FUNCTION american physiological society

FUNCTION, 2023, 4(6): zqad057

https://doi.org/10.1093/function/zqad057
Advance Access Publication Date: 4 October 2023
Perspectives

PERSPECTIVES

Breast Milk Epidermal Growth Factor Confers Paracellular Calcium Absorption in the Infant Small Intestine

Andreanna Burman[1] and Izumi Kaji[1,2,*]

[1]Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37232, USA and [2]Epithelial Biology Center, Section of Surgical Sciences, VUMC, Nashville, TN 37232, USA

*Address correspondence to I.K. (e-mail: izumi.kaji@vumc.org)

A Perspective on "Maternal Epidermal Growth Factor Promotes Neonatal Claudin-2 Dependent Increases in Small Intestinal Calcium Permeability"

In addition to the well-known roles of calcium (Ca^{2+}) as a cellular signaling molecule, the absorption of calcium by the intestine is critical for bone mineralization. The requirement of calcium is especially important in periods of growth, namely infancy, to allow for proper density in growing bones. The source of calcium necessary for bone mineralization is absorption from the diet through the intestinal epithelium. However, the mechanisms underlying calcium absorption in infancy had not been well understood.

Calcium absorption by the intestine is characterized into two distinct pathways: active transcellular and passive paracellular pathways. In transcellular transport of calcium, luminal calcium enters the enterocyte using transporters, including the transient receptor potential vanilloid 6 (TRPV6) and a voltage-dependent L-type calcium channel ($Ca_v1.3$). Calcium is then shuttled toward basolateral membrane by calcium-binding proteins and extruded to allow for calcium bodily absorption. Recently, the Alexander lab has published the mechanisms underlying transcellular absorption of calcium in the developing pre-wean mouse intestine and its effect on bone mineralization.[1] Using mice lacking functional *Trpv6* or *Cacana1d* ($Ca_v1.3$), this previous study illustrated the importance of both channels in calcium absorption in the jejunum and ileum of preweaning mice. Accompanying a decrease in ileal transcellular calcium absorption at postnatal day (PND) 14, $Ca_v1.3$-lacking mice exhibited delayed bone mineral accumulation. These studies illustrate mechanisms of active transcellular calcium absorption in the prewean mouse intestine, however, passive paracellular pathways remained unexplored.

As paracellular calcium transport occurs passively by the electrochemical gradient of calcium, this route of calcium absorption only occurs when luminal calcium is higher than circulating concentration. As calcium is especially enriched in breast milk, approximately 95 mM in mice,[2] the capacity for transcellular calcium absorption is high in pre-weaning pups. In addition to a favorable electrochemical gradient, passive calcium transport requires a cation-selective paracellular pore. Tight junctions between epithelial cells can allow for passive charge-selective ion transport, permitting epithelial paracellular permeability. Several tight junction transmembrane proteins, including claudin-2 (gene *CLDN2*) and claudin-12 (*CLDN12*), form paracellular pores to facilitate cation movement. Claudins-2 and -12 are expressed in the human and mouse intestinal epithelium. Overexpression of either of these claudins in Caco-2 cells increases cellular calcium permeability,[3] suggesting the importance of these claudins in passive calcium transport in epithelial monolayers.

Previous studies have indicated differences in active versus passive intestinal calcium absorption with age. In rats, proximal small intestine calcium permeability decreased with age and a transition toward active transport of calcium occurred at weaning age.[4] These data suggest that the passive transport of calcium could be predominant during preweaning/suckling period. Similarly, intestinal *Cldn2* mRNA expression decreases following weaning age in mice.[5,6] Intestinal epithelial cells

Submitted: 2 October 2023; **Accepted:** 2 October 2023

demonstrate the dynamic adaptation of calcium absorption pathways depending on the availability of luminal calcium. Until recently, there was a knowledge gap between the age-dependent changes and the regulatory mechanism of paracellular pathway in calcium absorption in infancy.

A study conducted by Beggs et al.[7] in the recent issue of *Function* investigated both the contribution of paracellular calcium transport in the intestinal epithelium during postnatal development and regulatory factors in breast milk to enhance paracellular calcium absorption. The authors assessed paracellular calcium permeability by measuring the unidirectional basolateral to apical flux of $^{45}Ca^{2+}$, which ignores transcellular calcium movement, and the bi-ionic diffusion potential, which can calculate absolute calcium permeability from the apical to the basolateral side. They first evaluated the higher capacity of calcium permeability across the different segments of small intestine in PND14 mice compared to adult 2-mo-old mice. Their experimental setup to specifically assess the paracellular pathway was confirmed by the mutant mice lacking functional Trpv6, which demonstrated decreased transcellular calcium transport, but maintained similar paracellular calcium permeability to wild-type mice. Mirroring the paracellular calcium transport capacity, mRNA and protein expressions of claudin-2 were greater in the jejunum and ileum, but not duodenum, of PND14 mice compared to adult mice. Examining early weaned mice, this study indicated that the claudin-2 expression was increased by breast milk intake in postnatal mice, and this finding was recapitulated by Caco-2 cells grown in media containing human breast milk. Concordantly, global *Cldn2* knockout mice lack the elevated calcium permeability seen in the wild-type and *Cldn12* knockout mouse jejunum and ileum at PND14. The decrease in early age intestinal calcium permeability was accompanied by a significant decrease in cortical bone volume and mineral density in PND14 *Cldn2* knockout mice. These data suggest the functional importance of specifically claudin-2 in paracellular calcium absorption in developing distal small intestine. Furthermore, the passive calcium absorption by small intestine is implicated in the postnatal bone mineralization.

Beggs and team have further explored molecular mechanisms of claudin-2 regulation that confer enhanced calcium permeability in pre-wean mice. Given the presence of epidermal growth factor (EGF) in both human and murine breast milk, the effect of EGF on claudin-2 expression and calcium permeability was determined in Caco-2 cells. Treatment with EGF or 2% milk similarly elevated CLDN2 expression and increased calcium permeability across Caco-2 monolayers. These effects of milk were blocked by Erlotinib treatment, an epidermal growth factor receptor (EGFR) inhibitor. Together these data suggest that breast milk-derived EGF promotes the increase in claudin-2 expression in the infant intestine, thereby promoting enhanced paracellular calcium absorption. EGF has been recognized to enhance nutrient and ion absorption in experimental animals[8] and pediatric patients with short-bowel syndrome.[9] However, target molecules downstream of EGFR in enterocytes have been largely unknown, especially in developing intestine.

The present study thoroughly demonstrates the importance of paracellular transport of calcium in the infant jejunum and ileum, this pathway's contribution to bone mineralization in early life, and the regulation of paracellular calcium transport by EGF in breast milk.[7] These data could provide important insights into the physiology of the developing intestine, which differs from adult tissues, and a potential therapeutic target to promote calcium absorption in health and disease.

Funding

A.B. is supported by the National Foundation of Science (NSF) GRP Grant. I.K. is supported by the National Institute of Health (NIH) R01 DK128190.

Conflict of interest

The authors disclose no relevant conflicts of interest.

References

1. Beggs MR, Lee JJ, Busch K, et al. TRPV6 and Ca(v)1.3 mediate distal small intestine calcium absorption before weaning. *Cell Mol Gastroenterol Hepatol*. 2019;**8**(4):625–642.
2. Reinhardt TA, Lippolis JD, Shull GE, Horst RL. Null mutation in the gene encoding plasma membrane Ca^{2+}-ATPase isoform 2 impairs calcium transport into milk. *J Biol Chem*. 2004;**279**(41):42369–42373.
3. Fujita H, Sugimoto K, Inatomi S, et al. Tight junction proteins claudin-2 and -12 are critical for vitamin D-dependent Ca^{2+} absorption between enterocytes. *Mol Biol Cell*. 2008;**19**(5):1912–1921.
4. Ghishan FK, Parker P, Nichols S, Hoyumpa A. Kinetics of intestinal calcium transport during maturation in rats. *Pediatr Res*. 1984;**18**(3):235–239.
5. Holmes JL, Van Itallie CM, Rasmussen JE, Anderson JM. Claudin profiling in the mouse during postnatal intestinal development and along the gastrointestinal tract reveals complex expression patterns. *Gene Expr Patterns*. 2006;**6**(6):581–588.
6. Tamura A, Hayashi H, Imasato M, et al. Loss of claudin-15, but not claudin-2, causes Na+ deficiency and glucose malabsorption in mouse small intestine. *Gastroenterology*. 2011;**140**(3):913–923.
7. Beggs MR, Young K, Plain A, et al. Maternal epidermal growth factor promotes neonatal claudin-2 dependent increases in small intestinal calcium permeability. *Function (Oxf)*. 2023;**4**(5):zqad033.
8. Opleta-Madsen K, Hardin J, Gall DG. Epidermal growth factor upregulates intestinal electrolyte and nutrient transport. *Am J Physiol*. 1991;**260**(6 Pt 1):G807–14.
9. Sigalet DL, Martin GR, Butzner JD, et al. A pilot study of the use of epidermal growth factor in pediatric short bowel syndrome. *J Pediatr Surg*. 2005;**40**(5):763–768.

Submitted: 2 October 2023; **Accepted:** 2 October 2023