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Luminal Glucose Does Not Enhance Active Intestinal Calcium Absorption in mice: Evidence Against a Role for Cav1.3 as a Mediator of Calcium Uptake During Absorption

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Abstract

Intestinal Ca absorption occurs through a 1,25 dihydroxyvitamin D₃ (1,25(OH)₂D₃)-regulated transcellular pathway, especially when habitual dietary Ca intake is low. Recently the L-type voltage-gated Ca channel, Ca_v1.3, was proposed to mediate active, transcellular Ca absorption in response to membrane depolarization caused by elevated luminal glucose levels following a meal. We tested the hypothesis that high luminal glucose could reveal a role for Ca_v1.3 in active intestinal Ca absorption in mice. Nine week-old male C57BL/6J mice were fed AIN93G diets containing either low (0.125%) or high (1%) Ca for 1 week and Ca absorption was examined by an oral gavage method using a ⁴⁵Ca-transport buffer containing 25 mmol/L of glucose or fructose. Transient receptor potential vanilloid 6 (TRPV6), Calbindin D_{9k} (CaBPD_{9k}) and Ca_v1.3 mRNA levels were measured in the duodenum, jejunum and ileum. TRPV6 and CaBPD_{9k} expression were highest in the duodenum, where active, 1,25(OH)₂D₃-regulated Ca absorption occurs while Ca_v1.3 mRNA levels were similar across the intestinal segments. As expected, the low Ca diet increased renal cytochrome p450-27B1 (CYP27B1) mRNA (p=0.003), serum 1,25(OH)₂D₃ (p<0.001) and Ca absorption efficiency by 2-fold with the fructose buffer. However, the glucose buffer used to favor Ca_v1.3 activation did not increase Ca absorption efficiency (p=0.6) regardless of the dietary Ca intake level. Collectively, our results show that glucose did not enhance Ca absorption and they do not support a critical role for Ca_v1.3 in either basal or vitamin D-regulated intestinal Ca absorption *in vivo*.

Keywords

mouse; Ca_v1.3 calcium channel; Ca absorption; transcellular Ca transport; 1,25-Dihydroxyvitamin D₃

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1. Introduction

Intestinal Ca absorption is part of a multi-tissue axis whose coordinated actions regulate whole body Ca metabolism to maintain serum Ca levels within a very narrow range [1]. The most important nutritional regulator of intestinal Ca absorption is habitual dietary Ca intake. Low Ca intake increases renal production of the vitamin D hormone, 1,25 dihydroxyvitamin D₃ (1,25(OH)₂D₃), which then works through the vitamin D receptor (VDR) to increase Ca absorption efficiency [2-4]. Ca absorption occurs through two routes, a paracellular, passive route and a transcellular, active pathway. The paracellular pathway is directly proportional to luminal Ca concentration and predominates when Ca intake is high [5]. The transcellular pathway is regulated by 1,25(OH)₂D₃ and is activated when habitual dietary Ca intake is low [6]. The facilitated diffusion model has been proposed to explain 1,25(OH)₂D₃-regulated, transcellular Ca absorption. In this model, Ca uptake at the apical membrane of the enterocyte is mediated by the transient receptor potential vanilloid-family member 6 (TRPV6). Once in the enterocyte, calbindin D_{9k} (CaBPD_{9k}) works as a ferry protein to transport Ca across the enterocyte. Finally, extrusion of Ca is mediated by a Plasma Membrane Ca ATPase, PMCA1b [7]. The mRNA levels for TRPV6, CaBPD_{9k}, and PMCA1b are all increased in response to 1,25(OH)₂D₃ treatment suggesting that their upregulation mediates the impact of vitamin D on Ca absorption [3]. Although intestine-specific transgenic expression of TRPV6 is sufficient to restore normal Ca absorption and rescue the abnormal bone phenotype of VDR knockout mice [8], the validity of the facilitated diffusion model has been challenged by studies showing that basal and vitamin D-regulated Ca absorption is not eliminated in TRPV6 [9] and CaBPD_{9k} [10] knockout and double knockout mice [11]. Similarly, we recently showed that while Ca absorption efficiency is significantly positively correlated with both TRPV6 and CaBPD_{9k} mRNA in a genetically diverse population of 11 inbred mouse lines, this association is weak [12]. Collectively, these data suggest that other proteins may contribute to the active intestinal calcium transport.

Recently, Kellett [13] proposed a model in which the L-type voltage-dependent Ca channel Ca_v1.3, mediates transcellular Ca transport by complementing TRPV6 action in the enterocyte. In this model, Ca absorption is regulated when Ca_v1.3 is activated by glucose-mediated membrane depolarization that occurs following meals. Traditionally, Ca absorption experiments are performed in fasted animals to remove the confounding influence of residual diet on the absorption tests. In these studies, the transport solutions used to assess active Ca transport contain fructose (10 mmol/L), a sugar that does induce membrane depolarization [14] rather than glucose or other depolarizing nutrients. Kellett [13] suggested that this approach emphasizes the role of TRPV6 and proposed that the role of Ca_v1.3 could be revealed if an adequate concentration of luminal glucose (20 mmol/L) was present in the Ca transport solution used for experiments. While this proposal is interesting, its physiological relevance has not been confirmed. Based on this proposed model we hypothesized that the use of a Ca transport buffer with 20 mmol/L glucose will induce membrane depolarization leading to an increase in Ca absorption through Ca_v1.3. To test this hypothesis we examined Ca absorption *in vivo* by administering a ⁴⁵Ca-transport buffer containing 25 mmol/L of glucose or fructose (control) by oral gavage in male

C57BL/6J mice. In addition, we fed the mice either a high or low Ca diet to induced changes in $1,25(\text{OH})_2\text{D}_3$ production and vitamin D-mediated active transport; the purpose of this manipulation was to determine whether the role of $\text{Ca}_v1.3$ would be seen under conditions where vitamin D signaling was low (i.e. the high Ca diet group) or high (i.e. the low Ca diet group). This work has allowed us to investigate the role of $\text{Ca}_v1.3$ in active intestinal Ca absorption under physiologically relevant conditions.

2. Methods and materials

2.1 Experimental Design

Male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were raised from weaning until 9 weeks of age on a commercial chow diet and then were switched to AIN93G-based diets (Research Diets, Inc., New Brunswick, NJ USA) (**Table 1**) containing 1000 IU vitamin D/kg, 0.4% P (4 g/kg), and low (0.125%; 1.25 g/kg) or high (1%; 10.2 g/kg) Ca for 1 week. We have previously shown that 1 week is sufficient to increase Ca absorption in response to changes in dietary Ca level in mice [15]. Food and water were provided *ad libitum* and mice were housed in an UVB light-free environment on a 12 h-light/dark cycle. Mice were deprived of food overnight prior to the experiment and Ca absorption was examined by oral gavage as described below. After the 10 min absorption test, mice were bled by cardiac puncture to obtain serum samples for analysis of ^{45}Ca and $1,25(\text{OH})_2\text{D}_3$. Intestinal segments were obtained and rinsed in Phosphate Buffered Saline + 5 mmol/L EGTA. Mucosal scrapings of intestinal sections and minced kidneys were collected into TriReagent (Molecular Research Center, Inc., Cincinnati, OH) and frozen in liquid nitrogen for later analysis of mRNA levels. Duodenum was defined as the 2 cm segment starting 0.5 cm after the pyloric sphincter; jejunum was the 3 cm segment starting 4.5 cm from the pyloric sphincter; distal ileum was the 4 cm segment starting 0.5 cm from the cecum and proximal ileum was the 4 cm segment starting 10 cm from the cecum (n = 10-12 mice per diet group). A small group of 4-month old mice (n=3) was used to evaluate the transit distance of the oral dosing solution through the intestine during the Ca absorption test. All of the experiments were approved by the Purdue University Animal Care and Use Committee.

2.2 Oral Gavage test for intestinal Ca absorption

Mice were deprived of food overnight prior to the evaluation of intestinal Ca absorption. On the morning of the test, mice were anesthetized with a cocktail of ketamine (22 mg/mL) and xylazine (33 mg/mL) (0.1 mL/20 g body weight). Ca absorption was examined by an oral gavage method originally reported by Van Cromphaut *et al.* [16] and used by us elsewhere [12]. Briefly, mice were given an oral gavage of a solution containing 0.1 mmol/L CaCl_2 , 125 mM NaCl, 17 mM Tris Base, enriched with 20 μCi $^{45}\text{CaCl}_2/\text{ml}$ (Perkin Elmer, Waltham, MA), and containing 25 mmol/L of either glucose or fructose, (10 L of buffer per g of body weight) (n=9-12 mice from each diet group per buffer). Blood was collected in live anesthetized animals 10 minutes after administration of the dosing solution using the GoldenRod lancet (Medipoint, Inc., Mineola, NY) to puncture the submandibular vein. Serum was isolated (10 μL) and bleached for 30 min, pH was neutralized and samples were analyzed using a liquid scintillation counter (Beckman LS 6500; Beckman Coulter Inc.,

Fullerton CA) for 1 min. The change in the serum calcium concentration was calculated from the ^{45}Ca content of the serum and the specific activity of the administered calcium.

2.3 Evaluation of the Transit distance for the oral dosing solution in the Ca absorption test

A role for $\text{Ca}_v1.3$ has been described in the mid and distal jejunum [17] while TRPV6 expression and function is most prevalent in the duodenum [8]. We evaluated whether the oral dosing solution was able to reach the segments of the intestine where $\text{Ca}_v1.3$ and TRPV6 function are maximal. Four month old mice were subjected to the oral gavage test using a buffer solution (0.1 mmol/L CaCl_2 , 125 mmol/L NaCl, 17 mmol/L Tris Base, and 10 mmol/L fructose) containing 5% Coomassie blue (pH 6.3) at a dose of 10 L per g body weight. After 10 minutes the animals were killed and the stomach and intestine were examined for the distance traveled by the dosing solution. The location of the Coomassie blue in the intestine was measured by visual inspection.

2.4 Gene Expression

RNA from kidney and intestine sections was isolated from TriReagent according to the manufacturer's directions (Molecular Research Center Inc., Cincinnati, OH). cDNA was prepared and real time PCR was conducted as we have reported elsewhere [8]. The primers and PCR conditions used to measure the mRNA level for the housekeeping and intestinal and renal genes have been previously reported: RPLP0 [8], TRPV6, CaBP_{9k} [3], $\text{Ca}_v1.3$ [18], Claudin 2, CLDN2 [19], Claudin 12, CLDN12 [20] and, cytochrome p450-27B, CYP27B1 [21].

2.5 Serum $1,25(\text{OH})_2\text{D}_3$

Serum $1,25(\text{OH})_2\text{D}_3$ was measured by radioimmunoassay (ImmunoDiagnostic Systems, Scottsdale, AZ) (n=12 per diet group) according to the manufacturer's instructions as we have previously reported [22].

2.6 Statistical Analyses

To exclude potential outliers, extreme values were identified using a z-score with a 2.5% cut off in either tail of the distribution of experimental groups. If data were not normally distributed, the BOXCOX procedure was performed to identify the optimal transformation to correct this defect. The following transformations were made: serum $1,25(\text{OH})_2\text{D}_3$ ($y^{0.25}$); and TRPV6 mRNA, CaBP_{9k} mRNA, CLDN2 mRNA, and CYP27B1 mRNA (natural log). Main effects for Ca absorption (dietary Ca, transport buffer sugar) and intestinal gene expression (dietary Ca, tissue effect), and their interaction were analyzed by two-way ANOVA using SAS enterprise 4.2 (SAS Institute, Inc., Cary, NC). Differences between individual means were determined by the Tukey-Kramer multiple comparisons test only if the overall F statistic was significant. For serum $1,25(\text{OH})_2\text{D}_3$ and renal CYP27B1 mRNA, the *student's t* test was used to determine significant differences between dietary groups. Differences were considered significant when $p < 0.05$. Data are expressed as means $\pm$ SEM. The sample size for each treatment group was calculated using variance estimates from our published data and $\alpha = 0.05$ and $\beta = 0.8$. Our analysis indicated that with n=12 mice

per group we could detect a 30% difference between groups for Ca absorption and with n=8 mice we could observe the same difference in gene expression across our groups.

3. Results

3.1 Transit distance for the oral dosing solution in the Ca Absorption test

Ten minutes after administration of the Coomassie blue-containing buffer by oral gavage the solution had traveled 11.8 ± 0.7 cm and was seen in the duodenum, jejunum and the start of proximal ileum (data not shown). This demonstrates that the segments proposed to contain TRPV6 or $\text{Ca}_v1.3$ were exposed to the transport solution.

3.2 The impact of dietary Ca on Vitamin D metabolism

Compared to the 1% Ca diet, the 0.125% Ca diet significantly increased CYP27B1 mRNA levels in the kidney (**Figure 1A**, $p=0.003$) and caused a 5-fold increase in serum $1,25(\text{OH})_2\text{D}_3$ (**Figure 1B**, $p<0.001$).

3.3 Intestinal Expression of Genes Proposed to Mediate Intestinal Ca Absorption

The expression of traditional $1,25(\text{OH})_2\text{D}_3$ -target genes (TRPV6, CaBPD_{9k}), two tight junction proteins proposed to mediate intestinal Ca absorption (CLDN2 [23] and CLDN12 [20]), and $\text{Ca}_v1.3$ were characterized in the duodenum, jejunum, proximal ileum, and distal ileum of mice fed the experimental diets for 1 week. Results from the 1% Ca diet group reveal the basal levels of gene expression in the intestine of growing mice. In this group, the highest expression of TRPV6 and CaBPD_{9k} mRNA was found in duodenum and very low expression was seen in the jejunum and ileum. CLDN2 mRNA levels were high in the distal ileum and duodenum while CLDN12 mRNA levels were moderately higher in the jejunum and had their lowest expression in ileum (**Figure 2**). In contrast, $\text{Ca}_v1.3$ mRNA levels were uniform across the small intestinal segments. When mice were placed on the low Ca diet (0.125% Ca), only the duodenal mRNA levels of TRPV6 and CaBPD_{9k} were strongly upregulated (26.6-fold and 4.1-fold, respectively, **Table 2**). In contrast, $\text{Ca}_v1.3$ mRNA was modestly upregulated only in the distal ileum (60% increase) (**Table 2**). While CLDN2 and CLDN12 mRNA expression has been reported to be increased by $1,25(\text{OH})_2\text{D}_3$ treatment *in vitro* [23], we did not observe an increase in CLDN2 or CLDN12 mRNA levels in response to the physiological increase in serum $1,25(\text{OH})_2\text{D}_3$ induced by low dietary Ca. These results are consistent with our published data showing that CLDN2 and CLDN12 mRNA levels in duodenum and colon of male mice were not affected by $1,25(\text{OH})_2\text{D}_3$ when fed a low Ca diet (0.25% Ca) from weaning to 3 months of age [24].

3.4 Impact of dietary Ca level and luminal sugars on Ca Absorption

Kellett hypothesized that a role for $\text{Ca}_v1.3$ will be revealed when glucose is used in luminal Ca transport solutions [13]. We tested this hypothesis under dietary Ca intakes that are known to increase (low Ca diet) or suppress (high Ca diet) vitamin D-regulated transcellular Ca absorption [3]. As we have previously reported [12], compared to high Ca intake (1% Ca), low Ca intake (0.125% Ca) significantly improved Ca absorption efficiency (+200%, **Figure 3**). However, Ca absorption was not significantly affected when a glucose-containing

buffer was used to promote membrane depolarizing conditions in mice fed either the high (p= 0.94) or low Ca diet (p=0.57) (Figure 3).

4. Discussion

Recently, the facilitated diffusion model has been challenged by observations from knockout mouse models [9, 11, 25]. These studies suggest that proteins other than those from the facilitated diffusion model may be involved in active intestinal calcium transport. One such candidate is the L-type voltage-gated Ca channel $\text{Ca}_v1.3$, a protein that was proposed to mediate Ca uptake into enterocytes after glucose mediated membrane depolarization [13]. In this study we used a high glucose Ca transport buffer to test whether $\text{Ca}_v1.3$ contributes to active intestinal Ca absorption. However, our data do not support this hypothesis. We found that although $\text{Ca}_v1.3$ is expressed throughout the small intestine, the high glucose buffer did not increase Ca absorption efficiency *in vivo*.

The $\text{Ca}_v1.3$ hypothesis was proposed based on several lines of evidence. First, some studies have shown that pharmacologic inhibition of L-type channels reduced Ca absorption in perfused rat jejunal loops [17, 26] or everted intestinal sacs [27, 28]. However, other studies show inconsistent [29, 30] or no effects of L-type channel inhibitors on intestinal Ca absorption [31, 32]. Second, $\text{Ca}_v1.3$ protein levels are highest from proximal jejunum to mid ileum of rats [17, 26] and thus, $\text{Ca}_v1.3$ could be the L-type channel mediating Ca absorption in these segments. Third, while the capacity to absorb Ca is highest in the duodenum [5], Morgan *et al.* [17] argued that due to the long residence time for Ca and the depolarizing environment in the distal small intestine, the high levels of $\text{Ca}_v1.3$ in the distal jejunum and ileum would permit high levels of Ca absorption in these segments.

A critical aspect of the $\text{Ca}_v1.3$ model is related to the reported ability of glucose to enhance Ca absorption [13]. Morgan *et al.* have shown that Ca uptake through $\text{Ca}_v1.3$ results from membrane depolarization induced by transport through the sodium-glucose co-transporter, SGLUT1 [17] and they have shown that 20 mmol/L of glucose is sufficient to induce membrane depolarization and to increase Ca absorption in perfused rat jejunal loops [33]. Traditionally, Ca absorption tests are performed in fasted animals using transport buffers containing 10 mmol/L fructose. While glucose absorption through SGLUT1 [34] results in depolarization of the intestinal epithelial cell membrane [14], fructose absorption is a passive, Na-independent process that occurs through GLUT5 [35, 36] and does not result in membrane depolarization [14]. Thus, Kellett [13] argued that the failure to appreciate the role of $\text{Ca}_v1.3$ in intestinal Ca absorption is an artifact of the methods used to study intestinal Ca absorption. However, despite our efforts to implement experimental conditions recommended as necessary to reveal the role of $\text{Ca}_v1.3$ in Ca absorption (i.e. transport buffers with 25 mmol/L glucose, exposure of intestinal segments with high $\text{Ca}_v1.3$ expression to the transport buffers), glucose did not increase Ca absorption compared to an equimolar amount of fructose. In addition, although studies on glucose-induced Ca absorption in diabetic rats suggest that the effect of glucose on Ca absorption is vitamin D-independent [37, 38], we were unable to observe a benefit of glucose on Ca absorption even when $1,25(\text{OH})_2\text{D}_3$ levels were low due to consumption of high Ca diets.

The central feature of the argument in favor of $\text{Ca}_v1.3$ in intestinal Ca absorption depends upon glucose-mediated membrane depolarization. However, the impact of physiological oral glucose levels on Ca absorption has been inconsistent across published studies. Wood *et al.* [39] reported that Ca absorption from a 7.2 mmol/L CaCl_2 solution was increased by 20% in healthy males and females when delivered orally along with 1.1 mol/L glucose. Similarly, Zheng and Wood [40] reported an increase in Ca absorption in rats when solutions containing 90 mmol/L CaCl_2 and 4.4 mol/L of glucose (up 67%) or a glucose polymer (up 75%) were administered by stomach gavage. However, since the luminal concentration of glucose in the small intestine is < 100 mmol/L even after a meal [41], the supra-physiological concentrations of glucose in these two studies could be an artifact resulting from sugar-induced hyperosmolarity within the lumen. This phenomenon was previously described by Pansu *et al.*, [42] who found that absorption of a 50 mmol Ca/L solution was significantly increased in rat jejunal loops by the presence of 200 mg/ml of glucose or xylose (1.1 mol/L and 1.3 mol/L, respectively) but not with lower concentrations of either Ca or the sugar. Since the effect was seen only for high Ca concentrations, the authors ascribed the effect of sugars to an impact on the paracellular, rather than the transcellular, Ca transport pathway. It is important to note that in our experiment we used a low Ca concentration (0.1 mmol/L of Ca) in our absorption buffers to minimize the role of passive Ca absorption. In a clinical trial using physiological doses of 56 and 222 mmol glucose/L, Knowles *et al.* [43] found that compared to a control absorption test conducted with no sugar, glucose increased fractional Ca absorption of a 5 mmol Ca/L solution in healthy females. However, these results are the opposite of what Francis and Peacock [44] observed in healthy postmenopausal women given a lower carrier load (0.5 mmol/L CaCl_2).

Several earlier observations also directly refute critical elements of the $\text{Ca}_v1.3$ hypothesis. For example, Carroll *et al.* [45] measured Ca transport in intact rat intestinal mucosa with Ussing chambers and found that glucose could increase Ca transport in duodenal tissue but not in the ileal tissue where $\text{Ca}_v1.3$ protein levels are high [17, 26] but where active Ca absorption been previously reported to be minimal [5]. Thus, at least one segment where $\text{Ca}_v1.3$ resides was not responsive to luminal glucose. Carroll *et al.* also reported that α -methylglucoside, a nonmetabolizable glucose analog that is exclusively transported by SGLUT1, did not stimulate Ca transport in intact duodenal mucosa or net Ca uptake by isolated enterocytes. This shows that membrane depolarization caused by SGLUT1-mediated glucose transport [34] is not sufficient to increase Ca uptake in enterocytes. Additionally, despite the fact that $\text{Ca}_v1.3$ is expressed in intestine and in osteoblasts, female $\text{Ca}_v1.3$ knockout mice have normal bones [46]. This shows that $\text{Ca}_v1.3$ does not have a major role in whole body Ca homeostasis. Since intestinal VDR deletion is known to dramatically reduce intestinal Ca absorption leading to severely abnormal bone growth in mice [22, 47], the $\text{Ca}_v1.3$ knockout mouse data indirectly suggests that $\text{Ca}_v1.3$ is not essential for intestinal Ca absorption.

While our data do not support an essential role for $\text{Ca}_v1.3$ in intestinal Ca absorption there are several limitations to our work. For example, we used only one glucose concentration and we did not extend the concentration to the full range that can be observed directly after a meal (i.e. 100 mmol/L). Related to this, we were unable to directly measure intestinal

membrane depolarization *in vivo*. Thus, while we used concentrations of glucose that others have reported cause intestinal membrane depolarization, we cannot exclude the possibility that higher, yet still physiologic luminal levels of glucose could enhance Ca absorption. It is also possible that our Ca absorption test was too short (10 min absorption period). However, Morgan *et al.* [17] showed that Ca absorption in rat jejunal perfusates increased significantly after 10 min incubation with 20 mmol/L glucose. These findings suggest that if polarization of the membrane and activation of the Ca_v1.3 was induced, we would have been able to detect differences in Ca absorption with our test.

In conclusion, while a logical argument regarding a potential role for Ca_v1.3 as a mediator of intestinal Ca absorption was proposed [13], our data does not support this hypothesis and we have rejected it. A careful review of the literature provides further support for our conclusion. The role of glucose on calcium absorption is more likely to be related to enhancement of paracellular movement due to an osmotic effect rather than due to activation of jejunal and ileal epithelial cell depolarization and transcellular Ca movement resulting from uptake through the Ca_v1.3 channel.

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ABBREVIATIONS

Ca_v1.3	Calcium voltage-gated channel 1.3
1,25(OH)₂D₃	1,25 dihydroxyvitamin D ₃
Ca	Calcium
VDR	Vitamin D receptor
TRPV6	Transient receptor potential vanilloid 6
CaBPD_{9k}	Calbindin D _{9k}
CLND2	Claudin 2
CLDN12	Claudin 12
CYP27B1	cytochrome p450-27B1
KO	Knock Out

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Highlights

- Activation of $\text{Ca}_v1.3$ has been proposed to increase active Ca absorption
- $\text{Ca}_v1.3$ activation depends upon glucose-mediated membrane depolarization
- We examined the $\text{Ca}_v1.3$ role and relevance in active Ca absorption *in vivo* in mice
- The presence of glucose did not increase active Ca absorption in mice
- $\text{Ca}_v1.3$ is not critical for active intestinal Ca absorption *in vivo* in mice

Figure 1. CYP27B1 mRNA levels in kidney (A) and serum 1,25(OH)₂D₃ (B) in response to dietary Ca

C57BL/6J mice were fed AIN93G diets with either 1% (high) or 0.125% (low) Ca content for one week. Serum for 1,25(OH)₂D₃ analysis and kidney tissue for gene expression were collected at harvest. Bars represent means $\pm$ SEM (n=10-12 mice per diet group). Different from the 1% Ca diet group, **p<0.01.

Figure 2. Characterization of target genes mRNA expression across the small intestine
 Each data point represents the normalized mRNA level means in each tissue segment $\pm$ SEM (n=10-12 mice per intestinal segment) for the specific target gene on mice fed the 1% Ca AIN93G diet for one week (duodenum mRNA expression = 100%). Statistical differences across intestinal segments are summarized in Table 2. Different from duodenum value within each target gene, *p<0.05.

Figure 3. Ca absorption measured in serum in response to dietary Ca and sugar

Mice were fed AIN93G diets with either 1% (high) or 0.125% (low) Ca content for one week. At the time of harvest, two different transport buffers enriched with Ca^{45} containing 25 mmol/L of either glucose or fructose were administered by oral gavage. 10 minutes after the gavage injection, blood samples were collected and percentage of Ca absorbed was estimated by serum counting. Results are expressed as means $\pm$ SEM ($n=9-12$ mice from each diet group per buffer). Different from the 1% Ca diet group, $**p<0.01$.

Table 1

Ingredient composition of the AIN93G-based experimental diets

	0.125 % Ca diet ¹ g/kg	1 % Ca diet g/kg
Macronutrient Composition		
Protein	20	20
Carbohydrate	66	63
Fat	7	7
Total Ca and P content		
Ca	1.25	10.2
P	4	4
Ingredient		
Casein, Lactic 80 mesh	200	200
L-cysteine	3	3
Corn starch	397.486	397.486
maltodextrin 10	132	132
Sucrose	115.684	93.324
Cellulose, BW200	50	50
Soybean Oil	70	70
t-Butylhydroquinone	0.014	0.014
Mineral Mix S10022C ²	3.5	3.5
Sodium Chloride	2.59	2.59
Calcium Carbonate (40% Ca)	2.64	25
Potassium Phosphate, Monobasic (22.8% P, 28.7 % K)	10	10
DiCalcium Phosphate (29.5 % Ca, 22.8% P)	0.526	0.526
Vitamin Mix V13203 ³	10	10
Vitamin D3 (100,000 IU/g)	0.01	0.01
Choline Bitartrate	2.5	2.5
FD&C Yellow Dye #5	0	0.025
FD&C Red Dye #40	0	0.025
FD&C Blue Dye #1	0.05	0
Total	1000	1000

¹ Research Diets product numbers: 0.125% Ca diet = D09102805; 1% Ca diet = D09102802

² Standard mineral mix for growing rodent diets (S10022G) without Sodium Chloride, Ca Carbonate, Potassium Phosphate, Monobasic and Potassium Citrate 1 H₂O. These ingredients were separately added to the diet.

³ Standard vitamin mix with no Vitamin D added.

Table 2

Characterization of mRNA expression of genes across the small intestine in response to dietary Ca.

Target gene	Tissue segment	Relative mRNA expression		Fold Change relative to 1% Ca diet	Tissue main effect	Diet main effect	Tissue × diet interaction
		1% Ca diet	0.125% Ca diet				
TRPV6	Dd	1 ^a	26.6±7.01 ^{**}	26.6	p<0.01	p<0.01	p<0.01
	Jej	0.004±0.001 ^b	0.009±0.005	2.3			
	Il Prox	0.0011±0.0002 ^c	0.0013±0.0002	1.14			
	Il Distal	0.001±0.0002 ^c	0.002±0.0003	2			
CaBP _{D9k}	Dd	1 ^a	4.1±0.6 ^{**}	4.1	p<0.01	p<0.01	p<0.01
	Jej	0.015±0.01 ^b	0.009±0.001	0.6			
	Il Prox	0.004±0.001 ^c	0.0007±0.0001	0.18			
	Il Distal	0.0065±0.001 ^b	0.045±0.01 ^{**}	6.9			
Ca _v 1.3	Dd	1 ^{ab}	1.1±0.07	1.1	p= 0.062	NS	p<0.05
	Jej	1.2±0.1 ^{ab}	1.05±0.1	0.87			
	Il Prox	0.93±0.08 ^b	1±0.14	1.1			
	Il Distal	1.02±0.07 ^a	1.6±0.2 [*]	1.6			
CLDN2	Dd	1 ^b	1.04±0.1	1.04	p<0.01	NS	NS
	Jej	0.33±0.04 ^c	0.34±0.04	1.01			
	Il Prox	0.18±0.03 ^d	0.14±0.02	0.78			
	Il Distal	2±0.2 ^a	1.8±0.2	0.9			
CLDN12	Dd	1 ^b	1.3±0.1	1.3	p<0.01	NS	p<0.05
	Jej	1.4±0.1 ^a	1.3±0.1	0.9			
	Il Prox	0.6±0.08 ^c	0.45±0.07	0.75			
	Il Distal	0.55±0.06 ^c	0.4±0.07	0.7			

Mice were fed high (1%) or low (0.125%) Ca diets (AIN93G) for 1 week. Each value is shown as the mRNA gene expression means ± SEM for each tissue normalized to Dd value for the 1% Ca diet (Dd 1% Ca=1) within a target gene (n= 10-12 mice per diet group). (Dd = Duodenum, Jej = Jejunum, Il Prox = Proximal Ileum, Il Distal = Distal Ileum). Different letters indicate significant differences across tissue segments within a target gene (Tukey-Kramer, p<0.05). Different from the 1% Ca diet group

^{**}
p<0.01

^{*}
p<0.05. NS = No significant.