.** Expression of genes for transepithelial calcium-transporting proteins in the ruminant gastrointestinal tract and in the small intestine of laboratory animals and human

Species	Segment	VDR	TRPV5	TRPV6	Calbindin-D <sub>9k</sub>	PMCA
Goat	Rumen	n.d.	n.d.	n.d.	n.d.	n.d.
	Small intestine	+ <sup>1,2</sup>	+ <sup>4</sup>	+ <sup>4</sup>	+ <sup>3</sup>	n.d.
	Colon	+ <sup>2</sup>	n.d.	n.d.	n.d.	n.d.
Sheep	Rumen	+ <sup>5</sup>	+ <sup>6</sup>	+ <sup>6</sup>	— <sup>5</sup>	n.d.
	Small intestine	+ <sup>5</sup>	+ <sup>6</sup>	+ <sup>6</sup>	+ <sup>5</sup>	+ <sup>10</sup>
	Colon	n.d.	n.d.	n.d.	n.d.	n.d.
Cattle	Rumen		n.d.	n.d.	n.d.	
	Small intestine	+ <sup>8</sup>	n.d.	n.d.	+ <sup>7,8</sup>	+ <sup>8</sup>
	Colon	+ <sup>9</sup>	n.d.	n.d.	— <sup>7</sup>	n.d.
Mouse	Small intestine	+ <sup>11</sup>	+ <sup>12</sup>	+ <sup>12</sup>	+ <sup>12</sup>	+ <sup>12</sup>
Rat	Small intestine	+ <sup>13</sup>	+ <sup>12</sup>	+ <sup>12</sup>	+ <sup>12,13</sup>	+ <sup>12</sup>
Pig	Small intestine	+ <sup>14</sup>	+ <sup>15,16</sup>	+ <sup>15,16</sup>	+ <sup>17</sup>	+ <sup>18,19</sup>
Human	Small intestine	+ <sup>20</sup>	— <sup>21</sup> + <sup>22</sup>	+ <sup>21</sup>	+ <sup>20</sup>	+ <sup>23</sup>

+, present; —, not present; n.d., not determined; TRPV5 (ECaC1, CaT2), TRPV6 (ECaC2, CaT1), Ca channels of the TRP super family; PMCA; plasma membrane Ca-ATPase.

<sup>1</sup>Schröder *et al.* (1990).

<sup>2</sup>Schröder *et al.* (1995).

<sup>3</sup>Rittmann (1996).

<sup>4</sup>K. Huber, personal communication; no differentiation between TRPV5 and TRPV6.

<sup>5</sup>Schröder *et al.* (2001).

<sup>6</sup>Wilkens *et al.* (2006).

<sup>7</sup>Yamagishi *et al.* (2002).

<sup>8</sup>Yamagishi *et al.* (2006).

<sup>9</sup>Goff *et al.* (1995).

<sup>10</sup>M. Wilkens and B. Schröder, unpublished data.

<sup>11</sup>Nakajima *et al.* (1992).

<sup>12</sup>Van Abel *et al.* (2003).

<sup>13</sup>Fukushima *et al.* (2005).

<sup>14</sup>Schröder *et al.* (1993).

<sup>15</sup>Hinterding *et al.* (2002).

<sup>16</sup>Brandenburger *et al.* (2004).

<sup>17</sup>Schröder *et al.* (1998).

<sup>18</sup>Timmermans *et al.* (1991).

<sup>19</sup>Brandenburger (2004).

<sup>20</sup>Delvin *et al.* (1996).

<sup>21</sup>Peng *et al.* (2001).

<sup>22</sup>Hoenderop *et al.* (2001).

<sup>23</sup>Howard *et al.* (1993).

Basolateral efflux of Ca occurs against a considerable electrochemical gradient, which is mainly mediated via Ca-ATPase activity ('plasma membrane Ca-ATPase', PMCA1b; Sepulveda and Mata, 2004), with only a minor contribution of the Na<sup>+</sup>/Ca<sup>2+</sup> exchange mechanism at least in pigs (Timmermans *et al.*, 1991; Kaune, 1992; Hoenderop *et al.*, 2000). Controversy exists about the influence of calcitriol on PMCA activity. Whereas van Corven *et al.* (1987) and Kaune *et al.* (1990) were unable to demonstrate calcitriol effects on duodenal PMCA in rats and pigs, respectively, Wasserman *et al.* (1992) demonstrated a 3-fold increase in Ca pump activity in the duodenum of vitamin D-repleted chicks. This could later be attributed to calcitriol-dependent PMCA gene upregulation (Pannabecker *et al.*, 1995). Studying coexpression of basolateral PMCA, calbindin-D<sub>9k</sub> and apical Ca channels in human duodenum, Barley *et al.* (2001) could

not demonstrate a relationship between plasma calcitriol levels and respective gene expression but they found that the expression of the Ca transport components were tightly linked and this appears to be useful for an effective directional Ca movement. A similar positive correlation between TRPV6 and calbindin-D<sub>9k</sub> expression was also shown for porcine proximal small intestine (Brandenburger, 2004).

Beside its well-known long-term genomic effects, calcitriol also initiates an acute enhancement of intestinal Ca transport, at least in the chick (Norman *et al.*, 1992). This rapid non-genomic calcitriol effect has been called 'transcaltachia' and was attributed to a putative membrane VDR (mVDR)-mediated event inducing transcytotic vesicular Ca transport (Nemere and Farach-Carson, 1998). However, from more recent work it was hypothesized that the physiological function of the mVDR may be to

provide relevant interaction with the classical nuclear VDR (nVDR) to fine-tune intestinal Ca absorption rather than to exert an alternative Ca transport mechanism (Nemere, 2005; Sterling and Nemere, 2005; Rohe *et al.*, 2005).

## Ruminants

### Sheep and goats

In sheep the quantitative proportions of the individual segments of the gastrointestinal tract in overall net Ca absorption have been studied in various balance experiments using single- or multi-fistulated animals (Pfeffer *et al.*, 1970; Grace *et al.*, 1974; Ben-Ghedalia *et al.*, 1975; Dillon and Scott, 1979; Rayssiguier and Poncet, 1980; Greene *et al.*, 1983a; Wylie *et al.*, 1985). The majority of these experiments showed net absorption of Ca before the duodenum in addition to the upper small intestines. Controversial data exist on net Ca absorption from the large intestines. From all balance studies cited above a mean daily Ca intake of 5.4 g and a mean daily faecal Ca excretion of 4.3 g can be calculated resulting in an overall net Ca absorption of  $1.1 \text{ g day}^{-1}$ . On average, proportions of about 50, 35 and 15% of this amount had been absorbed before the duodenum, from the small intestines and from the hindgut, respectively. In principle, the pre-duodenal absorption still has to be corrected for salivary Ca secretion and for the potential role of the abomasum, which has not yet been studied in detail with respect to its absorptive function for Ca. The potential role of the reticulorumen has also been examined by applying the washed rumen technique and a positive correlation between ruminal Ca concentration and Ca net absorption could be demonstrated (Höller *et al.*, 1988).

Active mechanisms have been shown to make significant contributions to Ca transport in caprine as well as ovine rumen, by measuring Ca flux rates in Ussing chambers (Schröder *et al.*, 1997, 1999; Wadhwa and Care, 2000; Uppal *et al.*, 2003). These mechanisms could only be detected when short chain fatty acids (SCFAs) were present in the luminal buffer solutions. This has been explained by the assumption that a  $\text{Ca}/\text{H}^+$  exchange mechanism could be involved in transepithelial Ca transport as it has already been discussed for rat colon (Lutz and Scharrer, 1991). Concerning this, it has been proposed that non-dissociated SCFAs can easily pass the apical membrane of the rumen epithelium via diffusion into the cytosol where they may readily dissociate to deliver protons which can be used for  $\text{Ca}/\text{H}^+$  exchange (Schröder *et al.*, 1999).

The contribution of classical vitamin D-dependent components in ruminal Ca absorption appears to be a matter of species affiliation. In goats, it was found that ruminal net Ca flux rates were significantly stimulated by more than 50% when the animals had been kept on a long-term low Ca diet (Schröder *et al.*, 1997). Due to this

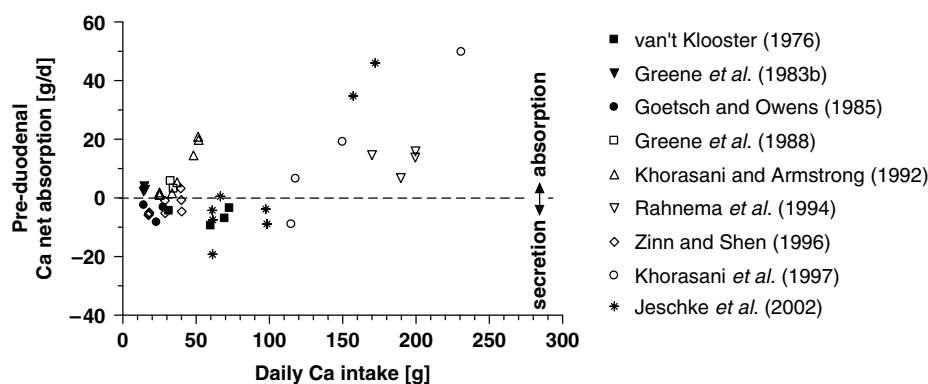
procedure, plasma calcitriol concentrations were elevated by about 80%, which suggests the presence of classical vitamin D-dependent regulatory processes for active Ca absorption from the rumen, the more so as net Ca absorption could be significantly inhibited by the Ca channel blocker verapamil. Thus, future experiments should focus on structural identification of Ca transport components in goat rumen. In contrast to the findings in goats, dietary Ca depletion and increased plasma calcitriol in sheep as well as pharmacological treatment of the animals with vitamin D<sub>3</sub> had no effect on ruminal net Ca absorption (Schröder *et al.*, 1999, 2001). In addition, calbindin-D<sub>9k</sub> could not be detected in ovine ruminal tissues indicating vitamin D insensitivity of active Ca transport across the rumen wall of sheep (Table 1).

Concerning Ca transport in the intestinal segments, it has often been suggested in the past and today from several *in vivo* studies that in analogy to the situation in pigs, Ca absorption is under the control of the vitamin D-hormone system (for reviews see Horst, 1986; Reinhardt *et al.*, 1988; Horst *et al.*, 2005; Goff, 2006). Two relevant studies supporting this hypothesis may be emphasized. Braithwaite (1978) treated lactating sheep with high doses of  $1\alpha$ -hydroxyvitamin D<sub>3</sub> and found an increase of Ca net absorption from the gastrointestinal tract of 40–90 mg per kg per day. Unfortunately, from the experimental design no conclusion could be drawn as to which segment might have been responsible for the effect. Hove and Kristiansen (1984) could demonstrate an approximately 3-fold increase of Ca net absorption from the intestinal tract in goats receiving calcitriol *per os* in a fatty acid matrix. Furthermore, the assumption of vitamin D-sensitive Ca absorption is supported by the detection of VDR and calbindin-D<sub>9k</sub> in goat small intestine (Schröder *et al.*, 1995; Rittmann, 1996). In addition, gene expression of epithelial Ca channels was demonstrated for the jejunum (Huber, unpublished observation). In sheep, VDR as well as calbindin-D<sub>9k</sub> was found in the jejunum (Schröder *et al.*, 2001) and more recently, the presence of TRPV5 and TRPV6 in duodenum and jejunum could be demonstrated on mRNA level with an *in situ* hybridization technique (Table 1; Wilkens *et al.*, 2006). But the relatively low net flux rates of Ca in the proximal small intestine of sheep and goats obtained from *in vitro* measurements in Ussing chambers, and the lack of significant stimulation of duodenal Ca transport by increased plasma calcitriol during dietary Ca depletion may argue against the 'monogastric concept' (Schröder *et al.*, 1997; Vössing 1997).

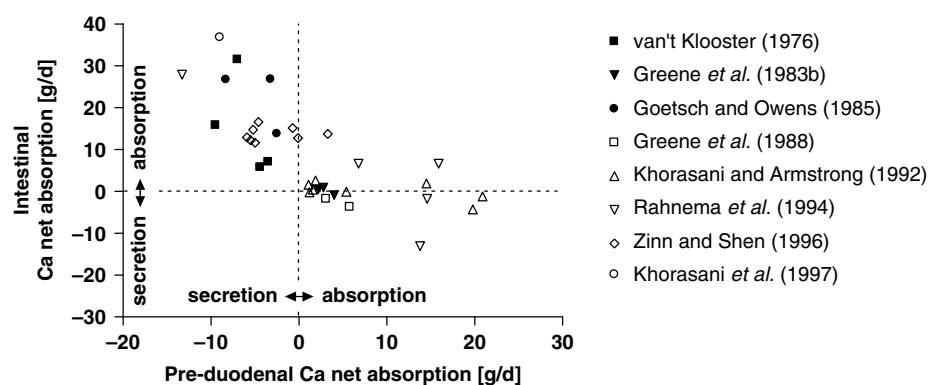
### Cattle

In analogy to the studies in fistulated sheep, considerable efforts have been made to characterize the quantitative proportions of the individual segments of the gastrointestinal tract in overall net Ca absorption in cattle. Similar to the situation in sheep, the pre-duodenal proportion seems to become more prominent if the daily





**Fig. 3.** Pre-duodenal (including reticulorumen, omasum and abomasum) Ca net absorption as a function of daily dietary Ca intake in cattle (analysis from the literature).



**Fig. 4.** Intestinal (including small intestines, cecum and colon) Ca net absorption as a function of pre-duodenal Ca net absorption (analysis from the literature).

dietary Ca intake exceeds a certain amount. From the analysis shown in Fig. 3, which is based on nine comparable studies, this 'set-point' may be in the order of a daily dietary Ca intake of 120 g. Concerning this, it should be noticed that Lean *et al.* (2006) demonstrated in a recent meta-analysis of data from 137 published trials involving 2545 calvings that prevention of milk fever appears to be possible at high Ca intake. This could mean that the absorption of Ca from the pre-duodenal compartments may play a significant role in prevention of milk fever at high Ca intake.

Although the presence of active mechanisms for Ca transport could also be verified for bovine rumen (Ricken, 2005), it is unknown at present to what extent passive and active pathways may contribute to overall Ca net absorption from pre-duodenal compartments. In another diagram, the proportion of the intestines in Ca net absorption as a function of the pre-duodenal proportion is evaluated (Fig. 4). It appears, that there is a negative correlation between pre-duodenal and intestinal Ca net absorption. This means that if there is Ca net secretion into the forestomachs/abomasum compartment, this will be at minimum compensated by respective intestinal Ca

uptake resulting in a positive Ca balance in most of those experiments. On the other hand, if there is already relevant Ca net absorption from the forestomachs, Ca absorption from the intestines was lowered. This again implies the contribution of regulatory mechanisms. As in small ruminants, this may be mediated by components of the vitamin D-hormone system, since expression of genes for transepithelial Ca-transporting proteins (VDR, calbindin- $D_{9k}$  and PMCA; Table 1) in bovine duodenum could be demonstrated (Yamagishi *et al.*, 2006). In addition, this assumption is supported by the findings that intramuscular application of calcitriol in cows could increase Ca levels in plasma of dairy cows (Gang *et al.*, 1979; Hove *et al.*, 1983; Yamagishi *et al.*, 2005).

## Conclusions

In summary, in the rumen, active Ca absorption may be exerted via vitamin D-sensitive and/or -insensitive mechanisms, and this appears to be species-dependent. Marked contribution of Ca net absorption via passive pathways particularly in the situation of a high daily

dietary Ca intake has to be kept in mind, and for principal reasons this is also true for the abomasum and particularly for the 'leaky' intestinal segments. Whether passive Ca uptake is a matter of regulation has to be investigated. With respect to the intestines, it is still a matter of debate whether the proximal small intestines are a major site for controlled active Ca absorption as it is in monogastric species. But from recent molecular studies, it now becomes stepwise clearer that at least the single components which are proposed to be involved in transcellular Ca movement are also present in ruminants. With these molecular tools, it appears to be more than promising to examine in comprehensive future studies the potential regulation of gastrointestinal Ca absorption in pigs and ruminants and the factors that may be involved.

### Role of the gastrointestinal tract in Ca homeostasis during the periparturient period in dairy cows

Provided an adequate dietary Ca supply, mechanisms for maintaining physiological plasma Ca concentrations in dairy cows function adequately most of the time, particularly during the dry period when the Ca homeostatic system is not further compromised (Horst *et al.*, 1994). The processes involved in maintaining Ca homeostasis may principally be assigned to short- (within minutes), middle- (within hours) and long-term (within days) events (Kurbel *et al.*, 2003). Small decreases of plasma Ca levels would be answered rather rapidly by an adequate increase of PTH secretion by the parathyroid glands (Fig. 1). PTH works as a kind of 'Ca saver' by stimulating renal Ca reabsorption which may restore the Ca homeostasis, subsequently PTH concentrations return to former low levels. If the Ca demand is more pronounced, PTH levels would remain elevated over a longer time, thereby leading to an increased activation of calcitriol synthesis in the kidneys. Main function of calcitriol is the stimulation (hours to days) of active Ca absorption across the small intestinal mucosa via a complex mechanism (Fig. 2). Another important function of PTH and calcitriol is their concerted action on Ca mobilization from bone which may take a few days. This may be concluded from the time courses of bone resorption markers in hypocalcemic dairy cows (Liesegang *et al.*, 1998). With respect to the PTH action on bone and kidneys, it appears worth mentioning that some evidence exists that the respective PTH receptor sensitivity is pH-sensitive. Lowering pH, as it can be induced by feeding low dietary cation anion difference (DCAD) diets during the prepartum period can increase the target tissue responsiveness to PTH thereby allowing stimulation of renal calcitriol production and bone Ca resorption (Horst *et al.*, 2005). This may permit the cow to successfully adapt to the Ca stress associated with the onset of lactation.

In principle, the Ca saving mechanisms hold even true for the non-lactating, pregnant dairy cow when daily fetal Ca transfer increases up to  $\sim 80 \text{ mg kg}^{0.75}$  by drain to the fetus in late pregnancy (Horst *et al.*, 2005). Although this for sure exhibits an additional stress for the Ca homeostatic system, the animals are normally able to adapt to the increasing requirements during gestation. Concerning this, it has to be kept in mind, that irrespective of their Ca intake, the animals are unable to absorb enough dietary Ca at least in late pregnancy (and early lactation) to meet the growing demands, and skeletal stores of Ca have to be mobilized to make good the deficit (Braithwaite, 1983; Liesegang *et al.*, 2005). At parturition, Ca homeostasis is abruptly burdened additionally by the need to provide huge amounts of Ca for lactation (Neville, 2005) which leads to daily Ca losses of  $\sim 500 \text{ mg kg}^{0.75}$  (Horst *et al.*, 2005). Particularly colostrum contains 1.5–2 g Ca per liter milk (Bojkovski *et al.*, 2005). To meet this demand, remarkable adaptive processes must occur. Regarding this, no quantitatively relevant effect of the kidneys can be expected since renal Ca losses between  $0.5$  and  $2 \text{ g day}^{-1}$  are already relatively low in cows under normocalcemic conditions (Horst *et al.*, 2005), and in addition renal Ca excretion is already further restricted during late gestation (Ulutas *et al.*, 2003). Thus, the only Ca stores which can be recruited are Ca mobilization from bone and increased Ca absorption from the gastrointestinal tract. However, both these mechanisms are already stressed during late gestation and particularly stimulation of gastrointestinal Ca absorption seemed to be somehow restricted. From this, it is not surprising that even under the physiological condition most of the cows develop an acute hypocalcemia during the first hours after parturition, which, however, is normally recovered by the Ca homeostatic system during the first week of lactation (Tucker *et al.*, 1992; Andersen *et al.*, 2005). If animals fail to adapt they develop strong hypocalcemia, which is referred to as milk fever. Milk fever generally occurs 12–24 h after parturition and is a metabolic disease almost exclusively in ruminants, particularly in dairy cows (Horst *et al.*, 2005).

The pathophysiology of this disease and current concepts for the prevention of milk fever have been described intensively elsewhere in the past and shall therefore not be recapitulated here (i.e. Horst, 1986; Horst *et al.*, 1994, 2005; Thilting-Hansen *et al.*, 2002). From a physiological viewpoint as was the intention of this review, it appears to be worthwhile in future studies to focus on the conditions and factors which determine and fine regulate Ca absorption in different compartments of the gastrointestinal tract of ruminants. This includes also such factors that may determine Ca bioavailability, which is often rather low, i.e.  $<50\%$  (Martz *et al.*, 1999), particularly in ruminants. This represents marked dietary Ca resources which potentially could be used more efficiently by the animals in case Ca homeostasis is impaired.

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