Characterization of the human intestinal calcium transporter, CaT1, stably expressed in CHO cells

Yoshihiko Takano, Ryuichiro Sato, Hideo Satsu and Makoto Shimizu*

Department of Applied Biological Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113-9657, Japan
(* Author for correspondence: E-mail: ams316@mail.ecc.u-tokyo.ac.jp)

Received 4 June 2003; accepted in revised form 13 October 2003

Key words: calcium uptake, CaT1, CHO cell, transporter

Abstract

The human calcium transporter, hCaT1, was cloned and analyzed. The obtained amino acid sequence was slightly different from the ortholog of hCaT1 which had been identified by Peng et al. (2000. *Biochem. Biophys. Res. Commun* 278: 326–332). An mRNA analysis of human gastrointestinal segments demonstrates that hCaT1 was expressed in the stomach, duodenum, jejunum, ileum, ileocecum, cecum, ascending colon, transverse colon, descending colon, and, at very low levels, in the esophagus and rectum. hCaT1 was transiently expressed by transfecting COS-1 cells and was stably expressed by the transfected CHO cells. The transfected cells expressed hCaT1 with a molecular mass of 75 kDa. Stable expression of hCaT1 in the CHO cells increased the cellular uptake of Ca^{2+} . hCaT1 was inhibited by La^{3+} , Gd^{3+} and Cd^{2+} , whereas Co^{2+} , Fe^{2+} , Mn^{2+} and Mg^{2+} showed no significant effects on the activity. Acidification of the extracellular solution to pH 5.5 reduced the $^{45}\text{Ca}^{2+}$ uptake by hCaT1 in the CHO cells. The addition of lactose and raffinose had no effect on the $^{45}\text{Ca}^{2+}$ uptake, whereas galactose and glucose increased the $^{45}\text{Ca}^{2+}$ uptake. CHO cells stably expressing hCaT1 will be useful to detect and analyze food substances that could modulate the hCaT1 activity.

Abbreviations CHO – Chinese hamster ovary; ECaC – epithelial Ca^{2+} channel; FBS – fetal bovine serum; hCaT1 – human calcium transporter 1; RT-PCR – reverse transferase-polymerase chain reaction

Introduction

The intestinal calcium absorption through the transcellular pathway in the duodenum, jejunum and ileum is thought to account for 30–40% of the total calcium absorption, whereas 60–70% of the absorption would be via the paracellular route (Karbach, 1992). When dietary calcium is limited, the transcellular transport of calcium is particularly important. 1,25-Dihydroxycholecalciferol ($1,25\text{-(OH)}_2\text{D}_3$) plays an important role in this pathway; i.e., it up-regulates the calcium-binding

protein, calbindin D_{9k} , which facilitates intracellular Ca^{2+} diffusion across intestinal epithelial cells and increases the Ca^{2+} efflux from the basolateral membrane via Ca^{2+} -ATPase (PMCA) and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) (Wasserman et al. 1992). Although the increased calcium absorption by $1,25\text{-(OH)}_2\text{D}_3$ has been well studied, the mechanism for the entry of Ca^{2+} across the apical membrane was not known for a long time. The calcium channel, ECaC (Hoenderop et al. 1999), and transporter, CaT1 (Peng et al. 1999), have recently been cloned from the rabbit and rat duode-

num, respectively. These transporters are now thought to be responsible for the calcium entry across the cell membrane, and their activity might be related to the Ca^{2+} absorption-enhancing effects of certain food substances. Human CaT1 has been cloned and expressed in *Xenopus laevis* oocytes, its distribution, gene localization and functional properties being examined by Peng et al. (2000). However, the regulatory characteristics of hCaT1, particularly the effect of food substances on the activity, have scarcely been studied.

It is well known that intestinal calcium absorption is influenced by various factors including food components. For example, enhancement of the intestinal calcium absorption by lactose has been reported in men (Condon et al. 1970; Kocian et al. 1973; Cochet et al. 1983) and in animals (Leichter et al. 1975; Armbricht et al. 1976; Schaafsma et al. 1980). However, it is not yet clear whether CaT1 is involved in the increased Ca^{2+} uptake by lactose. To examine the effect of food substances on CaT1, it is necessary to prepare a simple evaluation system for measuring the cellular Ca^{2+} uptake via CaT1. In this present study, we transfected a mammalian cell (CHO) with hCaT1 DNA, and constructed a system to measure the hCaT1-mediated Ca^{2+} entry by using this stably CaT1-expressing cell line. The effects of lactose and its monosaccharide components (glucose and galactose) were examined by this evaluation system.

Materials and methods

Cell culture

COS-1 cells were maintained in medium A [Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO, USA), 100 units ml^{-1} of penicillin, and 100 $\mu\text{g ml}^{-1}$ of streptomycin] supplemented with 10% fetal bovine serum (FBS) at 37 °C in a 5% CO_2 atmosphere. Caco-2 cells were maintained in medium A supplemented with 10% FBS and 1% nonessential amino acids at 37 °C in a 5% CO_2 atmosphere. The cells that had been cultured for 10 days after total confluency were considered as being differentiated. Chinese hamster ovary (CHO) cells were maintained in medium B [a 1:1 mixture of Ham's F-12 medium and Dulbecco's modified Eagle's medium (Sigma), 100 units ml^{-1} of penicillin, and 100 $\mu\text{g ml}^{-1}$ of streptomycin]

supplemented with 10% FBS at 37 °C in a 5% CO_2 atmosphere.

Cloning of human CaT1

Based on the mouse CaT1 sequence (GenBank Accession No. AB037373), a BLAST database search was performed (<http://www3.ncbi.nlm.nih.gov/BLAST>). This revealed the human-expressed sequence tag (GenBank Accession Nos. AA579526 from human prostate and AA078617 from human brain). Based on these sequences, a human-specific primer set was designed, and a reverse transferase-polymerase chain reaction (RT-PCR) was performed by using first-strand cDNA from Caco-2 cells and a human small intestinal cDNA library (Clontech Lab., Palo Alto, CA, USA). The resulting fragments were cloned in the pGEM-T-Easy vector (Promega, Madison, WI, USA) and sequenced with an automated sequencer (Perkin-Elmer, Boston, MA, USA).

Northern blot analysis

A membrane of human multiple-tissue MTN blots (human digestive system 12-lane MTN blot; Clontech) was used in the analysis. Probes for human CaT1 and 36B4, a kind of house-keeping gene used for normalizing the results (Inoue et al. 2001), were labeled with [α - ^{32}P]dCTP (3000 Ci mmol^{-1} ; Amersham Biosciences, NJ, USA) by using a random-primed DNA labeling kit (Mega-prime DNA labeling system; Amersham Biosciences). The membrane was hybridized with the radioactive cDNA probe, and the signals on the membrane were quantified by an image-analyzing system (FLA-2000; Fuji Film, Tokyo, Japan).

Antibody

A polyclonal antibody (RS08CAT) against human CaT1 was produced by immunizing rabbits with a fusion protein encoding six histidines followed by amino acids 636–725 of human CaT1. The fusion protein construct was cloned into a pET28(a) vector (Novagen, Darmstadt, Germany), expressed in *Escherichia coli*, and purified by Ni^{2+} -Sepharose affinity chromatography. The

horseradish peroxidase-conjugated antibody against rabbit immunoglobulin was obtained from Amersham Pharmacia Biotech.

Construction of the expression vector for human CaT1

The pME-His vector was generated by inserting synthetic oligonucleotides encoding an MRGS(H)₆ sequence into the *EcoRI* and *XhoI* sites of pME-18S. To generate pMEHishCaT1 (4-725), the *XhoI*-*NotI* PCR fragment coding human CaT1 (4-725) was inserted into the *XhoI* and *NotI* sites of pME-His.

Transient and stable transfection

COS-1 cells were transfected by the DEAE dextran method and then re-fed with medium A supplemented with 10% FBS. To obtain CHO-hCaT1 cells expressing His-tag-hCaT1 (4-725), CHO cells were transfected with pMEHishCaT1 (4-725) and pMAM (Funakoshi, Tokyo, Japan) by using LipofectAMINE (Invitrogen, CA, USA) and then re-fed with medium B containing 10% FBS with 8 $\mu\text{g ml}^{-1}$ of blasticidin. The surviving cells were cloned, and the one with the most stable cell line expressing His-tag-hCaT1 (4-725) most abundantly (CHO-hCaT1) was used in the subsequent experiments.

Western blot analysis for human CaT1

COS-1 cells and CHO cells were harvested in 10 mM Tris-HCl at pH 8.0, 150 mM NaCl, 1% Nonidet P-40, and a protease inhibitor cocktail (Sigma). After centrifugation at 20,000 *g* for 5 min at 4 °C, the supernatant was incubated with 50 μl of a 50% slurry of Ni resin (Qiagen, Hilden, Germany), and the Ni resin-bound protein was analyzed by SDS-PAGE. A western blot analysis was carried out by using anti-human CaT1 antibodies with a chemiluminescent substrate (ECL; Amersham Biosciences).

⁴⁵Ca²⁺ uptake in CHO-hCaT1 cells

CHO-hCaT1 cells were set up on day 0. On day 5, the cells were successively washed with phosphate-

buffered saline (PBS) and Hank's balanced salt solution (HBSS), and then incubated with an uptake solution (HBSS containing 5.28 μM ⁴⁵Ca²⁺ and 0.2 mM CaCl₂) for 10 min. The cells were washed twice with an ice-cold stop buffer (PBS containing 0.05% NaN₃ and 0.02 mM CaCl₂), and harvested with a solubilizing solution containing 1% Triton-X100. The cell lysate was dissolved in a scintillation liquid and its radioactivity counted.

Results

Cloning of human CaT1

The cDNA obtained in this study consisted of a 2172-bp transcript with an open reading frame of 2165 bp encoding a protein of 721 amino acids. The hydropathy plot predicted the presence of six transmembrane-spanning domains with a putative pore-forming region between transmembrane domains 5 and 6 (data not shown). Homology in the coding sequence between human CaT1 and rat CaT1 (GenBank Accession No. AF160798) was 85% at the DNA level, and that between hCaT1 and human ECaC (GenBank Accession No. AJ271207) was also 85%. However, the homology between the corresponding carboxyl-terminal 300 bp of hCaT1 and hECaC was only about 50%. The amino acid sequence of our hCaT1 and that reported by Peng et al. (2000) was different in three amino acid residues (Figure 1).

Tissue distribution of human CaT1

The distribution of hCaT1 mRNA in the human gastrointestinal tract was assessed by a northern blot analysis, using mRNAs from various intestinal segments. Transcripts were expressed in the stomach, duodenum, ileocecum, ileum, jejunum, ascending colon, descending colon, transverse colon, cecum and, at much lower levels, in the esophagus and rectum (Figure 2).

Detection of human CaT1 in the transfected cells

The anti-hCaT1 antiserum, RS08CAT, was prepared in a rabbit by injecting the carboxyl-termi-

hCaT1	MGLSLPKEKGLILCLWSKFCRWFORRESWAQSRDECDLQOKRIWESPLLLAAKDNDVQA	60
hCaT1'	..W.....N.....H.....N.....E.N...	56
rCaT1	..W.....N.....H.....N.....E.N...	60
hCaT1	LNKLLKYEDCKVHFRGAMGETALHIAALYDNLEAAMVLEAAPELVFEPMTSELYEGQTA	120
hCaT1'	D.....	116
rCaT1	..I...F.G.E...K.....	120
hCaT1	LHIAVVNQNMNLVRALLARRASVSARATGTAFRRSPCNLIYFGEHPLSFAACVNSEEIVR	180
hCaT1'		176
rCaT1	I...V.....G.....SV.HYR.H...Y.....G.....	180
hCaT1	LLIEHGADIRAQDSLGNITVLHILIQPNKTFACQMYNLLSYDRHGDHLQPLDLVPHQG	240
hCaT1'		236
rCaT1	-G...KS.E...N...	239
hCaT1	LTPFKLAGVEGNTVMFQHLMQKRKHTQWTYGPLTSTLYDLTEIDSSGDEQSLELIITTK	300
hCaT1'		296
rCaT1	I.....I.....D.....V.....	329
hCaT1	KREARQILDQTPVKELVSLKWKRYGRPYFCMLGAIYLLYIICFTMCCTIYRPLKPRTNRT	360
hCaT1'		356
rCaT1	V.....V.....IT...	359
hCaT1	SPRDNLLQOKLLQEAAYMTPKDDIRLVGELVTIGAIILLVEVPDIFRMGVTRFFGQTI	420
hCaT1'		416
rCaT1	N.....SIV..V.....I.....L.....	419
hCaT1	LGGPFHVLIIITYAFMVLVTVMRLISASGEVPMFALVLGWCNVMYFARGFQMLGPFTI	480
hCaT1'		476
rCaT1	I.V.....TNSD.....	479
hCaT1	MIQKMTFGDLRFCLWMAVVILGFASAFYIIFQTEDPEELGHFYDYPMALFSTFELFTI	540
hCaT1'		536
rCaT1	D.....	539
hCaT1	IDGPANYNDLPFMYSTITYAAFAIIATLLMLNLLIAMMGDTHWRVAHERDELWRAQIVAT	600
hCaT1'		596
rCaT1	D.....V.....	699
hCaT1	TVMLERKLPRCLWPRSGICGREYGLGDRWFLRVE	657
hCaT1'		653
rCaT1	R.....QQQDDLY..	659
hCaT1		717
hCaT1'		713
rCaT1	..E...G...MAR...GAY...F.T.....T.D...A.K.Q.....	719
hCaT1	GSMTYQ	725
hCaT1'		721
rCaT1	..G.....	727

Figure 1. Alignment of the amino acid sequences of human CaT1 and rat CaT1. Transmembrane segments are underlined. Unfilled boxes represent the differences among the amino acids of our hCaT1' and that reported by Peng et al. (2000). The filled box represents the region for preparing antibody.

Figure 2. Tissue distribution of hCaT1. Human multiple-tissue MTN blots (human digestive system 12-lane MTN blot; Clontech) were used in the analysis.

nal peptide of hCaT1 (Figure 1). The antibody titer was monitored by using an His-tagged hCaT1-C-terminus fusion protein expressed in

E. coli as an antigen. RS08CAT thus obtained was used for detecting CaT1 in mammalian cells. COS-1 and CHO cells were transiently and stably transfected, respectively, with expression plasmids for the pMEHishCaT1 (4-725) protein or unmodified pMEHis. The cell lysate was treated with Ni resin, and the bound fraction was applied to SDS-PAGE. A specific hCaT1 band (75 kDa) was detected in the cells expressing pMEHishCaT1 by immunoblotting with RS08CAT, whereas the CaT1 band was not detected in the control cells (expressing pMEHis only) (Figure 3). To confirm whether the anti-hCaT1 antibody really recognized the CaT1 protein, immunoblotting with an anti-RGS(H)₅ monoclonal antibody was also performed. A band of the same size (75 kDa) was

Figure 3. Western blot analysis of hCaT1 expression in COS-1 cells and CHO cells by using the RS08CAT antibody. The lysates of COS-1 and CHO transfected with pMEHis and pMEHishCaT1 were fractionated by SDS-PAGE (7.5% gel), electrotransferred to a polyvinylidene fluoride membrane, and then probed with the RS08CAT antibody (1:500). The expected CaT1 bands were demonstrated in all membranes in the hCaT1-transfected cells.

detected with the anti-RGS(H)₅ antibody in COS-1 and CHO cells that had been transfected with pMEHishCaT1 (data not shown), indicating that the antibody prepared in this study recognized the CaT1 protein expressed by the transfected cells.

Characterization of the functional properties of human CaT1 by a $^{45}\text{Ca}^{2+}$ uptake assay

Peng et al. (1999) have characterized CaT1 by using CaT1-injected oocytes, but the hCaT1 protein expressed in the mammalian cells had not previously been fully characterized. Figure 4 shows the $^{45}\text{Ca}^{2+}$ uptake in the CHO-hCaT1 cells. Since the uptake of $^{45}\text{Ca}^{2+}$ was observed in the control cells without expressing hCaT1 (Figure 4a), the CaT1-mediated $^{45}\text{Ca}^{2+}$ uptake was demonstrated by subtracting the value for the control cells. The CaT1-mediated $^{45}\text{Ca}^{2+}$ uptake in CHO-hCaT1 cells was linear for up to 10 min and then reached a plateau (Figure 4b). The Ca^{2+} uptake was concentration-dependent and saturable, with an apparent Michaelis constant (K_m) of 0.043 mM (Figure 5a). Inhibition studies on the $^{45}\text{Ca}^{2+}$ uptake by various di- and trivalent cations were performed to study the substrate specificity of hCaT1. La^{3+} , Gd^{3+} , Cd^{2+} and Co^{2+} markedly inhibited the calcium uptake, whereas Fe^{2+} , Mn^{2+}

Figure 4. $^{45}\text{Ca}^{2+}$ uptake by hCaT1-transfected cells as a function time. (a) Measurements were performed at the indicated times on the control (pMEHis) cells and CHO-hCaT1 cells. (b) The hCaT1-mediated $^{45}\text{Ca}^{2+}$ uptake was demonstrated by subtracting the values for the control cells. The experiments were performed three times, and each value is the mean - S.E.M. ($n = 3$).

and Mg^{2+} did not show any significant effects (Figure 5c). The CaT1-mediated calcium transport decreased with decreasing pH value, suggesting that the transport was not coupled to H^+ (Figure 5b).

Effect of various sugars on the hCaT1-mediated Ca^{2+} uptake

We investigated the effect of lactose and some sugars on the hCaT1-mediated calcium uptake by using CHO-hCaT1 cells. The cells were preincubated for 4 h with a sugar (204 mM) in a calcium-free Dulbecco's modified Eagle's medium

Figure 5. Functional characterization of hCaT1. (a) Concentration dependence of the Ca^{2+} uptake mediated by hCaT1. Measurements of the $^{45}\text{Ca}^{2+}$ uptake were performed for 10 min at various Ca^{2+} concentrations (0.01–1 mM). (b) The pH dependence of the hCaT1-mediated Ca^{2+} uptake was measured in the uptake solution with varying pH values. (c) Inhibition of the hCaT1-mediated Ca^{2+} uptake by divalent and trivalent metal ions (all at 100 μM , except for 10 mM Mg^{2+}) and choline chloride (ChoCl) in the uptake solution. The experiments were performed three times, and each value is the mean $\pm$ S.E.M. ($n = 3$).

Figure 6. Effect of various sugars on the hCaT1-mediated Ca^{2+} uptake. Each sugar concentration was adjusted to 204 mM, considering that the lactose concentration in human milk is about 7% (204 mM). Cells were preincubated with a sugar for 4 h. The uptake experiments were performed as described in the Materials and methods section. The experiments were performed three times, and each value is the mean $\pm$ S.E.M. ($n = 3$). * $p < 0.05$, ** $p < 0.01$ vs. control (ANOVA)

(Invitrogen) supplemented with 2.5% FBS and 2% L-glutamine, and then the calcium uptake activity of the cells was measured. The hCaT1-mediated $^{45}\text{Ca}^{2+}$ uptake was increased by incubating the cells with galactose and glucose, while lactose and raffinose each showed no effect (Figure 6).

Discussion

A variety of *in vivo* and *in vitro* methods have been used to estimate the availability or absorption efficiency of dietary calcium in the intestinal tract. For example, Fordtran et al. (1965) have developed a triple-lumen perfusion technique by using 30-cm segment of jejunum. A stable-strontium test (Milsom et al. 1987) and a method using a suspension of brush-border membrane vesicles (BBMV) prepared from the rat intestinal epithelium (Gunshin et al. 1991) have also been employed for the assay. However, there has been no simple and reliable method to distinguish the calcium absorption via the transcellular pathway from that via the paracellular one. Although there have been many reports demonstrating that the intestinal calcium absorption was affected by various factors including food substances, those effects have not been clearly explained because the

experimental methods used were not informative enough to reveal the mechanism. The discovery of CaT1 (Peng et al. 1999) has provided a new approach for studying the factors that would modulate the intestinal calcium absorption via the transcellular pathway. In this present study, we constructed a mammalian cell (CHO-hCaT1) system for stably expressing hCaT1. Although the background of calcium uptake was not negligible with this cell system, the hCaT1-mediated calcium transport could be clearly observed. The calcium uptake by transfected CHO cells occurred rapidly, reaching a plateau in 10 min (Figure 4b). Peng et al. (2000) have reported that the K_m value for hCaT1 expressed in *Xenopus laevis* oocytes was 0.25 mM. However, the K_m value was calculated to be 0.043 mM in our study (Figure 5a), indicating that CHO-hCaT1 cells had higher affinity than that expressed in oocytes. These results suggest that this system would be useful to evaluate the modulatory effects of dietary substances on the hCaT1-mediated transcellular calcium absorption.

Among the dietary substances that enhance intestinal calcium absorption, casein phosphopeptide (CPP) is well known. Binding of CPP with Ca^{2+} and the resulting solubilization of calcium in the neutral pH range in the lower small intestine are thought to constitute the mechanism (Sato et al. 1986). The enhancing effect of oligosaccharides is also thought to be due to the increased solubilization of calcium by lowering the intestinal pH value. Since oligosaccharides are metabolized by intestinal microflora, such organic acids as lactic acid and propionic acid are produced and acidify the intestinal content. These effects may be important for increasing the calcium absorption in the lower small intestine via the paracellular pathway.

The calcium absorption-enhancing effect of lactose is also well known (Pansu et al. 1970). However, unlike other oligosaccharides, the effect of lactose is still controversial. Some researchers have reported that the calcium absorption-enhancing effect of lactose was not due to lactose itself, but to its component sugars, glucose and galactose. (Birloues 1988) has shown that lactose-hydrolyzed milk resulted in increased calcium absorption. Schuette et al. (1989) have reported that a mixture of glucose and galactose could enhance the jejunal calcium absorption more markedly than lactose. On the other hand, Zittermann et al. (2000) have reported

that neither lactose nor a galactose/glucose mixture enhanced the calcium absorption in lactose-tolerant adults by using the stable-strontium test. Even if lactose is actually effective for calcium absorption, its mechanism is not likely to be due to the simple solubilization of calcium ions in the intestinal tract. Armbrrecht et al. (1976) have suggested the interaction between lactose and the brush-border membrane to be involved in the calcium absorption-enhancing effect of lactose. However, the detailed mechanism for this is still not known.

The results of our experiments demonstrate that lactose and raffinose did not significantly enhance the CaT1-mediated Ca^{2+} uptake, whereas glucose and galactose did (Figure 6). The calcium absorption-enhancing effect of lactose mediated by CaT1 might therefore have been due to its component sugars produced by intestinal digestion. Since hCaT1 is expressed in the ileum and jejunum, galactose/glucose would possibly stimulate intestinal calcium absorption via CaT1 *in vivo*. Vaughan et al. (1960) have reported that galactose showed a greater enhancing effect on the ileal Ca^{2+} absorption than glucose. The mechanism for the enhancing effect of galactose or glucose is however not known. Norman et al. (1980) have hypothesized that the calcium absorption-enhancing action of glucose was a consequence of water movement into the cells. Further studies on the response of the CaT1-mediated calcium uptake to galactose/glucose should be performed, and our cell system will be useful for this purpose. This system also enables the search for CaT1-modulatory dietary factors, which will be beneficial for the development of food with a calcium absorption-stimulatory function.

Acknowledgement

This study was partly supported by Special Coordination Funds of the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government.

References

- Armbrrecht H.J. and Wasserman R.H. 1976. Enhancement of Ca^{2+} uptake by lactose in the rat small intestine. *J. Nutr.* 106: 1265–1271.

- Birlouez A.I. 1988. Effect of lactose hydrolysis on calcium absorption during duodenal milk perfusion. *Reprod. Nutr. Dev.* 28(6A): 1465–1472.
- Condon J., Nassim J.R., Millard F.J.H., Hilbe A. and Stainton E.M. 1970. Calcium and phosphorus metabolism in relation to lactose tolerance. *Lancet* 1: 1027–1029.
- Cochet B., Jung A., Griessen M., Bartholdi P., Schaller P. and Donath A. 1983. Effects of lactose on intestinal calcium absorption in normal and lactase-deficient subjects. *Gastroenterology* 84: 935–940.
- Fordtran J.S., Rector F.C., Ewton M.F., Soter N. and Kinney J. 1965. Permeability characteristics of the human small intestine. *J. Clin. Invest.* 44: 1935–1944.
- Gunshin H., Noguchi T. and Naito H. 1991. Lactose-enhanced uptake of calcium by isolated brush border membrane vesicles from the rat small intestine. *Agric. Biol. Chem.* 55(7): 1919–1921.
- Hoenderop J.G., der Kemp A.W., Hartog A., der Graaf S.F., Van Os C.H., Willems P.H. and Bindels R.J. 1999. Molecular identification of the Apica; Ca^{2+} channel in 1,25-dihydroxyvitamin D₃-responsive epithelia. *J. Biol. Chem.* 274(13): 8375–8378.
- Inoue J., Kumagai H., Terada T., Maeda M., Shimizu M. and Sato R. 2001. Proteolytic activation of SREBPs during adipocyte differentiation. *Biochem. Biophys. Res. Commun.* 283(5): 1157–1161.
- Karbach U. 1992. Paracellular calcium transport across the small intestine. *J. Nutr.* 122: 672–677.
- Kocian J., Skala I. and Bakos K. 1973. Calcium absorption from milk and lactose-free milk in healthy subjects and patients with lactose intolerance. *Digestion* 9: 317–324.
- Leichter J. and Tolensky A.F. 1975. Effect of dietary lactose on the absorption of protein, fat and calcium in the postweaning rat. *Am. J. Clin. Nutr.* 28: 238–241.
- Milsom S., Ibbertson K., Hannan S., Shaw D. and Pybus J. 1987. Simple test of intestinal calcium absorption measured by stable strontium. *Br. Med. J.* 295: 231–234.
- Norman D., Morawski S. and Fordtran J. 1980. Influence of glucose, fructose and water movement on calcium absorption in the jejunum. *Gastroenterology* 78: 22–25.
- Pansu D. and Chapuy M.C. 1970. Calcium absorption enhanced by lactose and xylose. *Calc. Tissue Res.* 4(suppl): 155–156.
- Peng J.B., Chen X.Z., Berger U.V., Vassilev P.M., Tsukaguchi H., Brown E.M. and Hediger M.A. 1999. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. *J. Biol. Chem.* 274(32): 22739–22746.
- Peng J.B., Chen X.Z., Bergar U.V.M., Weremowicz S., Morton C.C., Vassilev P.M., Brown E.M. and Hediger M.A. 2000. Human calcium transport protein CaT1. *Biochem. Biophys. Res. Commun.* 278: 326–332.
- Sato R., Noguchi T. and Naito H. 1986. Casein phosphopeptide (CPP) enhances calcium absorption from the ligated segment of rat small intestine. *J. Nutr. Sci. Vitaminol. (Tokyo)* 32(1): 67–76.
- Schuette S.A., Knowles J.B. and Ford H.E. 1989. Effect of lactose or its component sugars on jejunal calcium absorption in adult man. *Am. J. Clin. Nutr.* 50: 1084–1087.
- Schaafsma G. and Visser R. 1980. Nutritional interrelationships between calcium, phosphorus and lactose in rats. *J. Nutr.* 110: 1101–1111.
- Vaughan O. and Filter J. 1960. The enhancing action of certain carbohydrates on the intestinal absorption of calcium in the rat. *J. Nutr.* 71: 10–14.
- Wasserman R.H., Chandler J.S., Meyer S.A., Smith C.A., Brindak M.E., Fullmer C.S., Penniston J.T. and Kumar R. 1992. Intestinal calcium transport and calcium extrusion processes at the basolateral membrane. *J. Nutr.* 122: 662–671.
- Zittermann A., Bock P., Drummer C., Scheld K., Heer M. and Stehle P. 2000. Lactose does not enhance calcium bioavailability in lactose-tolerant, healthy adults. *Am. J. Clin. Nutr.* 71: 931–936.