

## Molecular mechanisms triggered by low-calcium diets

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Ca is not only essential for bone mineralisation, but also for regulation of extracellular and intracellular processes. When the  $\text{Ca}^{2+}$  intake is low, the efficiency of intestinal  $\text{Ca}^{2+}$  absorption and renal  $\text{Ca}^{2+}$  reabsorption is increased. This adaptive mechanism involves calcitriol enhancement via parathyroid hormone stimulation. Bone is also highly affected. Low  $\text{Ca}^{2+}$  intake is considered a risk factor for osteoporosis. Patients with renal lithiasis may be at higher risk of recurrence of stone formation when they have low  $\text{Ca}^{2+}$  intake. The role of dietary  $\text{Ca}^{2+}$  on the regulation of lipid metabolism and lipogenic genes in adipocytes might explain an inverse relationship between dairy intake and BMI. Dietary  $\text{Ca}^{2+}$  restriction produces impairment of the adipocyte apoptosis and dysregulation of glucocorticosteroid metabolism in the adipose tissue. An inverse relationship between hypertension and a low- $\text{Ca}^{2+}$  diet has been described.  $\text{Ca}^{2+}$  facilitates weight loss and stimulates insulin sensitivity, which contributes to the decrease in the blood pressure. There is also evidence that dietary  $\text{Ca}^{2+}$  is associated with colorectal cancer. Dietary  $\text{Ca}^{2+}$  could alter the ratio of faecal bile acids, reducing the cytotoxicity of faecal water, or it could activate  $\text{Ca}^{2+}$ -sensing receptors, triggering intracellular signalling pathways. Also it could bind luminal antigens, transporting them into mucosal mononuclear cells as a mechanism of immunosurveillance and promotion of tolerance. Data relative to nutritional  $\text{Ca}^{2+}$  and incidences of other human cancers are controversial. Health professionals should be aware of these nutritional complications and reinforce the dairy intakes to ensure the recommended  $\text{Ca}^{2+}$  requirements and prevent diseases.

**Low- $\text{Ca}^{2+}$  diet: Intestine: Kidney: Bone: Hypertension: Lipids: Cancer**

### Introduction

Ca is a fundamental building block of bone and, hence, is essential for achieving optimal peak bone mass in the first two to three decades of life and for the maintenance of bone mass, later in life<sup>(1)</sup>. It is also important for many physiological processes such as nerve impulse transmission, muscle contraction, blood coagulation, secretory activity and apoptosis<sup>(2,3)</sup>. The dysregulation of Ca homeostasis appears to be a common factor linking conditions such as hypertension, insulin resistance and obesity<sup>(4)</sup>. Although some epidemiological studies have shown an inverse association between dietary  $\text{Ca}^{2+}$  and risk of breast and colon cancer, in prostate cancer, a high  $\text{Ca}^{2+}$  intake has been associated with higher risk<sup>(5–8)</sup>. As the diet is the only external source of  $\text{Ca}^{2+}$ , appropriate levels of the mineral intake according to age and sex are recommended, in order

to preserve bone health and metabolic balance. These requirements are higher in childhood, pregnancy and lactation. The intake of dairy products and  $\text{Ca}^{2+}$  supplements has been highly advertised for many years. However, most of the studies show that  $\text{Ca}^{2+}$  intake is much lower than the international recommendations in the majority of countries<sup>(9–11)</sup>, with a few exceptions such as Finland and Denmark<sup>(12,13)</sup>. Although adaptive mechanisms have evolved to control the amount of  $\text{Ca}^{2+}$  that is absorbed, the efficiency of this response involves metabolic changes, whose persistence with time may be deleterious.

### Effect of dietary $\text{Ca}^{2+}$ deficiency on $\text{Ca}^{2+}$ homeostasis and the metabolism of calcitropic hormones

Dietary  $\text{Ca}^{2+}$  deficiency provokes increments in the efficiency of intestinal  $\text{Ca}^{2+}$  absorption and in renal  $\text{Ca}^{2+}$

**Abbreviations:** BMD, bone mineral density; CaR, Ca-sensing receptor; CYP24, 24-hydroxylase; CYP27B1, 25-OH-cholecalciferol 1-hydroxylase; DASH, Dietary Approaches to Stop Hypertension; IGF-1, insulin-like growth factor-1; KO, knock-out;  $1,25(\text{OH})_2\text{D}_3$ , 1,25-dihydroxyvitamin  $\text{D}_3$ ;  $25\text{OHD}_3$ , 25-hydroxyvitamin  $\text{D}_3$ ; PTH, parathyroid hormone; TRPV6, transient receptor potential cation channel, subfamily V, member 6; VDR, vitamin D receptor.

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reabsorption<sup>(14)</sup>. This is an adaptation process performed as a compensation mechanism in order to cover the cation needs of the organism<sup>(15)</sup>. Serum  $\text{Ca}^{2+}$  levels may be normal or low, according to the extent and degree of  $\text{Ca}^{2+}$  deficiency. We have demonstrated hypocalcaemia in chicks after 10 d of low  $\text{Ca}^{2+}$  intake (0.1%)<sup>(16,17)</sup>. The mechanisms of adaptation to low- $\text{Ca}^{2+}$  diets depend on the vitamin D status, mainly of the rate of 1,25-dihydroxyvitamin  $\text{D}_3$  ( $1,25(\text{OH})_2\text{D}_3$ ) synthesis. Cholecalciferol is hydroxylated in two steps: the first hydroxylation occurs at the carbon at the 25 position in the liver, and the second hydroxylation takes place in the kidney at the level of the carbon at the 1 position<sup>(18)</sup>. An increase in the serum levels of  $1,25(\text{OH})_2\text{D}_3$  by a low- $\text{Ca}^{2+}$  diet has been shown in human subjects<sup>(19)</sup>, chicks<sup>(20)</sup>, ewes<sup>(21)</sup> and rats<sup>(22)</sup>, but not in hamsters<sup>(23)</sup>. On the contrary, dietary  $\text{Ca}^{2+}$  restriction in the presence of constant vitamin D intake may cause depletion of 25-hydroxycholecalciferol, as shown in the plasma of rats as a consequence of high activity of the renal enzyme 25-OH-cholecalciferol 1-hydroxylase (CYP27B1), which catalyses the transformation of 25-hydroxyvitamin  $\text{D}_3$  ( $25\text{OHD}_3$ ) into  $1,25(\text{OH})_2\text{D}_3$ <sup>(24)</sup>. In renal stone formers, identified as absorptive hypercalciuric or renal hypercalciuric, Gascon-Barré *et al.*<sup>(25)</sup> have observed lower circulating levels of  $25\text{OHD}_3$  when they were on a low- $\text{Ca}^{2+}$  diet as compared with those values shown with a normal- $\text{Ca}^{2+}$  diet. CYP27B1 mRNA has been found to be expressed in the duodenum, the site of maximal vitamin D-regulated intestinal  $\text{Ca}^{2+}$  absorption<sup>(26)</sup>, but at low levels relative to the kidney. Besides, it has been demonstrated that the intestinal enzyme is not altered by dietary  $\text{Ca}^{2+}$  restriction, which is the classical condition regulating the renal CYP27B1<sup>(26)</sup>. High levels of  $1,25(\text{OH})_2\text{D}_3$ , caused by low- $\text{Ca}^{2+}$  diets, modulate the adaptive changes in intestinal  $\text{Ca}^{2+}$  absorption and in renal  $\text{Ca}^{2+}$  reabsorption, apparently through vitamin D-mediated transcriptional activation<sup>(27)</sup>. An increment in the expression of proteins presumably involved in the  $\text{Ca}^{2+}$  movement through the cells has been reported, such as the  $\text{Ca}^{2+}$  channels Ca transport protein 1 (transient receptor potential cation channel, subfamily V, member 6; TRPV6) and Ca transport protein 2 (transient receptor potential cation channel, subfamily V, member 5; TRPV5)<sup>(28)</sup>, calbindin  $\text{D}_{9k}$ , calbindin  $\text{D}_{28k}$ <sup>(29)</sup>,  $\text{Ca}^{2+}$ -ATPase or the  $\text{Ca}^{2+}$  pump<sup>(29)</sup> and the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger<sup>(17)</sup>.

The effect of low- $\text{Ca}^{2+}$  diets on other metabolites derived from vitamin D is not well established. Fox *et al.*<sup>(30)</sup> did not show an increase in  $1,25(\text{OH})_2\text{D}_3$  catabolism, but they showed enhancement of the renal clearance of  $1,25(\text{OH})_2\text{D}_3$ . Goff *et al.*<sup>(31)</sup> found a 6- to 20-fold increase in 24-hydroxylase (CYP24) activity in animals exposed to  $\text{Ca}^{2+}$ -restricted diets as compared with those fed a  $\text{Ca}^{2+}$ -replete diet.

Parathyroid hormone (PTH) secretion is also stimulated by low- $\text{Ca}^{2+}$  diets<sup>(32)</sup>. Several studies have shown that the Ca-sensing receptor (CaR) of parathyroid cells is a key mediator of direct actions of extracellular  $\text{Ca}^{2+}$  on PTH secretion. CaR is a G protein-coupled receptor, whose activation by extracellular  $\text{Ca}^{2+}$  results in the suppression of PTH release<sup>(33,34)</sup>. CaR was first identified in bovine parathyroid cells, and successively found in neurons, osteoblasts, keratinocytes, enterocytes and mammary

epithelial cells. The sensitivity of parathyroid glands to small changes in serum Ca is remarkable. When hypocalcaemia is acute, the glands secrete PTH in a few seconds or minutes, this release being maintained between 60 and 90 min. A reduction in the intracellular PTH degradation also contributes to sustain this response. If hypocalcaemia is maintained for several hours or days, PTH gene expression is increased and if the same condition persists for several days or weeks, cellular proliferation in the glands is augmented<sup>(35)</sup>. In rabbits, it has been found that 6 weeks of a low- $\text{Ca}^{2+}$  diet produce parathyroid hyperplasia, characterised by increases in PTH secretion, glandular weight and proliferation and by a decrease in CaR mRNA<sup>(36)</sup>. Brown<sup>(37)</sup> defined the set-point of the PTH secretion:  $\text{Ca}^{2+}$  levels ratio, which is the Ca concentration producing half of the maximal inhibition of secretion. This relationship has been useful in the analysis of PTH from patients with secondary hyperparathyroidism due to renal failure and in other conditions. Secondary hyperparathyroidism as well as dietary  $\text{Ca}^{2+}$  deprivation are characterised by an increase in parathyroid epithelial cell number. By using rats fed a low- $\text{Ca}^{2+}$  diet for 8 weeks, it has been shown that endothelin-1 is significantly increased and bosentan, an endothelin-1 receptor antagonist, prevents any increase in the proliferation of parathyroid cells. Therefore, the blockage of endothelin receptors has been suggested to be an important strategy for preventing secondary hyperparathyroidism<sup>(38)</sup>. Miao *et al.*<sup>(39)</sup> have observed in PTH-deficient mice placed on a low- $\text{Ca}^{2+}$  diet that renal CYP27B1 expression increases despite the absence of PTH, leading to an increase in serum  $1,25(\text{OH})_2\text{D}_3$  levels, osteoclastogenesis, and a profound bone resorption. The authors think that although PTH is the first defence against hypocalcaemia,  $1,25(\text{OH})_2\text{D}_3$  can be mobilised in the absence of PTH, to protect against an intense  $\text{Ca}^{2+}$  deficiency. Recently, it has been suggested that oestrogen is also necessary for the full adaptive response to a low- $\text{Ca}^{2+}$  diet mediated by both PTH and  $1,25(\text{OH})_2\text{D}_3$ <sup>(40)</sup>.

Regarding calcitonin, another important calciotropic hormone, it has been shown that diets deficient in  $\text{Ca}^{2+}$  and vitamin D fed to weanling rats for 3 weeks do not change calcitonin mRNA levels, in contrast to the large increases in PTH mRNA levels. So, the authors conclude that calcitonin gene expression *in vivo* in the rat is not affected by changes in serum  $\text{Ca}^{2+}$ <sup>(41)</sup>. The lack of studies on this issue makes it difficult to have a precise idea about the effect of  $\text{Ca}^{2+}$  deficiency on this hormone and its action.

### Alteration of the intestinal function

Dietary  $\text{Ca}^{2+}$  deficiency exerts an important impact on the intestine and its function, mainly affecting the composition of intestinal plasma membranes and  $\text{Ca}^{2+}$  transport. Intestinal  $\text{Ca}^{2+}$  absorption seems to occur by two different mechanisms: transcellular and paracellular pathways. Both mechanisms are regulated by hormones, nutrients and other factors, which have been studied for many years due to their close relationship with osteoporosis and other disorders related to  $\text{Ca}^{2+}$  metabolism<sup>(15)</sup>. The transcellular pathway comprises three steps: entry across the brush-border membrane, intracellular diffusion and exit through the

basolateral membrane. As mentioned above, all the genes presumably involved in the transcellular pathway are enhanced by a low-Ca<sup>2+</sup> diet, probably by activation of the vitamin D endocrine system<sup>(16,17,27,28)</sup>. Furthermore, the enhancement in the activity and expression of the intestinal Ca<sup>2+</sup> pump and the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger caused by Ca<sup>2+</sup>-deficient diets occurs either in mature or in undifferentiated enterocytes<sup>(17)</sup>. However, vitamin D receptor (VDR) levels are decreased by low-Ca<sup>2+</sup> diets. Ferrari *et al.*<sup>(42)</sup> suggested that dietary Ca<sup>2+</sup> deficiency might have a dual effect on VDR gene expression because homologous stimulation of VDR gene expression by calcitriol does not occur on a low-Ca<sup>2+</sup> diet, as a result of a transcriptional suppression by a concomitant increase of PTH. In our laboratory, we found down-regulation of VDR expression by a low-Ca<sup>2+</sup> diet, an effect that was independent of the degree of cell maturation<sup>(17)</sup>. We think that high levels of serum 1,25(OH)<sub>2</sub>D<sub>3</sub> caused by low-Ca<sup>2+</sup> diets do not regulate the intestinal function by up-modulation of its nuclear receptor but promoting differentiation, which would produce cells more capable of expressing vitamin D-dependent genes required for Ca<sup>2+</sup> absorption.

Other biochemical changes produced by low-Ca<sup>2+</sup> diets in the intestine are related to the protein sulfhydryl groups and the lipid composition and fluidity of intestinal membranes. We have shown that the reactivity and availability of sulfhydryl groups from intestinal brush-border membrane proteins of chicks are increased by low-Ca<sup>2+</sup> diets<sup>(43)</sup>. Although the functional significance of this response remains unknown, it is quite possible that the sulfhydryl status of the brush-border membrane proteins might be involved in the vitamin D-dependent intestinal Ca<sup>2+</sup> absorption, as indicated by Mykkanen & Wasserman<sup>(44)</sup>. With regard to the lipid composition, we have shown minor changes in the fatty acid content of the intestinal basolateral membrane; however, lipid fluidity of diphenylhexatriene-labelled intestinal basolateral membrane from chicks is highly increased by the dietary Ca<sup>2+</sup> restriction as compared with that from the control group<sup>(16)</sup>. Thus, it appears that the Ca<sup>2+</sup> exit through the basolateral membrane from the enterocytes in chicks adapted to a low-Ca<sup>2+</sup> diet is greater than that from the control group; higher expression and activity of the Ca<sup>2+</sup> pump and the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger and changes in lipid composition and fluidity, which could affect the microdomains of ion transporters, would be the mechanisms responsible for these adaptive responses of the intestine<sup>(18)</sup>. The activity of alkaline phosphatase, another candidate molecule to be involved in intestinal Ca<sup>2+</sup> absorption, is highly increased in chicks by dietary Ca<sup>2+</sup> restriction, either in mature or immature enterocytes<sup>(17)</sup>. This could be a concomitant effect of higher levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> triggered by a low-Ca<sup>2+</sup> diet, but a real role in intestinal Ca<sup>2+</sup> absorption cannot be discarded.

Recent data showed that under low dietary Ca<sup>2+</sup> conditions there was a 4.1-, 2.9- and 3.9-fold increase in Ca<sup>2+</sup> transport in the duodenum of wild-type, TRPV6 knock-out (KO) and calbindin D<sub>9k</sub> KO mice, respectively. In the TRPV6/calbindin D<sub>9k</sub> double KO mice fed a low-Ca<sup>2+</sup> diet there was a 2.1-fold increase in the duodenal Ca<sup>2+</sup> transport. Therefore, this study shows that active intestinal Ca<sup>2+</sup> transport occurs in the absence of TRPV6 and

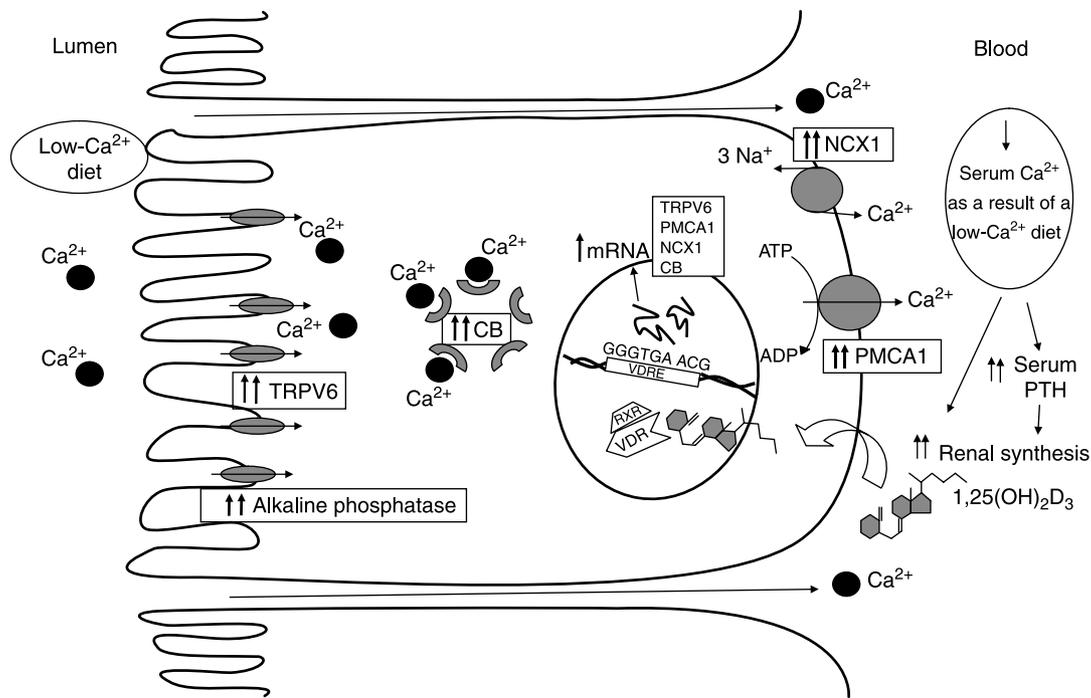
calbindin D<sub>9k</sub>, which challenges the dogma that both proteins are essential for vitamin D-induced active intestinal Ca<sup>2+</sup> transport<sup>(45)</sup>. The data would indicate that TRPV6 may not be the rate-limiting factor in the transcellular pathway or its function is partially compensated by an unknown factor. On the basis that insulin-like growth factor-1 (IGF-1) mRNA was significantly induced in the duodenum of double KO mice under low dietary Ca<sup>2+</sup> conditions, it is possible to think that IGF-1 may be the factor that contributes to the increment in intestinal Ca<sup>2+</sup> absorption.

Figure 1 shows a schematic representation of mechanisms triggered by dietary Ca<sup>2+</sup> restriction on the intestine as has been described above.

### Low-Ca<sup>2+</sup> diets and bone

Bone is highly affected by nutritional Ca<sup>2+</sup> deficiencies in different periods of life and under some physiological conditions or pharmacological treatments. Kalkwarf *et al.*<sup>(46)</sup> have demonstrated that women with low Ca<sup>2+</sup> intakes during childhood and adolescence have less bone mass later in life and a greater risk of fractures. Regarding puberty in experimental animals, bone accretion seems to be very influenced by Ca<sup>2+</sup> deficiencies. Kasukawa *et al.*<sup>(47)</sup> have shown during 2 weeks of pubertal growth that wild-type mice increase femur bone mineral density (BMD) 35 and 7% when fed normal- or low-Ca<sup>2+</sup> diets, respectively, which indicates that bone accretion is impaired during Ca<sup>2+</sup> deficiency. Furthermore, these effects were exaggerated in IGF-1 KO mice. Ca<sup>2+</sup> deficiency produced decreases in endosteal bone formation parameters and much greater enhancement in the resorbing surface of the endosteum and periosteum of the tibia from the IGF-1 KO mice as compared with the wild-type mice. Although the molecular mechanisms by which IGF-1 deficiency increases serum PTH levels are unknown, these alterations could partially explain the negative impact of the lack of IGF-1 on bone accretion. The anabolic effect of PTH is also altered by nutritional Ca<sup>2+</sup> deficiency. Steiner *et al.*<sup>(48)</sup> have demonstrated that the anabolic effect of human PTH (1–38) in animal bone is blunted by a low-Ca<sup>2+</sup> diet, which suggests that dietary Ca<sup>2+</sup> intake is critical during PTH treatment. It has also been demonstrated in experimental animals that accelerated bone resorption, caused by low-Ca<sup>2+</sup> diets, promotes the growth of breast cancer tumours implanted in bone, independently of PTH action<sup>(49)</sup>. High vitamin D<sub>3</sub> intake does not prevent bone loss induced by dietary Ca<sup>2+</sup> restriction, at least in growing rats and mice<sup>(26)</sup>.

Lactation produces a transient loss of bone to provide adequate Ca<sup>2+</sup> for milk production. A complete recovery of bone density in the post-weaning period occurs in adult mothers irrespective of dietary Ca<sup>2+</sup> levels. However, adolescent mothers with low-Ca<sup>2+</sup> diets recover bone loss, but the rate of bone accretion seems not to be sufficient to attain peak bone mass at maturity<sup>(50)</sup>. Furthermore, the *Apa I*, *Bsm I* and *Taq I* VDR gene polymorphisms are associated with bone mass and/or breast milk Ca<sup>2+</sup> in lactating adolescents with low Ca<sup>2+</sup> intakes. Those adolescent mothers carrying the genotypes *aa* and *tt* had a better bone status and those with the genotype *bb* had higher breast milk Ca<sup>2+</sup><sup>(51)</sup>.



**Fig. 1.** A diet poor in  $\text{Ca}^{2+}$  decreases serum  $\text{Ca}^{2+}$  levels, which can be accompanied by normal or low serum phosphate. Hypocalcaemia induces a high parathyroid hormone (PTH) secretion, which in turn promotes renal 1,25-dihydroxyvitamin  $\text{D}_3$  ( $1,25(\text{OH})_2\text{D}_3$ ) synthesis. This hormone binds to vitamin D receptors (VDR) in the intestine triggering transient receptor potential cation channel, subfamily V, member 6 (TRPV6), calbindin D (CB),  $\text{Ca}^{2+}$  pump (PMCA1),  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX1) and alkaline phosphatase synthesis. As a consequence, the efficiency of intestinal Ca absorption is enhanced. VDRE, vitamin D response element; RXR, retinoid X receptor.

Adequate  $\text{Ca}^{2+}$  intake has been demonstrated to reduce bone loss in peri- and postmenopausal women and to reduce fractures in women older than 60 years of age. Due to the fact that there is no accurate test to determine  $\text{Ca}^{2+}$  deficiency, it is considered that women must meet the recommended  $\text{Ca}^{2+}$  intake levels<sup>(52)</sup>. Although a low- $\text{Ca}^{2+}$  diet has been considered as a risk factor for developing osteoporosis, a recent article reviewing several databases for low BMD or for bone loss in healthy men aged 50 years or older has found that dietary  $\text{Ca}^{2+}$  is a weak risk factor for low BMD<sup>(53)</sup>. The interaction of dietary  $\text{Ca}^{2+}$  content and physical activity seems to be very important to determine adequate BMD. It has been found in Scottish women with low or medium  $\text{Ca}^{2+}$  intake that BMD was higher amongst the most active individuals<sup>(54)</sup>.

#### Alteration of the renal function

It is well known that low blood  $\text{Ca}^{2+}$  increases PTH levels, which act on the kidneys increasing  $\text{Ca}^{2+}$  reabsorption and  $1,25(\text{OH})_2\text{D}_3$  production through the activation of CYP27B1<sup>(55)</sup>. Overproduction of  $1,25(\text{OH})_2\text{D}_3$  in the kidneys is regulated by CYP24, which inactivates calcitriol by hydroxylation of the side chain at the carbon at the 24 position<sup>(56)</sup>. Anderson *et al.*<sup>(57)</sup> have used the technique of real-time RT-PCR to determine mRNA for both enzymes in the kidneys from animals exposed to different dietary  $\text{Ca}^{2+}$  concentrations. The levels of CYP27B1 mRNA were highest in the animals fed a low- $\text{Ca}^{2+}$  diet and, conversely, the CYP24 mRNA levels were highest in animals with higher

$\text{Ca}^{2+}$  intake. These authors did not find a correlation between PTH and renal CYP27B1 mRNA levels in animals fed a vitamin D-replete diet, which suggests that serum  $\text{Ca}^{2+}$  may regulate CYP27B1 directly in conditions of normal blood  $\text{Ca}^{2+}$  levels. Thus, the transcription of CYP27B1 *in vivo* seems to be enhanced by PTH only in hypocalcaemic rats, but not in normocalcaemic animals.

Endogenous production of  $1,25(\text{OH})_2\text{D}_3$ , induced by a low- $\text{Ca}^{2+}$  diet, can raise hormone levels four or five times above normal values without suppression of CYP27B1<sup>(31)</sup>. A decrease in the renal VDR content, which drops to 20% of control after prolonged dietary  $\text{Ca}^{2+}$  deficiency, could explain this effect<sup>(58)</sup>. Bajwa *et al.*<sup>(59)</sup> have determined the differences in renal cortex gene expression between rats fed a low- $\text{Ca}^{2+}$  diet (0.02%  $\text{Ca}^{2+}$ ) and those fed a normal- $\text{Ca}^{2+}$  diet (0.47%  $\text{Ca}^{2+}$ ) and treated with two sequential  $1\ \mu\text{g}$  doses of calcitriol, by using the GeneChip oligonucleotide microarray technology and real-time RT-PCR to confirm the data. CaR was unaffected whereas PTH receptor-1 was increased (1.8-fold) by the low- $\text{Ca}^{2+}$  diet. In contrast, intracellular vitamin D-binding protein, VDR, calbindin  $\text{D}_{28\text{k}}$ , osteopontin and 24-OHase were all low under the same mineral condition. As expected, the  $1\alpha\text{-OHase}$  gene was up-regulated by the low- $\text{Ca}^{2+}$  diet. Surprisingly, the expression of secreted phosphoprotein-24 and the transcription factors such as cAMP response element binding protein (CREB) and GATA binding protein globin 1 transcription factor 1 (GATA-1) were increased by the low- $\text{Ca}^{2+}$  diet. The authors hypothesised that the cAMP-protein kinase A pathway is a distinctive feature of low  $\text{Ca}^{2+}$  in response to

increased PTH. As VDR decreases by a low-Ca<sup>2+</sup> diet, epithelial cells of the proximal tubules become refractory to enhanced calcitriol synthesis.

Patients with renal lithiasis may be at higher risk of recurrence of stone formation when they have Ca<sup>2+</sup> intakes below the RDA. Although the restriction of Ca<sup>2+</sup> decreases urinary excretion of the cation, intestinal oxalate absorption increases and the formation of stones due to secondary hyperoxaluria is enhanced. Health professionals must be aware of this, mainly with female patients who may develop osteoporotic complications and bone fractures because of dietary Ca<sup>2+</sup> deficiency<sup>(60)</sup>. The response of the stone formers to low-Ca<sup>2+</sup> diets seems to be dependent on BMD. Pasch *et al.*<sup>(61)</sup> have observed that when these patients have low lumbar BMD, they exhibit a blunted response of PTH release and an enhanced production of 1,25(OH)<sub>2</sub>D<sub>3</sub> after a low-Ca<sup>2+</sup> diet. On the contrary, when patients have high lumbar BMD, PTH levels are highly increased after a dietary Ca<sup>2+</sup> restriction. The reason for a large concentration of 1,25(OH)<sub>2</sub>D<sub>3</sub> in the absence of a PTH response to a low-Ca<sup>2+</sup> diet in stone-former patients with low BMD has been assumed to be reminiscent of a dynamic bone disease. The authors speculate that these patients might have normal or exaggerated intestinal Ca absorption because of 1,25(OH)<sub>2</sub>D<sub>3</sub> enhancement, but they are not able to deposit the extra Ca<sup>2+</sup> into their bones due to the primary bone problem and, hence, calciuria increases, facilitating stone formation.

### Relationship between dietary Ca<sup>2+</sup> and lipid metabolism

As MacDonald says if asked about a link between milk intake and weight in the past, the likely conclusion was that dairy was ‘fattening’<sup>(62)</sup>. However, with the pioneering work of Zemel<sup>(63)</sup>, it has been understood that an energy-restricted diet with the inclusion of at least three servings of dairy per d will help to attain the ideal weight. Mirmiran *et al.*<sup>(64)</sup> have also shown that there is an inverse relationship between dairy intake and BMI. The role of dietary Ca<sup>2+</sup> on the regulation of lipid metabolism and lipogenic genes in adipocytes constitutes the molecular basis that might explain the results of nutritional trials. Several years ago, it was found that *Agouti*, an obesity gene expressed in human adipocytes, produces a protein which stimulates Ca<sup>2+</sup> influx and energy storage in human adipocytes by Ca<sup>2+</sup>-dependent stimulation of fatty acid synthase and inhibition of lipolysis<sup>(65,66)</sup>. Calcitriol treatment of human adipocytes has been proved to activate fatty acid synthase and to inhibit lipolysis in a similar way to that done by *agouti* protein in these cells<sup>(67)</sup>. Therefore, suppression of calcitriol with high-Ca<sup>2+</sup> diets would produce an anti-obesity effect. In fact, this has been demonstrated in transgenic mice overexpressing *Agouti* in adipocytes under the control of the *aP2* promoter, mimicking the human expression pattern. Those mice placed on a low-Ca<sup>2+</sup>-high-fat-high-sucrose diet for 6 weeks showed increases in adipocyte lipogenesis, decreases in lipolysis and increments in body weight and adipose tissue mass. All these responses were partially reversed by high-Ca<sup>2+</sup> diets, the reversion being more successful with dairy sources of Ca<sup>2+</sup> than with Ca<sub>2</sub>(PO<sub>4</sub>)<sub>3</sub>. In a recent

review about Ca<sup>2+</sup>-related obesity research<sup>(68)</sup>, the authors discussed the different milk or dairy components that contribute to the impact of dairy Ca<sup>2+</sup> on body weight. First of all, milk proteins are more satiating than fat and carbohydrates and are often found to suppress appetite and intake. Proteins of whey and casein reduce food intake, and stimulate biomarkers of satiety including gastrointestinal hormones, insulin and amino acids. Peptides derived from whey and casein are inhibitors of the renin-angiotensin system; this can explain the inverse relationship between blood pressure and dietary Ca<sup>2+</sup>. Thus, dairy proteins in addition to Ca<sup>2+</sup> content may have a role in glycaemic control and the metabolic syndrome.

Several pathologies associated with Ca<sup>2+</sup> deficiency have shown an increase in intracellular Ca<sup>2+</sup> concentration in the presence of a low serum Ca<sup>2+</sup>. This is referred to as the ‘calcium paradox’<sup>(69)</sup>. A possible explanation of this response would be that the increase of 1,25(OH)<sub>2</sub>D<sub>3</sub>, promoted by a low-Ca<sup>2+</sup> diet, produces stimulation of Ca<sup>2+</sup> influx in the cells, as shown by Zemel *et al.*<sup>(67)</sup> in cultures of human adipocytes. This increase in intracellular Ca<sup>2+</sup> concentration stimulates fat storage by the activation of fatty acid synthase and inhibition of lipolysis by the activation of phosphodiesterase 3B, which leads to a decrease in cAMP, reducing the ability of agonists to stimulate hormone-sensitive lipase<sup>(70)</sup>.

Another mechanism triggered by low-Ca<sup>2+</sup> diets is an impairment in adipocyte apoptosis, which is attributed to high levels of 1,25(OH)<sub>2</sub>D<sub>3</sub>. In contrast, mice fed high-Ca and/or high-dairy diets exhibit a marked enhancement in adipocyte apoptosis. This is apparently contrary to many published reports showing pro-apoptotic effects of calcitriol in other tissues<sup>(71,72)</sup>. Sun & Zemel<sup>(73)</sup> attribute this discrepancy to dosing differences because the pro-apoptotic effects of calcitriol are as a result of employing supra-physiological concentrations of calcitriol (≥ 100 nM), while the anti-apoptotic effect of calcitriol on human adipocytes was observed with physiological concentrations. Furthermore, the anti-apoptotic effects, apparently due to suppression of uncoupling protein 2 expression, were reversed by pharmacological doses of calcitriol in human adipocytes<sup>(73)</sup>. It is of interest to note that adipocyte apoptosis has not been extensively studied. One of the main difficulties is that adipocytes have a very low nuclear:cytoplasmic ratio, which restricts the identification of apoptotic cells and comparisons of apoptotic rates<sup>(74)</sup>. Nevertheless, adipocyte apoptosis has been demonstrated to occur by leptin treatment, which would act through NF-κB activation and increased levels of PPARγ inducing transcription of pro-apoptotic factors<sup>(75)</sup>.

The dysregulation of glucocorticosteroid metabolism is another alteration that has been proposed to be triggered by low-Ca<sup>2+</sup> diets in adipose tissue. Morris & Zemel<sup>(76)</sup> have demonstrated an increase in 11-β-hydroxysteroid dehydrogenase type I activity in human adipocytes treated with 1,25(OH)<sub>2</sub>D<sub>3</sub>. This enzyme converts cortisone to active cortisol. Its expression is greater in visceral adipose tissue than in subcutaneous fat. Consequently, these authors propose that dietary Ca<sup>2+</sup> restriction might contribute to visceral fat through an increment in cortisol production induced by 1,25(OH)<sub>2</sub>D<sub>3</sub>, in addition to the fatty acid synthase activation already mentioned. A strong inverse

association between  $\text{Ca}^{2+}$  intake and abdominal adiposity (total abdominal fat, abdominal visceral fat, abdominal subcutaneous fat, waist circumference) was found in black men and white women of the HERITAGE (Health, Risk factors, exercise Training And Genetics) Family Study<sup>(77)</sup>. A similar finding was reported for women of the Québec Family Study<sup>(78)</sup>. However, not all studies found an inverse association between  $\text{Ca}^{2+}$  intake and adiposity<sup>(79)</sup>. Recently, Heiss *et al.*<sup>(80)</sup> determined lean and fat mass by dual-energy X-ray absorptiometry (DXA) and defined abdominal fat as fat mass between the top of the iliac crest and L1 on the DXA scan in Caucasian postmenopausal women. They observed that there was a significant inverse relationship between Ca intake and percentage body fat and abdominal fat mass, but there was no significant correlation between Ca intake and BMI, fat mass, lean mass, waist circumference or waist:hip ratio. They found that total fat was greater in the low dairy intake group *v.* the high dairy intake group, but they did not find significant differences between the groups in other body composition variables.

#### Association between hypertension and dietary $\text{Ca}^{2+}$ deficiency

The history that dietary  $\text{Ca}^{2+}$  might have a meaningful impact on blood pressure regulation started a long time ago<sup>(81,82)</sup>. In the early 1980s, two publications by McCarron *et al.*<sup>(83,84)</sup> showed that low- $\text{Ca}^{2+}$  diets were associated with hypertension and that dietary  $\text{Ca}^{2+}$  consumption by US adults was inversely related to the possibility of developing hypertension. A meta-analysis of forty-two clinical trials demonstrated significant blood pressure reduction by increasing  $\text{Ca}^{2+}$  intake either in non-pregnant populations as well as pregnancy-induced hypertension and pre-eclampsia<sup>(85)</sup>. The anti-hypertensive effect of  $\text{Ca}^{2+}$  appears to be paradoxical because  $\text{Ca}^{2+}$  supplementation leads to a reduction in systolic and diastolic blood pressure<sup>(86)</sup>, whereas an increase of intracellular  $\text{Ca}^{2+}$  enhances vascular smooth muscle tone, peripheral vascular resistance and blood pressure. The protective effect of  $\text{Ca}^{2+}$  on blood pressure could be explained through the stimulation of the vitamin D endocrine system. Low- $\text{Ca}^{2+}$  diets increase circulating levels of  $1,25(\text{OH})_2\text{D}_3$ , which stimulates  $\text{Ca}^{2+}$  influx into vascular smooth muscle cells, thereby increasing vascular tone and blood pressure. In contrast, high- $\text{Ca}^{2+}$  diets reduce the stimulus for  $\text{Ca}^{2+}$  influx by suppressing  $1,25(\text{OH})_2\text{D}_3$  production. Certain heterogeneity of response in blood pressure to dietary  $\text{Ca}^{2+}$  has been noted, salt-sensitive patients being those who have most consistently exhibited anti-hypertensive responses<sup>(87,88)</sup>. The Dietary Approaches to Stop Hypertension (DASH) study has demonstrated that a food consumption pattern rich in low-fat dairy products and in fruits and vegetables produces hypotensive effects comparable with those found in pharmacological trials of mild hypertension<sup>(89)</sup>. This study tested the effects of dietary patterns rather than individual nutrients on blood pressure. The authors think that the inconsistency of data from different trials may result from analysing a single nutrient, which could produce small blood pressure-lowering effects. They showed that either in subjects with hypertension or in those without hypertension,

the combined diet reduced blood pressure more than the fruits-and-vegetables or the control diets. Furthermore, the interaction between hypertensive status and diet was higher for systolic blood pressure than for diastolic blood pressure. Thus, adoption of the DASH combination diet might prevent or delay the initiation of drug therapy in individuals at the threshold for drug treatment. Recently, a large prospective cohort study of middle-aged and older women has shown an inverse association between low-fat dairy product intake and the subsequent risk of hypertension. This association was moderate and independent of other risk factors for hypertension, but it was not observed between high-fat dairy intake and the risk of hypertension<sup>(90)</sup>. Previously, by using a validated semi-quantitative FFQ, Alonso *et al.*<sup>(91)</sup> had shown in Spanish adult men and women that the highest quintile of low-fat dairy consumption was associated with a reduction of 54% in the risk of hypertension, while high-fat dairy consumption was not associated with the incidence of hypertension. The reason for this remains unclear. High-fat dairy products might hinder  $\text{Ca}^{2+}$  absorption because  $\text{Ca}^{2+}$  forms soaps with the fatty acids, reducing the bioavailability of  $\text{Ca}^{2+}$ . Another possibility is that changes in the nutritional composition of the skimmed milk and whole milk during processing and preparation (less amount of fat, loss of  $\text{Ca}^{2+}$  in soluble form, proteins and appearance of encrypted peptides with hypotensive potential)<sup>(92,93)</sup> could explain the differences, but this has not been confirmed<sup>(90)</sup>. Regarding molecular mechanisms, it has been shown that dietary  $\text{Ca}^{2+}$  may lower the activity of the renin-angiotensin system<sup>(94)</sup> and inhibit vascular smooth muscle cell constriction<sup>(95)</sup>. Besides,  $\text{Ca}^{2+}$  facilitates weight loss<sup>(96)</sup> and stimulates insulin sensitivity<sup>(97)</sup>, which contribute to decreases in blood pressure.

It remains unclear whether the  $\text{Ca}^{2+}$  content alone or in combination with several components such as other minerals or proteins, peptides or amino acids are responsible for the anti-hypertensive effects of dairy products<sup>(96)</sup>. High levels of  $\text{Ca}^{2+}$ ,  $\text{K}^+$  and  $\text{Mg}^{2+}$  seem to be favourable but data are not conclusive<sup>(98)</sup>. It is quite possible that interactions among different minerals present in milk or dairy products as well as additive effects of milk compounds on hypertension could explain the benefits of dietary patterns (DASH study) as compared with supplementations of isolated nutrients.

#### Nutritional $\text{Ca}^{2+}$ and risk of colon, breast, prostate and ovarian cancer

Evidence that dietary  $\text{Ca}^{2+}$  is associated with colorectal cancer has come from case-control studies, prospective cohort studies and some clinical trials<sup>(99)</sup>. Cohort studies have detected that milk and dairy products have a protective effect on colorectal cancer<sup>(99)</sup>. The mechanisms involved in this effect would be a decrease in cell proliferation or promotion in cell differentiation<sup>(100)</sup>. Three cohort studies demonstrated a modest effect of  $\text{Ca}^{2+}$  on colorectal cancer risk reduction<sup>(101-103)</sup>. Another study demonstrated that high consumption of milk might reduce colon cancer risk, but not because of the  $\text{Ca}^{2+}$  and vitamin D content<sup>(104)</sup>. Hofstad *et al.*<sup>(105)</sup> have found that a 3-year intervention with  $\text{Ca}^{2+}$  and antioxidants had no effect on polyp growth, but it

might have a protective effect in avoiding formation of new adenomas. Grau *et al.*<sup>(106)</sup> have found that the combination of Ca<sup>2+</sup> and vitamin D reduces the risk of colorectal adenomas. Case-control studies have produced contradictory data. Some of them have demonstrated that Ca<sup>2+</sup> intake is associated with a reduced risk of colorectal cancer<sup>(107,108)</sup>, but others have not<sup>(109,110)</sup>. Recent pooled analysis and epidemiological studies have demonstrated an inverse relationship between Ca<sup>2+</sup> intake and colorectal cancer or adenoma risk<sup>(111,112)</sup>. Furthermore, randomised clinical trials have shown that Ca<sup>2+</sup> supplementation reduces adenoma risk<sup>(113,114)</sup>.

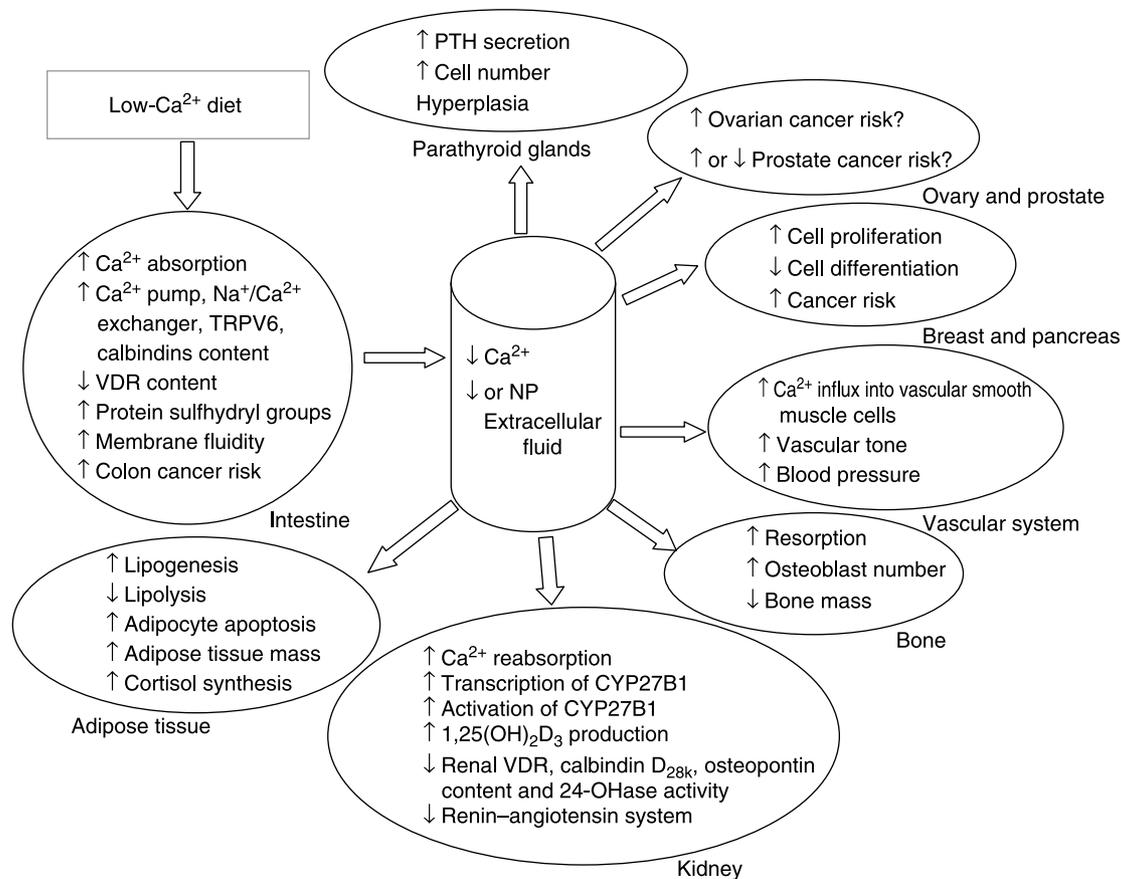
Induction of colonic hyperproliferation and expansion of an epithelial cell population containing atypical nuclei have been found in experimental animals such as rats and mice fed Western-style diets (high fat, low Ca<sup>2+</sup>, low vitamin D, low fibre)<sup>(115)</sup>. When this feeding was prolonged, markers of incipient tumorigenesis such as dysplastic lesions and focal hyperplasia appeared. Cyclo-oxygenase (Cox)-2 protein, an inducible enzyme that is frequently overexpressed in inflamed tissues and in colorectal cancer, has also been found to be enhanced by dietary Ca<sup>2+</sup> deficiency, mainly in females<sup>(115)</sup>. Although the molecular mechanisms by which dietary Ca<sup>2+</sup> influences colonic health remain unknown, Pele *et al.*<sup>(116)</sup> propose three possibilities: (1) dietary Ca<sup>2+</sup> could alter the ratio of faecal bile acids, decreasing the water-soluble bile acids and reducing the cytotoxicity of faecal water; (2) Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> particles could bind luminal antigens, transporting them into mucosal mononuclear cells as a mechanism of immunosurveillance and promotion of tolerance; and (3) dietary Ca<sup>2+</sup> could activate CaR, triggering intracellular signalling pathways, among them proliferative and apoptotic pathways. Several pathways are activated by Ca<sup>2+</sup> through CaR, including activation of the p38 mitogen-activated protein kinase cascade, promotion of E-cadherin (tumour suppressor) and suppression of β-catenin/T cell factor binding (a process that promotes a malignant phenotype)<sup>(117,118)</sup>.

Data relative to nutritional Ca<sup>2+</sup> and incidences of human cancers of the breast, prostate and pancreas are also controversial<sup>(119)</sup>. In a large prospective study, a high intake of Ca<sup>2+</sup> and low-fat dairy products was found to be associated with a moderately lower risk of developing postmenopausal breast cancer as compared with women with the lowest intake levels. Interestingly, supplemental Ca or higher levels of total Ca (diet plus supplements) or vitamin D were not related to overall breast cancer risk<sup>(5)</sup>. Human mammary epithelial (HME) cells exhibit VDR and CYP27B1 and show growth inhibition after exposure to physiological doses of 25OHD<sub>3</sub>, which indicates that autocrine or paracrine vitamin D signalling is involved in the maintenance of differentiation and quiescence of the mammary epithelium<sup>(120)</sup>. Oncogenic transformations of HME cells through introduction of known oncogenes (SV40 T antigens and H-rasV12) were associated with a reduction in mRNA and protein levels of VDR and CYP27B1. In addition, the transformation was also found to be associated with a reduction in 1,25(OH)<sub>2</sub>D<sub>3</sub> synthesis and in the cellular sensitivity to growth inhibition caused by either 1,25(OH)<sub>2</sub>D<sub>3</sub> or 25OHD<sub>3</sub>. These changes indicate that disruption of the vitamin D signalling pathway occurs early

in cancer development<sup>(120)</sup>. Although the mechanisms involved in breast carcinogenesis induced by low-Ca<sup>2+</sup> diets remain unknown, it is quite possible that dietary Ca<sup>2+</sup> deficiency causes a dysregulation in the vitamin D endocrine system, which may result in a reduction in the response of breast cells to calcitriol and a promotion of oncogenic transformation. Calcitriol synthesis is increased by a secondary hyperparathyroidism provoked by hypocalcaemia<sup>(32)</sup> and the continuous high exposure of breast cells to calcitriol may reduce the sensitivity of cells to the hormone. This could be due, at least in part, to down-regulation of VDR expression. High levels of circulating PTH would promote bone resorption. Growth factors such as transforming growth factor β and IGF might be released from the bone matrix, promoting tumour growth<sup>(49)</sup>. The activation of proto-oncogenes and/or inactivation of tumour suppressor genes as local carcinogenic stimuli could also contribute to the initiation of transformation of normal breast cells into malignant breast cells. Further experiments are needed in order to know the mechanisms by which dietary Ca<sup>2+</sup> deficiency could contribute to the early steps of breast cancer development. As mentioned above, the growth of breast cancer tumours implanted in bone was promoted in experimental animals with accelerated bone resorption caused by low-Ca<sup>2+</sup> diets<sup>(49)</sup>.

Giovanucci *et al.*<sup>(6)</sup> have found an association between high Ca<sup>2+</sup> intake and a higher risk of high-grade prostate cancer (Gleason histological grade > 7), but not with well-differentiated organ-confined cancers. However, neither the development nor progression of prostate tumours in mice was enhanced by high-Ca<sup>2+</sup> diets<sup>(121)</sup>. Through a randomised controlled clinical trial, Baron *et al.*<sup>(122)</sup> demonstrated that there was no increase in prostate cancer risk associated with Ca<sup>2+</sup> supplements, and they raised the possibility that Ca<sup>2+</sup> may instead lower the risk of this cancer. Recently, Tornainen *et al.*<sup>(123)</sup> showed some evidence for low-fat milk as a potential risk factor for prostate cancer in patients from Nordic countries. Bonjour *et al.*<sup>(124)</sup> concluded that the link between Ca<sup>2+</sup> and the development of prostate cancer remains a hypothesis, which is not supported so far by clinical data.

Epidemiological studies indicate that low-Ca<sup>2+</sup> diets are risk factors for pancreatic cancer<sup>(125)</sup>. However, two recent prospective cohort studies have demonstrated that the inverse relationship between Ca<sup>2+</sup> intake and the risk for pancreatic cancer is attenuated by adjusting for vitamin D intake. In contrast, vitamin D consumption has a significant inverse relationship with pancreatic cancer risk<sup>(126)</sup>. Clinical characteristics of patients need to be carefully controlled in these studies. Stolzenberg-Solomon *et al.*<sup>(127)</sup> conducted a prospective nested case-control study in male Finnish smokers (aged 50–69 years at baseline) to test whether prediagnostic 25OHD<sub>3</sub> concentrations were associated with lower pancreatic cancer risk. Contrary to their expectations, they found that subjects with higher vitamin D status had an increased pancreatic cancer risk. Recently, they did not confirm that strong association between 25OHD<sub>3</sub> and pancreatic cancer, adjusting for smoking and BMI, but they found an association between low estimated annual residential solar UVB exposure and cancer risk<sup>(128)</sup>.



**Fig. 2.** Metabolic changes caused by low- $\text{Ca}^{2+}$  diets on different organs and tissues. TRPV6, transient receptor potential cation channel, subfamily V, member 6; VDR, vitamin D receptor; PTH, parathyroid hormone; NP, normal phosphate; CYP27B1, 25-OH-cholecalciferol 1-hydroxylase;  $1,25(\text{OH})_2\text{D}_3$ , 1,25-dihydroxyvitamin  $\text{D}_3$ .

The association between risk of ovarian cancer and milk or dairy food intake is not clear either. The disaccharide lactose, naturally present as a component of milk and dairy products, is hydrolysed by the intestinal lactase into glucose and galactose. It was thought that galactose levels could be related to the risk of ovarian cancer because high circulating galactose may impair ovarian feedback to the pituitary gland, increasing gonadotropin secretion, which may increase oestrogenic stimulation, resulting in proliferation of ovarian epithelium<sup>(129)</sup>. This has been named the galactose–gonadotropin hypothesis. However, several authors did not find any correlation between the risk of developing ovarian cancer and dairy food intake, daily galactose consumption or the prevalence of low activity of galactose-1-phosphate-uridylyltransferase or lactase persistence<sup>(130,131,132)</sup>. In a case–control study in Hawaii and Los Angeles to examine several dietary hypotheses related to the aetiology of ovarian cancer, it was found that the intake of low-fat milk,  $\text{Ca}^{2+}$  or lactose may reduce the risk of ovarian cancer<sup>(130)</sup>. An association between ovarian cancer risk and a high whole milk intake, but not low-fat dairy product intake, suggested that fat, and not galactose, was the component that increases the cancer risk<sup>(133)</sup>. Contrarily, a cohort study on diet and cancer carried out in The Netherlands<sup>(134)</sup> did not find an association between dairy products or lactose intake and ovarian cancer risk. A modest

increase in the risk of ovarian cancer with lactose intake at the level of three or more glasses of milk per d was observed in a pooled analysis of twelve cohort studies<sup>(135)</sup>. The authors of this study suggested that dairy product consumption in relation to ovarian cancer risk should be further examined. Based on considerations described above, a schematic representation of the main metabolic changes caused by low- $\text{Ca}^{2+}$  diets is summarised in Fig. 2.

### Concluding remarks

Although low- $\text{Ca}^{2+}$  diets trigger adaptive mechanisms in order to maintain extracellular  $\text{Ca}^{2+}$  concentration and ensure  $\text{Ca}^{2+}$ -dependent cellular functions, they also alter many metabolic pathways, whose persistence may lead to pathological conditions. The efficiency of intestinal  $\text{Ca}^{2+}$  absorption is increased due to the increment in renal calcitriol synthesis induced mainly by high levels of serum PTH. This calciotropic hormone promotes bone loss and, when  $\text{Ca}^{2+}$  deficiency occurs in childhood and adolescence, the attainability of peak bone mass is not reached. The association of low- $\text{Ca}^{2+}$  diets with osteoporosis development is very weak, but, apparently, interactions between nutritional  $\text{Ca}^{2+}$  and vitamin D with physical activity seem to be important along the lifespan either to increase peak bone mass or to delay bone loss in the elderly. Lipid

metabolism and lipogenic genes are altered in adipocytes as well as cortisol production, which might contribute to increased visceral fat. Circulating levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> stimulate Ca<sup>2+</sup> influx into vascular smooth muscle cells, thereby increasing vascular tone and blood pressure. Proliferative and apoptotic pathways might be dysregulated, leading to the development and progression of malignancies such as colon, breast, prostate and ovarian cancers. Health professionals should be aware of these nutritional complications and reinforce the dairy intakes in individuals of all ages to ensure the recommended Ca<sup>2+</sup> requirements and prevent diseases associated with poor Ca<sup>2+</sup> intake.

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