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Dietary and pharmacological compounds altering intestinal calcium absorption in humans and animals

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Abstract

The intestine is the only gate for the entry of Ca to the body in humans and mammals. The entrance of Ca occurs via paracellular and intracellular pathways. All steps of the latter pathway are regulated by calcitriol and by other hormones. Dietary and pharmacological compounds also modulate the intestinal Ca absorption process. Among them, dietary Ca and P are known to alter the lipid and protein composition of the brush-border and basolateral membranes and, consequently, Ca transport. Ca intakes are below the requirements recommended by health professionals in most countries, triggering important health problems. Chronic low Ca intake has been related to illness conditions such as osteoporosis, hypertension, renal lithiasis and incidences of human cancer. Carbohydrates, mainly lactose, and prebiotics have been described as positive modulators of intestinal Ca absorption. Apparently, high meat proteins increase intestinal Ca absorption while the effect of dietary lipids remains unclear. Pharmacological compounds such as menadione, DL-butionine-*S*,*R*-sulfoximine and ursodeoxycholic acid also modify intestinal Ca absorption as a consequence of altering the redox state of the epithelial cells. The paracellular pathway of intestinal Ca absorption is poorly known and is under present study in some laboratories. Another field that needs to be explored more intensively is the influence of the gene × diet interaction on intestinal Ca absorption. Health professionals should be aware of this knowledge in order to develop nutritional or medical strategies to stimulate the efficiency of intestinal Ca absorption and to prevent diseases.

Key words: Intestinal calcium absorption: Transcellular and paracellular pathways: Hormonal effects: Nutritional factors

Introduction

Ca is the main mineral component of bone and, hence, is essential for achieving optimal peak bone mass in the first decades of life and for maintaining bone mass, later in life[1]. It also plays an important role in many physiological processes[2–5]. The dysregulation of Ca homeostasis is not only associated with bone disorders, but also with hypertension, insulin resistance, obesity and the metabolic syndrome[6–9]. Epidemiological and experimental studies have shown an inverse relationship between dietary Ca and risk of breast, colon, prostate and ovarian cancer[10–13]. Therefore, an appropriate Ca homeostasis preserves bone integrity, metabolic balance and avoids epithelial cancers.

Ca metabolism is predominantly regulated by the intestine, kidney, bone and parathyroid glands. Because of their coordinated work, serum Ca concentration is maintained within a narrow range[14]. Intestinal Ca absorption is an essential process that occurs through an active transcellular pathway and a passive non-saturable route, named the paracellular pathway[15]. Both routes are regulated by hormones, nutrients and many other factors.

The transcellular pathway is a saturable process, which is prevalent in the proximal small intestine (duodenum and jejunum), vitamin D being the main modulator. This mechanism is energy dependent and implicates Ca movement from the mucosal to serosal side of the intestinal barrier occurring against a concentration gradient. In contrast, the paracellular mechanism occurs throughout the length of the intestine. It is a non-saturable and passive transport and is a linear function of Ca concentration in the lumen[16].

Ca ions are absorbed mainly in the small intestine, which is responsible for about 90 % of overall Ca absorption. The longer residence time in the ileum as compared with the other

Abbreviations: 1,25(OH)2D3, 1,25-dihydroxycholecalciferol; Al, aluminium ions; AP, alkaline phosphatase; BMD, bone mineral density; BSO, dl-buthionine-(S,R)-sulfoximine; CB, calbindin; CPP, caseinophosphopeptides; ER, oestrogen receptor; FCA, fractional Ca absorption; FGF-23, fibroblast growth factor-23; GC, glucocorticoid; GSH, glutathione; IGF-1, insulin-like growth factor-1; KO, knockout; MEL, melatonin; MEN, menadione; NaDOC, sodium deoxycholate; NCX1, intestinal Na^+/Ca^{2+} exchanger; OVX, ovariectomised; PMCA, plasma membrane Ca^{2+} ATPase; PTH, parathyroid hormone; TRP, transient receptor potential; TRPV, transient receptor potential vanilloid; UDCA, ursodeoxycholic acid; VDD, vitamin D deficiency; VDR, vitamin D receptor.

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segments of the small intestine favours Ca absorption in that segment[16]. In rat ileum the transit half-time is about 100–120 min, whereas in the duodenum it is about 2–6 min[17]. The colon is responsible for less than 10 % of the total Ca absorbed; minor amounts of Ca ions are absorbed from the stomach and large intestine[16]. The major contributors to the amount of Ca absorbed are the residence time and the absorption rate in each intestinal segment. The order of Ca absorption rate is: duodenum > jejunum > ileum[16]. Ca absorption in the colon is probably very important in pathological conditions such as short bowel syndrome[18].

Intestinal Ca absorption also depends on the physiological needs of Ca. When the requirements increase and/or the intakes are low, there is an improvement in the efficiency of Ca absorption[19]. Ageing occurs with a decrease in intestinal Ca absorption[20], while growth, pregnancy and lactation promote cation absorption[21–24]. There is little information about the mechanism of lactation-induced intestinal hyperabsorption. Increases have been found in the villous height, villous width and crypt depth with an expansion of the absorptive surface area in the duodenum of 21 d lactating rats. An enhancement in claudin 15 has been also demonstrated in the same animal model[25]. Recently, Teerapornpuntakit *et al.*[26] have developed a custom-designed cDNA microarray (CalGene Array) to study the expression of genes related to duodenal nutrient transport, among them those related to bone and Ca metabolism. They have determined the transcriptome responses of duodenal epithelial cells in pregnant and lactating rats; data were subsequently validated by quantitative real-time PCR. They have found that pregnancy and late lactation alter the expression of several transcripts, among them those belonging to Ca transporters.

Transcellular pathway

Epithelial calcium channels

TRPV6 (previously named ECaC2 or CaT1) and TRPV5 (previously named ECaC1 or CaT2) are the two epithelial Ca channels involved in Ca entry to the enterocytes. These channels are homologous members of the transient receptor potential (TRP) superfamily, belonging to the vanilloid subfamily (TRPV), which is different from the canonical (TRPC) and melastatin (TRPM) subfamilies[27]. TRPV6 and TRPV5 are co-expressed in the human kidney and intestine, but the first one is highly expressed in the intestine and the latter is the major isoform in the kidney. TRPV6 seems to be a major contributor to apical, intestinal Ca absorption, as suggested by a significant reduction in Ca absorption and serum Ca shown in TRPV6 knockout (KO) mice[28,29]. Both channels are also expressed in the pancreas, prostate, and mammary, sweat and salivary glands[27]. They present a similar structure to other members of the TRP family: six transmembrane domains, a short hydrophobic stretch between segments 5 and 6 involved in the Ca pore and large intracellular N and C terminal tails. The intracellular segments contain phosphorylation sites, post-synaptic density protein motifs and ankyrin repeat domains; all of them are involved in the regulation of channel activity and trafficking[30]. It has been demonstrated that the tetrameric structure of TRPV6 and TRPV5 can be combined with each other to form different heterotetrameric channel complexes[31]. Both channels have 75 % homology, share several properties, but have different N and C terminal tails. They are regulated by calcitriol, oestrogen and dietary Ca. Both are inactivated by intracellular Ca, but with a different kinetics. In addition, the affinity of TRPV5 for the inhibitor ruthenium red is 100-fold that of TRPV6[32].

TRPV6 transcripts have been found in duodenum, but not in ileum, human biopsies. The duodenal expression of TRPV6 in men was detected to be vitamin D dependent, whereas in elderly women the TRPV6 and vitamin D receptor (VDR) expressions were low and not vitamin D dependent. This finding could explain, at least in part, the lower intestinal Ca absorption in elderly postmenopausal women[33]. In rats, the basal mRNA expression of TRPV6 has been found to be the highest in the duodenum, followed by the colon (46 % of duodenum), and negligible in the jejunum and ileum. The rank order of the basal levels of TRVP6 protein was duodenum > colon (72 % of duodenum) > ileum (25 % of duodenum)[34].

Calbindins

These proteins appear to be responsible for carrying Ca from the apical side of the enterocyte to the basal region of the cell. Calbindin (CB) CB_{9k} is present in the intestine of mammals and CB_{28k} in that from avian species[35]. CB_{9k} has four α-helical regions forming an EF-hand pair consisting of a canonical and a non-canonical/pseudo EF-hand domain, which are joined by a linker region. These EF-hands organised in tandem domains are the physiological relevant structures and two Ca ions bind with positive cooperativity[36]. CB_{28k} has six EF-hand domains, four of which bind Ca with medium/high affinity[37]. EF-hand 2 is non-functional and under physiological conditions EF6 most probably is as well. The four medium/high-affinity sites[38] are considered Ca specific.

CB also buffer Ca^{2+} ions by keeping intracellular Ca^{2+} concentrations below 10^{-7} M, which contribute to the prevention of premature cell death by apoptosis. When there is a down-regulation of CB, an excess of Ca^{2+} is provoked that may trigger apoptosis in the epithelial cells[39]. Furthermore, it has been reported that CB_{28k} also inhibits apoptosis in osteoblastic cells[40] and in germ cells from Robertsonian mice[41,42]. In addition, it has been shown in kidney that CB_{28k} regulates the Ca^{2+} concentration in the vicinity of the TRPV5 pore by a direct association with the channel[43]. This might occur in the intestine and in other tissues with important movements in intracellular Ca^{2+} concentrations.

Genetic studies have provided information that confused the understanding of CB on Ca homeostasis. Mice with ablation of the CB_{28k} gene do not exhibit calcemic abnormalities[44]. In CB_{9k}-null mutant mice as well as mice lacking the epithelial Ca channel TRPV6 it has been demonstrated that the regulation of active intestinal Ca absorption is independent of CB_{9k} and TRPV6. The authors think that in the KO mice there is compensation by another Ca channel or protein and that other novel factors are involved in intestinal Ca absorption[45]. It has

been found that an ablation of CB_{9k} alters the expression of paracellular tight junction genes. The compensatory expression of paracellular tight junction genes in the duodenum was associated with CB_{9k}, but not with CB_{28k}[46]. This interaction between the transcellular and paracellular pathways might partially explain the variety of gut responses to absorb Ca under different pathophysiological conditions.

Calcium pump and Na^+/Ca^{2+} exchanger

Plasma membrane Ca^{2+}-ATPase (PMAC) 1 is an ATP-dependent transporter that pumps Ca out of the cytosol. This protein was detected in erythrocyte membranes and found to have a high Ca affinity[47]. It presents four isoforms (PMCA1–4), which are divided into several subtypes by alternative splicing. PMCA1 is considered as the housekeeping isoform because its mRNA is in all tissues. The correlation between the regulation of Ca homeostasis by TRPV6 and PMCA1 and duodenal and renal function is not well known. Nevertheless, some reports have described a role for TRPV6 and PMCA1 in the uterus, duodenum, kidney and brain[48–52]. PMCA has a relative molecular mass (M_r) of 130 kDa and a K_m for Ca of 0.2 μM in the presence of calmodulin[53]. In the intestine, PMCA is located in the caveolae, which can exist in open and closed forms that control Ca efflux from the cell[54]. The predominant form in the intestine is the isoform $PMCA_{1b}$. We have found that its expression and activity are higher in enterocytes from the villus tip than in those from the villus crypt, supporting the idea that mature enterocytes have the greatest capacity for transcellular Ca movement[55].

Another novel protein seems to be crucial in the transcellular Ca pathway. This is the protein 4.1R, which was also first identified in the erythrocyte membrane skeleton and is expressed in the epithelia of the intestine. So far, its physiological function remains unknown. Liu *et al.*[56] have detected that 4.1R co-localises with $PMCA_{1b}$. These authors have shown that 4.1R KO mice exhibit deterioration in intestinal Ca absorption. In 4.1R KO mice, the expression of $PMCA_{1b}$ in enterocytes was decreased. This finding that the deficiency in the adaptor protein 4.1R produces an impaired intestinal Ca absorption suggests that many yet to be defined molecules might also play important functions in epithelial Ca transport.

Apparently, the intestinal Na^+/Ca^{2+} exchanger (NCX1) is responsible for about 20 % of Ca exit. However, this protein has received little attention and many recent reviews ignore it as another molecule involved in the Ca^{2+} exit from the intestine. The activity of the intestinal Na^+/Ca^{2+} exchanger depends on the gradient created by Na^+/K^+-ATPase[57]. There are several isoforms that result from three different genes[58], but in the intestine NCX1 is present mainly in the enterocytes. It has been detected in rats[57], mice[59], chicks[55], horses[60] and dogs[61], but not in rabbits[62]. NCX1 has a stoichiometry of 3 Na^+:1 Ca^{2+} and can function in either a forward mode (Ca^{2+} extrusion) or in a reversed mode (Ca^{2+} entry), depending on the Na^+ and Ca gradients and the membrane potential[63]. We have found that the expression and activity of NCX1 are quite similar between mature and immature enterocytes from chick duodenum, but are slightly higher in the villus tip cells[55]. Recently, it has been found that the gene expression of NCX1, $PMCA_{1b}$ and CB_{9k} was down-regulated, whereas NCX1 expression was unchanged in the duodenum of a model of hypoxia in pregnant rats that shares clinical similarities with humans suffering from pre-eclampsia or other metabolic diseases. The authors have also found alterations in Ca transporters from the placenta and kidney and showed that these changes caused Ca deficiencies associated with pre-eclampsia[64]. As they pointed out, it is quite possible that this study may contribute to a better understanding of the interrelationship between Ca imbalances and metabolic disturbances during pre-eclampsia pathogenesis.

Paracellular pathway

The intestinal epithelium is formed by a continuous layer of individual cells with very narrow spaces between them through which small molecules and ions diffuse[65]. The epithelium must regulate this paracellular pathway for the maintenance of selective permeability. The movement of molecules and ions through this pathway is regulated by tight junctions. They are intercellular structures where plasma membranes of adjacent enterocytes have very close contact. The tight junction proteins are synthesised in the adjacent cells and they include occludin (Ocln) and claudins (Cldns). The latter is a protein family with more than twenty members. Both Ocln and Cldns are integral proteins having the capability of interacting adhesively with complementary molecules on adjacent cells and of co-polymerising laterally[66]. Ca^{2+} movement through the tight junctions is a passive process that depends on the concentration and the electric gradient across the epithelium. This transport is non-saturable and mainly occurs in the jejunum and ileum under conditions of adequate or high Ca intake[67]. When Ca intake is high, the sojourn time in the intestine is short and there is down-regulation of proteins involved in the transcellular pathway, which switches on the paracellular route[68] (see Fig. 1).

Hormonal effects

Calcitriol

Calcitriol or 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) is the main stimulus for intestinal Ca absorption. It induces changes in the structure and function of intestinal epithelial cells, which enhance Ca transport across the intestine. Calcitriol acts through genomic and non-genomic pathways, after binding to a VDR. Most of the studies have been focused on the effect of calcitriol on the intestinal transcellular Ca pathway. It has been found that the expression or the activity of all molecules presumably involved in this route is increased by calcitriol in experimental animals and even in human subjects[69–72]. Recently, it has been shown in mice that a single administration of $1,25(OH)_2D_3$ produced a 30-fold maximal increase in the ileal TRPV6 mRNA at 9 h. Multiple dosing of $1,25(OH)_2D_3$ increased the ileal TRPV6 to 200- to 600-fold, being the highest changes observed at the 3rd and 4th doses. TRPV6 protein levels were increased 1.5-fold throughout the duodenum and ileum after the 3rd and 4th doses, while levels in the colon were increased after

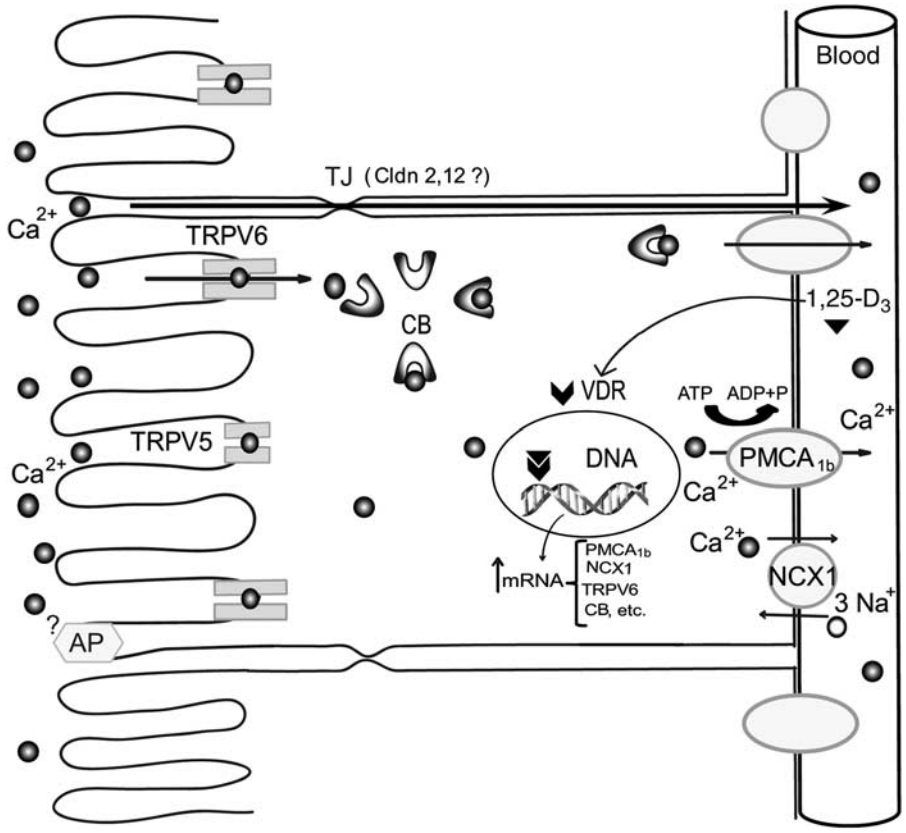

Fig. 1. Schematic model of transepithelial and paracellular calcium transport in the small intestine. The paracellular calcium pathway is carried out through tight junctions (TJ) by an electrochemical gradient (long arrow between cells). The transcellular calcium pathway consists of three steps: (1) apical entry of calcium through epithelial calcium channels TRPV5 and TRPV6 (the second one is the most abundant in intestine); (2) cytosolic diffusion bound to calbindins (CB); and (3) extrusion across the basolateral membranes by plasma membrane Ca^{2+}-ATPase ($PMCA_{1b}$) and Na^{+}/Ca^{2+} exchanger (NCX1). Calcitriol (1,25-D_3) stimulates the individual steps of transcellular calcium transport. Calcitriol molecules bind to their nuclear receptors (vitamin D receptors; VDR), and the complex 1,25-D_3–VDR interacts with specific DNA sequences inducing transcription and increasing the expression levels of $PMCA_{1b}$, NCX1, TRPV6 and CB. The real role of the intestinal alkaline phosphatase (AP) enzyme inintestinal calcium absorption has not been elucidated yet. Cldn, claudin.

the 4th dose. These changes in protein correlate with a high TRPV6 mRNA induction in the ileum at the same period[34]. The $1,25(OH)_2D_3$-enhanced Ca transport in mice was reported to be inhibited by FGF-23 as well as Ca transport in the colon cancer Caco-2 cells. Fibroblast growth factor-23 (FGF-23) produced an abolishment of the enhanced transcellular active Ca fluxes in both models. Despite the Arrhenius plot indicating that FGF-23 decreased the potential barrier of paracellular Ca movement, FGF-23 was found to modestly down-regulate the $1,25(OH)_2D_3$-enhanced paracellular Ca transport[73]. VDR-null mice adapt to pregnancy by up-regulating duodenal TRPV6 and intestinal Ca absorption, which enables a rapid normalisation of bone mineral content. These mice lactate normally and fully restore bone mineral content after weaning. In other words, VDR seems not to be required for skeletal adaptation during pregnancy, lactation and after weaning[74]. In the elderly, there is an age-related decrease in Ca absorption and a higher Ca intake is needed. It seems that increasing Ca intake from dairy products and Ca-fortified foods is much better than supplements. The combination of vitamin D intake to 800 IU (20 μg) daily together with a total Ca intake of 1000 mg daily is a simple and inexpensive strategy that could reduce fractures in aged individuals by 30 %[75].

Two studies have demonstrated that calcitriol increases paracellular fluxes across the intestine, mainly in the jejunum and ileum[76,77]. Fujita *et al.*[78] have demonstrated that $1,25(OH)_2D_3$ significantly increases claudin 2 and claudin 12 mRNA levels in Caco-2 cells. They have also shown that mRNA and protein levels for these proteins were lower at 12 weeks in the jejunum of VDR KO mice in comparison with wild-type mice, and small interfering RNA against these claudins decreased Ca permeability in the Caco-2 cells.

An intestinal calcistat has been hypothesised in order to explain vitamin D deficiency (VDD) with and without the clinical disease. Not all individuals with varying degree of VDD present secondary hyperparathyroidism and decreased bone mineral density (BMD). The intestinal calcistat would control Ca absorption, independently of parathyroid hormone (PTH) levels. A protein called Ca receptor (CaR) would dampen the production of active vitamin D metabolites in intestinal cells and diminish transcellular Ca transport, but would increase the paracellular Ca pathway. This local adaptation would adjust the fractional Ca absorption (FCA) according to the body's needs. When this local adaptation fails due to decreased Ca intake, decreased *25-hydroxyvitamin* D_3 ($25(OH)D_3$), CaR mutation, the systemic adaptation comes into play. The systemic adaptations

are an increase in PTH and in active vitamin D metabolites. A rise in PTH is the first indication of VDD with a decrease in BMD depending on the duration of VDD. Therefore, individuals with VDD with normal PTH and BMD should be called subclinical VDD[79]. The beneficial effect of vitamin D supplementation on this group of patients needs to be explored.

Parathyroid hormone

The action of PTH on intestinal Ca absorption occurs by the stimulation of renal CYP27B1 and, hence, increases $1,25(OH)_2D_3$-dependent intestinal Ca absorption. Direct effects of PTH on Ca uptake by enterocytes from rat duodenum have been shown. PTH stimulates enterocyte Ca influx, which could be blocked by the Ca channel antagonists verapamil and nitrendipine[80]. PTH/PTHrP receptors have been localised in intestinal epithelial cells along the villus[81]. PTH promotes nuclear effects such as a regulation of gene transcription and cell proliferation of enterocytes[82]. So far, a direct effect of PTH on the global process of intestinal Ca absorption has not been reported yet.

Thyroid hormones

Cross *et al.*[83] have demonstrated that thyroid hormone and vitamin D have a cooperative effect on intestinal Ca transport. They have also observed that thyroid hormones increase the genomic action of calcitriol in the intestine. Kumar *et al.*[84] have reported that hyperthyroid rats show larger Ca uptake by brush-border membrane vesicles and Ca efflux from the baso-lateral membranes of enterocytes than hypothyroid rats. The authors have also found that the Ca^{2+}-ATPase activity is not altered by thyroid hormones, while NCX1 activity is highly increased.

Growth hormone and insulin-like growth factor-1

Growth hormone (GH) can promote intestinal Ca absorption, which would occur indirectly mediated through an activation of renal CYP27B1 and the increase of serum $1,25(OH)_2D_3$ concentration[85]. Fleet *et al.*[86] have shown that GH treatment increases intestinal Ca absorption and duodenal CB_{9k} levels in aged rats without increasing serum $1,25(OH)_2D_3$ levels. The effect of GH on Ca absorption is mediated through insulin-like growth factor-1 (IGF-1) and there is evidence that this effect does not depend on vitamin D signalling. Intestinal Ca absorption in adult men has been shown to be positively correlated with IGF-1 and the age-related declines in IGF-1 have a negative impact on Ca absorption, which could not be explained by a decrease in serum $1,25(OH)_2D_3$[87]. Since the vitamin D-independent mechanism by which the GH/IGF-1 axis may regulate intestinal Ca absorption is not clear, this issue needs to be investigated.

Oestrogen

Oestrogen corrects the decline in the efficiency of intestinal Ca absorption at the onset of menopause, as suggested by cell culture studies[88], but the mechanisms that underlie this effect remain unknown. Colin *et al.*[89] have shown that oestrogen acts independently of $1,25(OH)_2D_3$ in the intestine, while others suggest that oestrogen alters intestinal Ca absorption through the vitamin D endocrine system[88]. Most of the oestrogen studies have been done in ovariectomised (OVX) animals. This ablation decreases endogenous oestrogen, but not totally since the adrenal androgens can be aromatised to oestrogen[90]. Oestrogen receptor (ER)α KO mice showed a decrease in duodenal TRPV6 mRNA expression, without changes in CB_{9k}, $PMCA_{1b}$ and VDR levels. In contrast, ERβ KO mice did not alter the genes for intestinal Ca absorption. It seems that the genomic effects of oestrogen in mice are mainly mediated by ERα. This idea should not be extrapolated to humans because it has been shown that in normal colon and cancer colon cells the subtype β is the predominant form of the ER[91]. In OVX rats treated with oestradiol, van Abel *et al.*[92] have found increased duodenal gene expression of TRPV5, TRPV6, CB_{9k} and $PMCA_{1b}$. They used CYP27B1 KO mice to analyse the calcitriol dependency of the stimulatory effects of oestradiol on intestinal Ca absorption and demonstrated that the oestradiol treatment increased mRNA levels of duodenal TRPV6.

The effect of two dietary phyto-oestrogens (coumestrol and apigenin) as well as ipriflavone, a synthetic phyto-oestrogen, on Ca absorption has been studied in the human Caco-2 cell line[93]. A direct effect of these compounds on intestinal Ca absorption was not observed. These controversial results indicate that the mechanism(s) triggered by oestrogen in the intestine requires further investigation[94].

Glucocorticoids

Osteoporosis is one of the most important side effects after long-term glucocorticoid (GC) treatments. Despite a reduced intestinal Ca absorption being part of the pathogenesis of GC-induced osteoporosis[95], the mechanisms triggered by GC on the intestine are not clear. A short-term GC treatment in young animals does not affect the expression of genes involved in intestinal Ca absorption[96], but a sustained dexamethasone suppresses mouse duodenal CB_{9k} expression through the GC receptor pathway[97]. It has been also reported that prednisolone for 10 d diminishes rat intestinal Ca absorption through a decreased expression of the active Ca transporters, which occurs independently of $1,25(OH)_2D_3$[98].

Nutritional factors affecting intestinal calcium absorption

Dietary calcium

Dietary Ca affects the composition of intestinal plasma membranes and alters intestinal Ca transport. All the genes involved in the transcellular pathway are enhanced by a low-Ca diet, which occurs by the activation of the vitamin D endocrine system[19,55,99,100]. The increase in the expression and activity of the intestinal Ca pump and NCX1 caused by a Ca-deficient diet occurs in both mature and immature enterocytes. However, VDR levels are decreased by low-Ca diets, independently of the degree of cell differentiation[55]. We think that high levels of

serum calcitriol provoked by low-Ca diets promote differentiation, which would produce cells more capable of expressing vitamin D-dependent genes required for Ca absorption. The activity of intestinal alkaline phosphatase (AP) is concomitantly increased by dietary Ca restriction, but a real role of this protein in intestinal Ca absorption cannot be discarded[55]. Benn *et al.*[101] have found that a low-Ca diet increases intestinal Ca absorption in wild-type, TRPV6 KO and CB_{9k} KO mice. This study indicates that the active intestinal Ca absorption occurs in the absence of TRPV6 and CB_{9k}, which challenges the dogma that both proteins are necessary for vitamin D-induced active intestinal Ca transport. Probably, TRPV6 is not the rate-limiting factor in the transcellular pathway or another factor/s is/are involved in its absence to compensate partially its function.

We have also observed that a low-Ca diet increases the reactivity and availability of sulfhydryl groups from intestinal brush-border membrane proteins of chicks[102]. It is quite possible that the sulfhydryl status of the brush-border membrane proteins is involved in the vitamin D-dependent intestinal Ca absorption. With regard to the lipid composition, we have detected minor changes in the fatty acid content of the basolateral membrane, but the lipid fluidity of these membranes is highly increased by dietary Ca restriction[19].

Recently, it has been reported that luminal Ca controls the intestinal Ca absorption through the modification of intestinal AP activity[103]. Authors have found that luminal Ca concentration increases the activity of AP and simultaneously decreases percentage Ca absorption, acting as a minute-to-minute regulatory mechanism of Ca entry.

Some studies have found an inverse association between Ca intake and adiposity[104–106]. However, not all studies have observed this relationship[107]. The mechanisms underlying these responses are not quite clear.

Most patients with idiopathic hypercalciuria show an increased intestinal Ca absorption. This has been demonstrated in studies using radiolabelled Ca and comparing the intestinal Ca absorption in patients with idiopathic hypercalciuria with that in normal subjects[108]. One possible mechanism for the increased Ca absorption might be the highest levels of serum calcitriol found in these patients as compared with normal individuals[65]. Apparently, this response is independent of Ca intake and reflects an enhancement in active Ca transport by the intestine[109].

With regard to the risk of kidney calculi formation in postmenopausal women, it is not clear if it is associated with Ca intake and vitamin D supplements. Haghighi *et al.*[110] have shown that the administration of 1000 mg/d of dietary Ca and vitamin D had a weak association with the formation of kidney calculi (only 1.9 % of fifty-three patients). Jackson *et al.*[111] have reported that in a study with 36 282 postmenopausal women treated with 1000 mg of Ca and 400 IU (10 μg) of vitamin D/d, a 17 % higher rate of kidney calculi was shown in the treated group after 7 years of follow-up. In contrast, another study contradicted this result, suggesting no association between Ca and vitamin D consumption and kidney calculi formation[112]. A low-Ca diet enhances the absorption of oxalate in the gut from normal individuals and kidney stone formers leading to an increase in urinary oxalate excretion, which has an important role in Ca stone formation[113,114].

There is evidence that dietary Ca restriction is a risk factor for cancer incidence, particularly for colorectal cancer[115]. By contrast, a high intake of dairy products and Ca intake protects against the distal colon and rectal tumours as compared with the proximal colon and reduces the risk of colon cancer[116]. It is also quite possible that a low Ca intake contributes to the development of renal, gastric, pancreatic, ovarian, endometrial and lung cancer as well as multiple myeloma, but there is no conclusive evidence[117].

Several studies have shown that a poor Ca intake is associated with an increased risk of breast cancer[118–120]. Vergne *et al.*[121] have recently demonstrated that Ca intake is associated with a high DNA repair capacity level, which in turn decreases the development of breast cancer.

Observational studies have identified an inverse relationship between maternal Ca intake and the incidence of pre-eclampsia[122,123]. When Ca supplements are administered during pregnancy, the incidence of pre-eclampsia and its consequences are reduced, including severe maternal morbidity and death[124].

Several meta-analyses of randomised controlled trials have also indicated that Ca supplementation can lead to a small reduction in systolic and diastolic blood pressure; therefore, Ca supplements might be beneficial in patients with hypertension[125,126]. Varenna *et al.*[127] have confirmed an association between hypertension and osteoporosis. There is a higher prevalence of hypertension in women with osteoporosis and a higher prevalence of osteoporosis in women with hypertension. The authors suggest that a low dairy Ca intake could be associated with an increased risk of both diseases and be a possible pathogenic link between the two conditions.

It is well known that a reduced intestinal Ca absorption is a risk factor for osteoporosis. There is evidence that the use of Ca supplements decreases bone turnover by about 20 %, and this is associated with a reduction in bone loss in postmenopausal women[128]. Low Ca intake was significantly associated with low BMD and increased risk of osteoporosis. However, the association between Ca and BMD was not consistently linear, and a sufficient vitamin D level might compensate for the negative influence of low Ca intake on bone[129]. Zhou *et al.*[130] have observed that a higher cumulative Ca and vitamin D intake in adult women was associated with better bone health, as indicated by BMD at multiple sites.

Other ions

Aluminium. Several authors have reported that aluminium ions (Al), at pharmacological doses, are able to reduce intestinal Ca absorption via the vitamin D-dependent transcellular pathway in the small intestine of humans and animals[131–135]. Orihuela *et al.*[135] have suggested that Al might interfere with Ca uptake by enterocytes through an effect on cell membranes. In addition, Al would decrease the intestinal glutathione (GSH) level affecting CB function and/or synthesis, which would lead to a reduced transcellular Ca absorption. Apparently, the inhibitory effect of Al varies according to thyroid hormone status[136]. In late pregnancy and mainly during the middle lactation of rats, Al has also been shown to reduce transcellular Ca absorption in the duodenum

by interfering with the mechanisms of Ca transport partially mediated by a high serum level of oestrogen and prolactin[137].

Phosphate. Severe dietary P deficiency is rare in humans and occurs only in conditions of severe starvation. Nevertheless, P deficiency can occur in alcoholics, patients with malabsorption syndromes, and those taking excessive amounts of Ca supplements[138]. Dietary P deficiency affects intestinal Ca absorption. Under this deficiency, serum $1,25(OH)_2D_3$ content increases[139,140] as well as the levels of CB and CB mRNA synthesis[141]. However, in some fashion, a low-P diet can stimulate CB formation and intestinal Ca absorption in the absence of an increased production of $1,25(OH)_2D_3$[142]. The reactivity and availability of sulfhydryl groups of chick intestinal brush-border membranes have been also shown to increase by a low-P diet[102], but it remains unknown whether this response is related to increased intestinal Ca absorption. Meyer *et al.*[143] have demonstrated that chickens fed a low-P diet displayed an increase in intestinal VDR mRNA. The authors have reported that a complex regulation of VDR expression occurs in that low-P restriction enhances VDR mRNA levels, possibly via increased serum $1,25(OH)_2D_3$.

Magnesium. For several decades, it has been known that there is interaction between Ca and Mg^{2+} in the intestine, which varies throughout the intestine. Mg^{2+} has been found to inhibit Ca absorption primarily in the duodenum, whereas Ca inhibits Mg^{2+} transport in the ileum but not in the duodenum[144]. In studies of short-term uptake in rat duodenal mucosa, O'Donnell & Smith[145] have demonstrated that Mg^{2+} inhibited the time-dependent uptake of Ca, but Ca did not alter Mg^{2+} uptake. Increasing the Mg^{2+} concentration to 1.25 mmol/l decreased the mucosal-to-serosal flux of Ca by 50 % and abolished net Ca absorption, mainly due to a depression in the paracellular pathway[146]. In patients with idiopathic hypercalciuria and renal Ca stone disease, oral supplementation of Mg^{2+} has been shown to be favourable because it decreases Ca absorption and increases Mg^{2+} absorption, which may reduce risk factors for renal Ca stone formation[147]. By contrast, Mg^{2+} deficiency significantly increased enterocyte content of Ca[148]. The mechanism is difficult to understand because Mg^{2+} deprivation has been associated with a low production of $1,25(OH)_2D_3$, the main stimulator of intestinal Ca absorption[149]. However, some studies contradict the previous data. Fine *et al.*[150] have found that increasing dietary Mg^{2+} had no effect on Ca absorption. Kosakai *et al.*[151] have demonstrated in sheep that the apparent Ca absorption tended to increase when the dietary Mg^{2+} content was increased, which was accompanied without an alteration in the plasma Ca concentration and increased urinary Ca excretion. Bae *et al.*[152] have shown that the consumption of seaweed Ca extract or inorganic calcium carbonate with Mg^{2+} oxide in OVX rats produced a similar intestinal Ca absorption, but only the seaweed Ca extract caused an increase in femoral BMD and strength in OVX rats. They concluded that seaweed Ca extract is a good Ca and Mg^{2+} source for improving bone health as compared with synthetic Ca and Mg^{2+} supplementation.

Lipids

At present, the effects of dietary lipids on intestinal Ca absorption are not clear. Hessov *et al.*[153] have demonstrated that low-fat-diets increase intestinal Ca absorption in nine patients with fat malabsorption. Steatorrhoea has been associated with an inhibition of intestinal Ca absorption[154]. Jewell *et al.*[155] have found that both the paracellular Ca transport and transcellular Ca transport across monolayers of Caco-2 cells were significantly increased after exposure to conjugated linoleic acid. Murphy *et al.*[156] have found that zona occludens-1, Ocldn, and Cldn-4 were all up-regulated while Cldn-1 was down-regulated by *trans*-10, *cis*-12-conjugated linoleic acid, which explains the increase in the paracellular route. However, they did not find any effect on genes involved in the transcellular pathway, which should be further explored. Fatty acids directly contribute to increasing intestinal Ca absorption via a cation exchange mechanism between cellular H^+ for luminal Ca favoured by exchanger activity $2H^+/Ca^{2+}$[157]. Fatty acids would increase the proliferation of colonic epithelial cells, producing a trophic effect on the mucosa; they contribute to increasing the absorptive surface[158,159].

Recently, in high-fat diet-fed mice, Xiao *et al.*[160] have found a marked decrease in the intestinal Ca absorption, which is accompanied by redox imbalance and increased duodenal oxidative damage, an effect that was avoided by lipoic acid or Ca supplementation. In addition, they have also shown that a high-fat diet down-regulates the gene expression of molecules involved in the transcellular pathway of intestinal Ca absorption, independently of calcitriol regulation. The authors think that the main cause for high-fat-diet-induced inhibitory intestinal Ca absorption is not Ca soap but duodenal oxidative stress.

Carbohydrates

Lactose is a disaccharide found in milk and dairy products that enhances Ca homeostasis, which should be beneficial for bone health[161]. It is well established that lactose stimulates the intestinal Ca absorption that seems to occur by passive transport in the small intestine[162]. Apparently, lactose promotes intestinal Ca absorption, independently of the vitamin D endocrine system. Natsuko *et al.*[163] have found that lactose alters intestinal AP in rats not only in a direct way, but also indirectly through a regulation of intestinal AP expression, mainly in the jejunum. Epilactose (a rare disaccharide in cows' milk) has been demonstrated to increase Ca transport in everted small-intestinal sacs by promoting the generation of SCFA and other organic acids[164]. Later on, the same group of investigators has found that the epilactose-mediated promotion of intestinal Ca absorption involves the paracellular route in the rat small intestine through the induction of myosin regulatory light chain phosphorylation via myosin light chain kinase and Rho-associated kinase[165]. They have also shown that epilactose improves intestinal Ca absorption in gastrectomised rats. They think that the resulting SCFA production by intestinal microbes is responsible for this effect, as well as the increase in the caecal mucosa area and the soluble Ca concentration[166].

Some sugar alcohols such as erythritol, xylitol, sorbitol, maltitol, palatinit or lactitol have been found to enhance Ca transport from rat small and large intestine epithelium *in vitro*. Differences in Ca transport were shown in different segments of the intestine, but not between the sugar alcohols tested[167]. Recently, Xiao *et al.*[168] have found that mannitol improves the absorption and retention of Ca and Mg^{2+} in growing rats, an effect that occurs through the fermentation of mannitol in the caecum.

Prebiotics

The fortification of milk with milk Ca or Ca salts is among the strategies suggested to increase Ca intake or absorption or both, but the availability of Ca salts in milk has not been well characterised[169]. In addition, several food ingredients such as fructo-oligosaccharides and caseinophosphopeptides (CPP) have been proposed as enhancers of the absorption of Ca from milk or other foods. Fructo-oligosaccharides belong to the group of non-digestible oligosaccharides (NDOs), which includes inulin, oligofructose and galacto-oligosaccharides. They can be digested by the colonic microflora, which produces SCFA that decreases intestinal pH and increases Ca solubility leading to an enhancement of paracellular and transcellular Ca transport[170,171]. It has also been suggested that NDOs increase active Ca transport by the activation of CB_{9k}[172]. Fukushima *et al.*[173] have demonstrated in rats that fructo-oligosaccharide consumption increases the gene expression of TRPV6 and CB_{9k} through the SCFA formed in the fermentation. The fibres formed mainly by inulin have positive chronic effects on Ca metabolism related to changes in the intestine, resulting in improvement of bone health[174]. In addition, galacto-oligosaccharides have also potential for improving mineral balance and bone properties[175]. With regard to CPP, originating from casein digestion, it has been demonstrated that their addition to Ca-fortified milk increases intestinal Ca absorption in growing rats[176], but the mechanisms involved in this response were not elucidated. Erba *et al.*[177] have studied the influence of different four CPP/Ca ratios and three mineral concentrations on the amount of passive Ca absorbed across the everted distal small intestine of rats. The positive effect was dependent on the relative amount of both species in the intestinal lumen, the ratio 15 being the most efficient at increasing mineral transport. Nevertheless, in a randomised cross-over trial undertaken in fifteen adults, no effect of CPP was found on intestinal Ca absorption[178]. Cosentino *et al.*[179] have demonstrated that both intestinal human HT-29 and Caco-2 cells have the ability to take up extracellular Ca under CPP stimulation. Recently, Colombini *et al.*[180] did not find effects of CPP on paracellular Ca absorption and on TRPV6 mRNA expression in intestinal human HT-29 and Caco-2 cell lines.

A recent study has shown that the daily intake of soluble maize fibre, a well-tolerated prebiotic fibre, increases short-term Ca absorption in adolescents consuming less than the recommended amounts of Ca[181].

Probiotics

Probiotics are viable microbes that alter the microflora in a compartment of the host exerting beneficial health effects in this host[182]. Most probiotic products contain lactic acid-producing bacteria, which mainly belong to the genera *Lactobacillus* and *Bifidobacterium*. In growing rats, it has been demonstrated that probiotic yoghurt containing strains of *Lactobacillus casei*, *L. reuteri* and *L. gasseri* increase intestinal Ca absorption and bone mineral content[183]. Besides, it has been observed that in Caco-2 cells, the probiotic *L. salivarius* causes an increase in Ca uptake[184].

Synbiotics

Synbiotics are defined as products containing prebiotics and probiotics, in which the prebiotic compound favours the probiotic compound[182]. It has been shown in OVX rats that intestinal Ca absorption tended to be higher in the synbiotic group and was significantly higher in the prebiotic group in comparison with the control group[185].

Proteins

Proteins are essential to bone, but the Ca-wasting effect of a high protein intake constitutes a point of debate. It has been known for many years that increasing dietary protein enhances urinary Ca either in human subjects or in rats[186]. The idea was that the additional Ca excretion was of skeletal origin as a result of buffering in bones the metabolic acid load imposed by higher protein intake[187]. The theory was that a high-protein diet, mainly meat, creates a higher acid load due to the high content of amino acids containing sulfur. This acid load cannot be neutralised by the kidneys and the body pulls Ca from the skeleton to balance pH at the expense of bone, causing an enhancement of urinary Ca[188]. However, clinical studies have demonstrated that a short-term high-protein diet (2.1 g/kg) significantly increases the intestinal Ca absorption as compared with a medium-protein diet (1 g/kg) and the increment in urinary Ca is quantitatively explained by an increase in intestinal Ca absorption efficiency[189]. The transcellular route, the paracellular pathway or a combination of both mechanisms of intestinal Ca absorption might be involved in response to high dietary protein. By using duodenal brush-border membrane vesicles, Gaffney-Stomberg *et al.*[190] have demonstrated that the transcellular component of Ca absorption was accelerated in rats fed a high-protein diet, which was due to an enhancement in maximum velocity, without affecting the Michaelis–Menten constant. However, they did not study whether the gene or protein expression of the molecules involved in the transcellular pathway was modified. They did not find increased bone resorption or changes in serum PTH and calcitriol levels.

Since more research is necessary to resolve the protein debate, it has been suggested not to reduce the protein intake below the dietary reference intake because it could be detrimental to bone health, especially in old individuals[191], and protein intakes and balance of different protein sources with a variety of different foods constitutes appropriate dietary advice[192].

Black tea

Black tea (*Camellia sinensis*) is a medicinal plant with a rich flavonoid content and a plethora of health-promoting effects[193,194].

Das *et al.*[195] have studied the ability of black tea extract as a suitable alternative adjunct for Ca supplementation in treating an OVX rat model of early osteoporosis. The results suggest that black tea could stimulate intestinal Ca absorption, which is associated with an increased activity of AP and Ca^{2+}-ATPase. Black tea's effectiveness in maintaining bone health was detected to be similar to 17β-oestradiol. Therefore, this study suggests that a simultaneous use of black tea is promising as a prospective candidate for adjunctive therapies for Ca supplementation in the early stage of menopausal bone changes.

Coffee

Coffee drinking is a popular habit worldwide. It is consumed in considerable amounts every day. Caffeine, a methylxanthine present in coffee, has been considered to be responsible for an increased risk of osteoporosis in coffee drinkers[196,197]. At present, data are inconsistent[198–200], hence, the effect of caffeine on intestinal Ca absorption is not well established. It has been demonstrated in rats that intestinal Ca absorption is stimulated by the increase in $1,25(OH)_2D_3$ production after chronic administration of caffeine[201]. In addition, urinary and faecal excretion is also increased. In postmenopausal osteoporotic women, a coffee intake in excess of 1000 ml could induce an extra Ca loss of 1.6 mmol Ca/d, while 1–2 cups of coffee/d would have little impact on Ca balance[202]. Metabolic balance studies show a weak negative effect of caffeine on the efficiency of intestinal Ca absorption. However, the effect of caffeine is small enough to be fully offset by 1–2 tablespoons (15–30 ml) of milk[138]. A recent study in OVX rats has shown that low to moderate caffeine intake may exert some beneficial effects on the skeleton, increasing bone mineralisation, and improving the strength and structure of cancellous bone and the mechanical properties of compact bone; however, it did not cause any significant effect in rats with normal oestrogen levels[203]. The continuous debate has weakened interest in the study of coffee as a risk factor for osteoporosis, which has been reinforced by the non-inclusion of coffee in the list of risk factors in the predictive scale for fracture informed by the WHO[204]. The understanding of physiological effects of coffee consumption is difficult because of the vast array of components included in the brewed product and the varied effects of each compound. Recently, an unfavourable effect of trigonelline, an alkaloid present in coffee, has been demonstrated on bone mechanical properties in oestrogen-deficient rats, but not in control rats[205]. It is quite possible that the effects on intestinal Ca absorption and the Ca economy by caffeine or other bioactive compounds present in coffee depend on the amount and frequency of coffee intake. This is another issue that merits to be more investigated due to the considerable number of coffee drinkers and the rising life expectancy that will increase bone disorders in the next years.

Pharmacological compounds altering intestinal calcium absorption

Almost two decades ago, we reported that the intactness of the steady-state levels of intestinal glutathione (GSH) seemed to be critical for Ca absorption. By using DL-buthionine-(*S*,*R*)-sulfoximine (BSO), a specific inhibitor of γ-glutamylcysteine synthetase, we have shown that the Ca transfer from lumen to blood in vitamin D-supplemented chicks was inhibited, an effect that did not occur in vitamin D-deficient chicks. At that time we concluded that the effects of BSO on intestinal Ca absorption were dependent on the vitamin D status of the animal. One explanation that we gave was that GSH depletion might increase the reactive oxygen species and other substances that could deteriorate intestinal Ca absorption[19]. Since intestinal AP was one of the best candidates to suffer oxidative stress[206], we later studied the effect of BSO on the activity of this enzyme in chicks fed a commercial diet. In fact, the AP activity declined after BSO treatment, which was dose and time dependent. The effect occurred either *in vivo* or *in vitro* but was not direct; it was first necessary to deplete GSH in order to produce free hydroxyl radicals and an increment in the protein carbonyl content. The reversibility of the BSO effect was proved by the addition of GSH monoester to the duodenal loop[207].

Menadione (MEN) is another pharmacological compound that alters chick intestinal Ca absorption. It is a quinone that is clinically relevant because of its anti-tumour properties[208] and its use in the treatment of osteoporosis[209]. MEN metabolism involves redox cycling, resulting in the release of various reactive oxygen species including free hydroxyl radicals[210]. We have demonstrated 30 min after a single large dose of MEN that the intestinal Ca absorption was inhibited, which lasted for 9 h. The inhibition affected the transcellular pathway as judged by the inhibition of Ca pump activity, the main protein involved in Ca extrusion from the enterocyte to the lamina propria. Intestinal AP activity was also inhibited, but not that from other brush-border membrane enzymes. GSH depletion, enhancement in the protein carbonyl content as well as the appearance of free hydroxyl radicals were indications that MEN caused oxidative stress provoking deleterious consequences on intestinal Ca absorption. The oral administration of GSH monoester prevented the inhibition of intestinal Ca absorption and the GSH deprivation produced by MEN[211]. As mitochondria are the major source of reactive oxygen species[212], we have investigated the role of these organelles in the inhibition of the intestinal Ca absorption caused by MEN. The quinone produced mitochondrial dysfunction as shown by inhibition of enzymes from Krebs' cycle, DNA fragmentation, release of cytochrome c, alteration of membrane potential and enhancement of Mn^{2+}-superoxide dismutase activity. The mitochondrial dysfunction would be a consequence of mitochondrial GSH depletion, which would alter the membrane permeability triggering the release of apoptotic molecules leading to DNA fragmentation. The oxidant effects would alter the transcellular Ca pathway affecting the global process of intestinal Ca absorption[213]. Since the flavonol quercetin has antioxidant properties[214], we have studied the ability of quercetin to protect the chick intestine against the inhibition of intestinal Ca absorption caused by MEN. Effectively, quercetin abrogated the inhibitory effect of MEN on chick intestinal Ca absorption through the restoration of intestinal redox state, blockage of alterations in the mitochondrial membrane permeability, and abolition of the FasL/Fas/caspase-3 signalling pathway activation[215].

In addition, the hormone melatonin (MEL) was also able to restore chick intestinal Ca absorption inhibited by MEN. MEL is known as a direct scavenger of free radicals with the ability to remove singlet oxygen, the superoxide anion radical and hydroperoxide. It has also an indirect antioxidant action through a modulation of antioxidant enzyme activities. MEL by itself did not alter intestinal Ca absorption and other variables influencing that process. The MEL protective mechanism seems to be switched on under oxidative stress conditions produced by MEN, leading cells to the normal redox status. MEL administration after MEN injection returned rapidly the intestinal GSH and protein carbonyl contents to control values as well as the SOD and CAT activities. Concomitantly, intestinal Ca absorption went up to normal values, suggesting that the restoration of redox status of the gut by MEL allowed the recovering of the intestinal capability to absorb the cation properly. MEL not only normalised the redox status of the enterocytes but also rescued the epithelial cells from MEN-induced apoptosis[216]. Therefore, MEL could be a potential drug of choice for the treatment of impaired intestinal Ca absorption caused by oxidative stress and exacerbated apoptosis, which occurs in certain pathophysiological conditions (ageing, coeliac disease, intestinal bowel disease, cancer and others) or after intake of drugs causing oxidation.

We have also shown that a single high concentration of sodium deoxycholate (NaDOC) inhibits intestinal Ca absorption through a down-regulation of proteins involved in the transcellular pathway, as a consequence of triggering oxidative stress and mitochondria-mediated apoptosis[217]. This inhibitory effect on intestinal Ca absorption produced by NaDOC has been shown to be abolished by the concomitant use of the antioxidant quercetin, which clearly indicates that the response of NaDOC was mediated by the oxidative stress. Deoxycholic acid or its salt, NaDOC, is the major secondary bile acid in humans and is toxic in high concentrations causing liver damage during cholestasis and acting as a promoter of colon cancer in experimental animals[218]. It is well known that its concentration varies according to the diet; a high-fat diet is associated with an increased secretion of NaDOC[219]. This bile salt perturbs the membrane structures by alteration of membrane microdomains and decreases the transepithelial electrical resistance in the Caco-2 cell line through reactive oxygen species generation and other signalling mechanisms[220,221]. Therefore, the tight junctions constitute another target of NaDOC in the intestine, suggesting that the paracellular pathway of intestinal Ca absorption might be also affected by this bile salt. Based on the knowledge that a minor bile acid, ursodeoxycholic acid (UDCA), has beneficial effects of protection against cytotoxicity due to more toxic bile acids, we have tried to ascertain the potentiality of UDCA to prevent the inhibition of intestinal Ca absorption caused by NaDOC. In addition, we have studied the effects of UDCA alone on intestinal Ca absorption either in chicks or rats. The data have shown that UDCA not only prevented the inhibition of intestinal Ca absorption caused by NaDOC either in chicks or rats, but also UDCA alone enhanced that process. This was an unpredictable finding, which together with the previous data indicate that NaDOC is a bad whereas UDCA is a good bile acid for intestinal Ca absorption. The interesting point is that the combination of both bile acids neutralises the response of each other, probably because UDCA protects the intestine against the GSH depletion and protein carbonyl increment produced by NaDOC. Both NaDOC and UDCA altered protein and gene expression of molecules involved in the transcellular pathway of intestinal Ca absorption, but in the opposite way. NaDOC decreased the protein expression of PMCA1b, NCX1and CB, whereas UDCA increased the protein expression of all of them. The expression of these molecules was identical to those from the control group when the combined treatment was used. The gene expression of *pmca1b*, *ncx1* and *cb* was increased by UDCA and UDCA + NaDOC. In contrast, NaDOC decreased the gene expression of *pmca1b* and *cb* without modifying that of *ncx1*. UDCA also increased the protein and gene expression of VDR, which suggests that VDR is involved in the enhancement of intestinal Ca absorption produced by UDCA[222]. The relationship between UDCA and VDR is not surprising because it has been demonstrated that VDR also binds bile acids[223,224] and the increase in the cathelicidin expression in biliary epithelial cells from human liver caused by UDCA is mediated by VDR activation, an effect that is blunted by a small interfering RNA strategy[225].

There are conflicting data with regard to the effects of proton pump inhibitors and osteoporotic fracture risk. Presumably, they increase the risk through hypochlorhydria and decreased FCA. Hansen *et al.*[226] have evaluated the effect of proton pump inhibitor therapy on FCA using the dual stable isotope method. Participants underwent three 24 h FCA studies; two of them were accomplished 1 month apart to establish the baseline of FCA, the third one was after taking omeprazole (40 mg/d for 30 d). The data revealed that age, gastric pH, serum omeprazole levels, adherence to omeprazole and 25-hydroxyvitamin D levels were not related to changes in FCA between visits 2 and 3. The level of serum $1,25(OH)_2D_3$ was the only variable associated with the change in FCA between visits 2 and 3. More studies are necessary to elucidate the mechanisms by which proton pump inhibitors increase osteoporotic fracture risk.

Wahl *et al.*[227] have demonstrated reduced FCA in patients under anticonvulsant treatment. The possible mechanism underlying this process is complex. It has been demonstrated that phenytoin and carbamazepine inhibit active Ca transport from the apical to the basolateral side of Caco-2 cells under physiological Ca conditions and vitamin D improves the antiepileptic drug-induced decrease in Ca permeability[228].

Restraint stress significantly down-regulates the mRNA expressions of TRPV6 and $Ca_v1.3$, CB-D_{9k}, and $PMCA_{1b}$, but not the expression of TRPV5 or NCX1. In contrast, the mRNA expressions of paracellular genes, ZO-1, occludin and claudin-3, are not modified by restraint stress. Since several antidepressant or anxiolytic drugs alleviate stress-induced depressive and anxiety symptoms, Charoenphandhu *et al.*[229] have hypothesised that these drugs might also enhance Ca transporter gene expression in stressed rats. In fact, a 4-week daily administration of 10 mg/kg fluoxetine, 10 mg/kg reboxetine or 10 mg/kg venlafaxine differentially increased the duodenal Ca transporter genes in stressed rats, whereas 2 mg/kg diazepam had no such effect. These findings might be applied to help ameliorate

the stress-induced bone loss and osteoporosis by restoring intestinal Ca absorption.

Octyphenol, a degradative product used to produce rubber, pesticides and paints, and bisphenol A (BPA), an organic compound used for manufacturing polycarbonate plastic and epoxy resins, are known as endocrine disruptors. The effect of both on serum Ca levels and expressions of Ca transport genes in the duodenum and kidney was studied in pregnant mice. Either octyphenol or BPA decreased serum Ca levels. Both drugs decreased the levels of TRPV5 and CB_{9k} in the kidney and the levels of TRPV6 in the duodenum. Gene expression and protein expression of CB_{9k} were decreased in the duodenum by BPA but increased by octyphenol at high doses. These results indicate that decreased serum Ca levels caused by these disruptors might be a consequence of the alteration in the expression of genes related to Ca transport[230].

Gene × diet interactions influence intestinal calcium absorption

Dietary Ca restriction increases the efficiency of intestinal Ca absorption, but the impact of genetics on this adaptive response is not clear. In humans, the efficiency of intestinal Ca absorption varies from 7 to 75 %[231]. The large variation is probably owing to the influence of multiple physiological factors (for example, growth, pregnancy, lactation, ageing) and environmental variables (for example, dietary Ca intake, vitamin D). Little information is available for the impact of genetics on the efficiency of intestinal Ca absorption and the adaptive up-regulation of Ca absorption to a low dietary Ca intake. Two laboratories have studied the efficiency of intestinal Ca absorption in different mice and have found that it is higher in C3H/HeJ mice in comparison with C57BL/6J mice, which suggests that genetic background might influence this trait[232]. In addition, racial differences in the ability of adolescent girls to increase Ca absorption efficiency during a low Ca intake also indicate that this adaptive response has a genetic component[233,234] In agreement with this concept, adolescent black girls have been shown to exhibit higher intestinal Ca absorption as compared with white girls[235], and this may contribute to the higher bone deposition found in black girls[236].

Replogle *et al.*[237] have examined eleven inbred lines of mice fed on defined diets containing either high or low Ca concentration from weaning to 12 weeks of age. The authors have shown that genetic variation and gene × diet interactions affect not only the active intestinal Ca absorption, but also its relationship to bone. These interactions are partially explained by variations in the traditional cellular mediators (i.e. TRPV6, CB_{9k}, $PMCA_{1b}$ mRNA) and in the main hormonal regulator, $1,25(OH)_2D_3$, of intestinal Ca absorption. This field is relatively new, hence many efforts are required to bring more light for the understanding of this knowledge.

Conclusions

Ca is an ion involved in multiple physiological functions. Absorption through the intestinal epithelium is a complex process regulated by an intricate network of hormones and nutritional factors. At present, the Western diet model adopted is poor in Ca content and at the same time interferes with the proper absorption of the cation. Some diseases such as osteoporosis, hypertension and cancer are associated with dietary Ca restriction. Current recommendations are to obtain Ca from the diet in preference to supplements since dietary Ca intake has not been associated with the adverse effects of supplements, probably because Ca is provided in smaller boluses absorbed more slowly[238]. Milk and dairy products are the best sources of Ca. There have been advances in food industry attempts to compensate for the Ca shortage through the introduction of prebiotics and probiotics in the basic diet. Certain dietary habits such as an increased protein intake remain a point of debate. It is important to take into account that alterations in the redox state of the intestinal epithelium produced by some medications such as MEN, BSO and UDCA also modify the intestinal Ca absorption. Therefore, intestinal Ca absorption must be carefully attended, so it is necessary to consider not only the intake, but also possible interactions with other ions, the genetic background, the effect of diet and the use of certain medications. Health professionals should be aware of this knowledge in order to develop nutritional or medical strategies to stimulate the efficiency of intestinal Ca absorption and to prevent diseases.

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