

Calcium and vitamin D in obesity

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Abstract

New and more effective nutritional measures are urgently needed for the prevention of obesity. The role of Ca and vitamin D in obesity has been recently implicated. Low Ca intake and low vitamin D status have been linked with an increased risk of obesity in epidemiological studies; however, clinical intervention trials designed to test this association have produced controversial results. The suggested anti-obesity mechanisms of Ca and vitamin D include the regulation of adipocyte death (apoptosis), adipogenesis and lipid metabolism. Dietary Ca has been also shown to increase faecal fat excretion. The potential role of Ca and vitamin D in shifting energy balance towards a more negative state is an area of considerable interest. Ultimately, a review of recent research findings does not allow the reaching of a definitive conclusion that increasing Ca intake and rising vitamin D status will influence fat mass and body weight or decrease the risk of obesity and overweight.

Key words: Calcium: Vitamin D: Obesity: Apoptosis: 1,25-Dihydroxyvitamin D₃: Intracellular Ca²⁺: Adipocytes

Introduction

Obesity is an emerging health problem of growing importance, and new and more effective nutritional measures are urgently needed for the prevention of this disease. While the aetiology of obesity is multifactorial, the major factors in the modern obesity epidemic are of dietary origin^(1,2).

Although the regulation of energy balance is the most critical factor in maintaining body composition, emerging evidence suggests that Ca and vitamin D may play a role in the regulation of weight gain, particularly when included in an energy-restricted diet. However, the results of clinical studies are inconclusive and the possible mechanisms linking dietary Ca intake and vitamin D status with obesity remain undefined. The aim of the present review is to provide a comprehensive assessment of the role of Ca and vitamin D in weight gain, overweight and obesity.

Obesity and adipose tissue

Obesity is associated with multiple disease outcomes, including heart disease, diabetes, stroke, the metabolic syndrome and a number of cancers^(3,4), as well being linked to increased mortality rates⁽⁵⁾. BMI, which estimates human body fat based on an individual's weight and height, is commonly used as a surrogate measure of overall obesity⁽⁶⁾. Although other factors such as muscularity may affect BMI,

the amount of body fat significantly contributes to weight excess or deficiency. Therefore, studies usually report body weight and BMI as outcomes.

Lifestyle and behavioural factors such as energy intake together with levels of physical activity play critical roles in the obesity epidemic. The United States Department of Agriculture reported that total daily energy intake increased from 9071 kJ (2168 kcal) in 1970 to 11 184 kJ (2673 kcal) in 2008⁽⁷⁾. In modern urban-industrial society, a sedentary lifestyle is commonly found in both the developed and developing countries. At least 60% of the world's population does not engage in the recommended amount of physical activity⁽²⁾. Inactivity is associated with increasing age, smoking, poverty, less education, and is more common among women than men. In addition, a number of genes are known to have contributions in defining the obese phenotype. Genetic and environmental inheritance may account for 70% of the population variation in BMI⁽⁸⁾. Current obstacles for the development of the effective prevention and treatment approaches to obesity include lack of understanding of genomic, epigenetic, metabolic and signalling pathways underlying this condition/disease.

Obesity is characterised by the accumulation of adipose tissue mass, which can result from both hypertrophy, an increase in adipocyte size, and hyperplasia, an increase in adipocyte number⁽⁹⁾. An obese individual can accumulate more than 70% of body mass as fat⁽¹⁰⁾. There are two types of

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; 25(OH)D₃, 25-hydroxyvitamin D₃; UCP, uncoupling protein; VDR, vitamin D receptor.

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adipose tissue: white adipose tissue and brown adipose tissue. The white adipocytes contain a single large lipid droplet, which squeezes the nucleus into a thin rim at the periphery. They express insulin, growth hormone, adrenergic, glucocorticoid, retinoid and vitamin D receptors (VDR). Brown adipocytes contain numerous smaller lipid droplets and a large number of mitochondria. They are especially abundant in newborns and in hibernating mammals⁽¹¹⁾. Adipose tissue depots are found in specific locations – subcutaneous, intra-abdominal and perirenal – and they may perform different functions^(12,13). An excess of visceral fat that is packed in between internal organs has a strong association with several health disorders such as heart disease, cancer and stroke⁽¹⁴⁾.

Adipose tissue performs important functions, with long-term energy storage being an essential function⁽¹⁵⁾. Adaptive increases of adipose tissue play a key role for birds and mammals living in a cold environment. Bergmann's rule indicates the tendency for the body size of birds and mammals to be larger in cooler climates⁽¹⁶⁾. Adipose tissue also stores cholesterol and lipid-soluble vitamins, in particular vitamin D⁽¹⁷⁾. It is now appreciated that white adipose tissue serves as an important endocrine organ by secreting hormones such as leptin, resistin, cytokines (including TNF α), adiponectin and steroids⁽¹⁸⁾. Brown adipose tissue plays a key role in generating heat (instead of ATP) by uncoupling mitochondria⁽¹⁹⁾.

Vitamin D and Ca play a critical, interlinked role not only in performing their classical functions related to mineral metabolism, but also in a number of signalling pathways that regulate a variety of cellular and physiological processes, including those in adipose tissue. Recently, the role of Ca and vitamin D in obesity has been implicated, and increasing dietary Ca intake and vitamin D status has been proposed as a promising strategy for the prevention of obesity.

Calcium and obesity

Calcium as a cellular regulator

Ca performs its regulatory functions at the cellular level in the form of Ca²⁺ ions. Ca²⁺ is considered as the most versatile, ubiquitous intracellular messenger^(20–23). It reversibly binds to specific proteins that act as Ca²⁺ sensors to decode its information before passing it on to targets. The membrane Ca²⁺ transport systems control the cellular homeostasis of Ca²⁺. Remarkably, Ca²⁺ is a universal, ambivalent signalling agent. It carries information to virtually all processes important to cell life, but also transmits signals that promote cell death. Spatial and temporal characteristics of the Ca²⁺ signal determine the type and magnitude of biological responses; for example, oscillations of cytosolic Ca²⁺ in pancreatic β -cells underlie the oscillatory pattern of insulin release^(24,25).

Calcium intake

The dietary reference intake for Ca was updated by the United States Food and Nutrition Board of the Institute of Medicine in 2010, with the biggest change made being the conversion from adequate intake to estimated average requirement and RDA^(26,27). The RDA varies from 700 mg/d for younger children to 1300 mg/d for adolescents; 1200 mg/d should be considered sufficient for most groups. The nutritional guidelines provided by the European Union Commission are similar⁽²⁸⁾.

The best source of Ca is dairy foods⁽²⁹⁾. Although some individuals are intolerant to dairy products or do not consume dairy products for ethical reasons, other good sources of Ca exist. However, Ca deficiency is still an important issue. Adolescents and older individuals are the most likely groups to be deficient in Ca⁽³⁰⁾, and Ca deficiency is considered in the USA to warrant a national effort to increase average intake levels⁽³¹⁾.

Calcium and body mass

A growing body of literature suggests that a low Ca intake is associated with a greater fat mass. The potential effect of Ca on weight loss has been shown in animal models^(32–35). Importantly, the results obtained with murine models of obesity appear to translate to humans. Several observational studies (mainly cross-sectional and retrospective cohort studies)^(36–42) have demonstrated a negative relationship between Ca intake and body weight. Furthermore, this association has been observed across multiple ages and ethnicities and in both sexes. However, it is necessary to consider that the overall energy density of diets characterised by a low Ca content can be higher than comparable diets characterised by a high Ca content⁽⁴¹⁾. Moreover, daily food consumption in experimental animals fed a high-Ca diet *ad libitum* can be lower than that of animals fed diets with normal or low Ca levels. These observations may provide an additional explanation of the possible effect of Ca on body weight.

The intervention studies examining the effects of Ca on body weight are summarised in Table 1. The studies conducted during an energy-restriction programme demonstrated that Ca intake has no significant effect on body weight^(43–48). Importantly, the studies conducted without requiring energy restriction failed to demonstrate the effect of Ca intake on body-weight loss as well^(49,50). Several studies showed no effect of a high Ca intake on body-weight loss^(44,46,47,49,50), although one of these studies demonstrated a significant increase in fat oxidation⁽⁴⁶⁾. The meta-analysis review that included randomised controlled trials also failed to find evidence of a beneficial effect of Ca on body weight⁽⁵¹⁾. A study conducted by Torres *et al.*⁽⁵²⁾ found that subjects consuming a diet containing 1200–1300 mg Ca per d exhibited a greater reduction in waist circumference and waist:hip ratio as compared with subjects

Table 1. Intervention studies examining the effects of calcium on body weight

Reference	Population	Design	Intervention	Results
Yanovski <i>et al.</i> ⁽⁴⁹⁾	<i>n</i> 340; 72% female; age 38.8 (SD 10.5) years; BMI > 25 kg/m ²	24 months, no energy restriction	1500 mg/d from supplement (<i>n</i> 170); placebo (<i>n</i> 170)	Dietary supplementation with elemental Ca had no effect on body weight
Faghih <i>et al.</i> ⁽⁴⁵⁾	<i>n</i> 85; 100% female; age 20–50 years; BMI > 25 kg/m ²	2 months, energy-restricted diet (500 kcal/d; 2092 kJ/d)	800 mg/d from supplement (<i>n</i> 22); 800 mg/d from milk (<i>n</i> 22); 800 mg/d from soya milk (<i>n</i> 21); control diet (500–600 mg/d; <i>n</i> 20)	Milk decreased body weight and BMI. Soya milk and Ca supplement had no effect on body weight or BMI
Wagner <i>et al.</i> ⁽⁴⁴⁾	<i>n</i> 58; 100% female; age 19–53 years; BMI 26–41 kg/m ²	3 months, energy-restricted diet (500 kcal/d; 2092 kJ/d); exercise three times per week	Control diet + 800 mg/d from Ca lactate (<i>n</i> 12); control diet + 800 mg/d from calcium phosphate (<i>n</i> 16); control diet + 800 mg/d from milk (<i>n</i> 17); control diet (750 mg/d; <i>n</i> 13)	Ca did not enhance the loss of body weight or fat
Torres <i>et al.</i> ⁽⁵²⁾	<i>n</i> 50; 90% female; age 22–55 years; BMI 30–34.9 kg/m ²	16 weeks, energy-restricted diet (800 kcal/d; 3347 kJ/d)	High-Ca diet (1200–1300 mg/d from milk; <i>n</i> 25); low-Ca diet (< 500 mg/d; <i>n</i> 25)	Ca had no effect on body weight, but showed effect on waist circumference and waist:hip ratio
Teegarden <i>et al.</i> ⁽⁴⁶⁾	<i>n</i> 24; 100% female; age 18–31 years; BMI 25–34.9 kg/m ²	3 months, energy-restricted diet (500 kcal/d; 2092 kJ/d)	900 mg/d from supplement (<i>n</i> 6); 900 mg/d from dairy (<i>n</i> 9); < 500 mg/d (<i>n</i> 9)	Ca increased fat oxidation, but did not change total energy expenditure
Riedt <i>et al.</i> ⁽⁴⁷⁾	<i>n</i> 44; 100% female; age 38 (SD 6.4) years; BMI 27.7 (SD 2.1) kg/m ²	6 months, weight-loss or weight-maintenance diet	Weight-loss diet with 1800 mg/d (<i>n</i> 14); weight-maintenance diet with 1000 mg/d (<i>n</i> 13); weight-loss diet with 1000 mg/d (<i>n</i> 17)	Ca did not enhance loss of body weight
Chailurkit <i>et al.</i> ⁽⁵⁰⁾	<i>n</i> 236; 100% female; age 60–97 years; BMI 25.0 (SD 3.5) kg/m ²	24 months, no energy restriction	500 mg/d from supplement (<i>n</i> 175); placebo (<i>n</i> 161)	Ca had no effect on body weight
Thomas <i>et al.</i> ⁽⁴⁸⁾	<i>n</i> 29; 100% female; age 36.8 (SD 4.8) years; BMI 29.1 (SD 2.1) kg/m ²	16 weeks, energy-restricted diet (250 kcal/d; 1046 kJ/d) and resistance training 3 d/week	≤ 500 mg low-Ca diet/d (<i>n</i> 15); ≥ 1200 mg Ca/d from dairy (<i>n</i> 14)	Dairy Ca had no added benefit on body-fat loss

who consumed a similar diet with a low Ca content (<500 mg/d). However, there were no statistically significant differences between groups with respect to body weight. Faghih *et al.*⁽⁴⁵⁾ found an inverse relationship between dairy Ca consumption and body weight, but there were no significant differences between body weight and BMI in the soya milk, Ca supplement and control groups.

Generally, results of the reviewed studies failed to demonstrate the direct effect of dietary Ca on body weight; however, several studies suggest the role of Ca in increasing fat oxidation and decreasing waist circumference. The inconsistency between observational studies and randomised controlled trials might arise from missing data on energy intake and physical activity in most observational studies (we found only one study demonstrating that, in adolescents, a lower Ca intake is accompanied by a higher intake of fat and energy⁽⁴¹⁾).

Vitamin D and obesity

Vitamin D as a hormonal and cellular regulator

The biological effects of vitamin D₃ result from its sequential metabolism in the liver into 25-hydroxyvitamin D₃ (25(OH)D₃), and then in the kidney into the steroid hormone 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃)⁽⁵³⁾. 1,25(OH)₂D₃ is considered as the principal Ca²⁺-regulatory hormone, which exerts its regulatory effects by increasing intestinal Ca²⁺ absorption, bone Ca²⁺ mobilisation and renal Ca²⁺ reabsorption^(21,53,54). 1,25(OH)₂D₃ regulates not only Ca²⁺ metabolism, but also a wide spectrum of cellular processes, including proliferation, differentiation, development, apoptosis and secretion^(21,25,55,56). 1,25(OH)₂D₃ produces its biological effects via both receptor-mediated regulation of nuclear events and rapid actions independent of genomic pathways^(21,55,57). The genomic responses utilise signal-transduction pathways linked to the nuclear/cytosolic VDR, while the rapid responses utilise signal-transduction pathways linked to the membrane VDR localised in the plasma and, possibly, endoplasmic reticulum membranes⁽⁵⁸⁾ as well as to the plasma membrane-associated rapid response steroid hormone-binding protein^(59,60).

Nuclear and membrane VDR have been demonstrated in many (over forty) tissues, including adipose tissue^(21,53,61). In these target tissues, the VDR functions both as a transcriptional factor to influence more than 3% of the human genome and a modulator of a number of cellular signal-transduction pathways, including Ca²⁺ signalling. Analogues of vitamin D that can act as agonists of genomic and non-genomic pathways have been identified^(62–64). It appears that Ca²⁺ signals (transient and prolonged) triggered by 1,25(OH)₂D₃ can be linked to both membrane and nuclear VDR^(22,65).

Significant evidence indicates that biological actions of 1,25(OH)₂D₃ are mediated or influenced by intracellular Ca²⁺-signalling events. Rapid (within seconds to minutes)

effects of 1,25(OH)₂D₃ on intracellular Ca²⁺ have been demonstrated in mammary and intestinal epithelial cells, pancreatic β-cells, osteoblasts and adipocytes^(21,25,55,56,66–69). It has been also shown that 1,25(OH)₂D₃ rapidly induces Ca²⁺ uptake, activates Ca²⁺ channels and stimulates phosphoinositide turnover in different cell types^(55,57,63). Thus, activation of the intracellular Ca²⁺-signalling pathways is essential for the actions of 1,25(OH)₂D₃. It is interesting to note that, in turn, Ca²⁺ can interact with the VDR via Ca²⁺-binding γ-carboxyglutamate residues of the receptor protein, which results in down-regulation of the vitamin D-mediated biological responses^(61,70,71).

Determination of vitamin D status

Vitamin D status is determined by measuring the circulating concentration of its transport form, 25-hydroxyvitamin D (25(OH)D). The serum concentration of 25(OH)D is approximately 1000 times higher than that of 1,25(OH)₂D, and 25(OH)D has a half-life of 2–3 weeks compared with that of 4–6 h for the hormone⁽⁷²⁾. The circulating concentration of 25(OH)D is considered to be the most reliable indicator of vitamin D production, intake and stores⁽⁷³⁾.

Currently, there is no consensus on how the circulating levels of 25(OH)D should be classified to describe vitamin D nutritional status. The concentration of 25(OH)D in severe vitamin D deficiency is 'undetectable' (i.e. < 1–5 ng/ml); normal range is traditionally defined as 20–50 ng/ml; levels below 10–20 ng/ml may indicate vitamin D insufficiency, while levels above 100 ng/ml represent a risk of toxicity^(26,27). A higher range of normal or 'optimal' 25(OH)D concentrations have been also suggested (for example, 30–70 ng/ml)⁽⁷⁴⁾.

Vitamin D status depends on sunlight exposure and dietary intake. The skin has a large vitamin D₃ production capacity and is capable of supplying body requirements in vitamin D. Latitude, season, skin pigmentation, sunscreen use and ozone air pollution influence the cutaneous production of vitamin D₃. The levels of UVB irradiation to produce a significant increase in the serum 25(OH)D concentration are not possible to achieve in winter at latitudes above and below 40°, thus limiting endogenous production of vitamin D₃ for several months of the year⁽⁷⁵⁾. Consumption of dietary or supplemental vitamin D or artificial UV irradiation should be considered under these circumstances⁽²⁶⁾.

The dietary reference intake allowance of vitamin D – the value sufficient to meet the needs of virtually all individuals – recommended in 2010 by the United States Food and Nutrition Board of the Institute of Medicine is 15 μg/d (600 IU/d)⁽²⁶⁾. For infants, adequate intake is 10 μg/d (400 IU/d). Estimated average requirement of vitamin D is 400 IU/d for all life-stage groups (it is not established for infants). Possible benefits of vitamin D intake at the level of 2000–4000 IU/d (25–100 μg/d) are actively

debated⁽⁷⁴⁾. The Institute of Medicine does not recommend such an increase⁽²⁶⁾. The recommended upper intake level of vitamin D for adults is 4000 IU/d (100 µg/d)⁽²⁶⁾; long-term intake at the level of 5000–10 000 IU/d may represent a risk of toxicity. The nutritional guidelines for vitamin D set forward by the European Union Commission are similar⁽²⁸⁾. The US and European Union recommendations are based on the established actions of vitamin D on Ca and bone metabolism.

Vitamin D status and obesity

The potential link between vitamin D and obesity was first observed in 1971 by Rosenstreich *et al.*⁽⁷⁶⁾. They demonstrated the association between increased body fat and low serum 25(OH)D concentrations and attributed this to sequestration of the fat-soluble vitamin D in the adipose tissue. Zemel *et al.*⁽⁷⁷⁾ showed that dietary Ca can reduce adiposity and suggested that the regulation by Ca of the production of 1,25(OH)₂D plays a role. These two studies laid the groundwork for examining the connection between vitamin D and body composition as well as for elucidating the underlying mechanisms.

Recently, a growing body of epidemiological evidence has emerged suggesting the role of vitamin D in obesity, including observational studies that demonstrated the association between vitamin D intake and body composition^(42,75,78–100). In these studies, a statistically significant inverse correlation suggesting that a low vitamin D status is associated with a larger fat mass as well as a greater risk of weight gain over time has been reported. However, it is important to mention that obese individuals have limited mobility and avoid outdoor activity⁽¹⁰¹⁾. In those individuals, exposure of skin to UVB irradiation may become inadequate to maintain optimal vitamin D status⁽¹⁰²⁾. It is also important to mention that vitamin D₃ is sequestered in the obese adipose tissue⁽¹⁰³⁾. These should be taken into consideration in explaining the inverse correlation between vitamin D status and body weight demonstrated in observational studies.

The effect of vitamin D supplementation (often together with an increased dietary Ca intake) on body fat in human subjects has been evaluated in several randomised controlled trials (Table 2)^(104–109). The 4-year study by Caan *et al.*⁽¹⁰⁹⁾ demonstrated that the consumption of 1000 mg Ca plus 400 IU vitamin D per d has a small effect on the prevention of weight gain, which was observed primarily in women who reported inadequate Ca intakes. In contrast, in five studies no effect was demonstrated^(104–108). For example, the 15-week weight-loss study by Major *et al.*⁽¹⁰⁶⁾ showed that the consumption of 600 mg Ca plus 1100 IU vitamin D per d enhanced the beneficial changes in HDL-cholesterol, TAG and total cholesterol in overweight or obese women with a low daily Ca intake, but had no effect on body weight. The study by Zhou *et al.*⁽¹⁰⁸⁾ demonstrated that percentage of trunk fat was significantly different

between the Ca intervention groups and the placebo group, but vitamin D supplementation had no additional effect. Furthermore, the 1-year study conducted by Zittermann *et al.*⁽¹⁰⁴⁾ in Norway revealed that a significant weight reduction in overweight and obese subjects is unlikely to occur with vitamin D supplementation.

The results of observational studies and randomised controlled trials do not provide conclusive causal or mechanistic insights into whether or how vitamin D (and Ca) might regulate body weight. It is necessary to note that confounding effects could explain the association between vitamin D levels and fat stores in a non-casual or reverse-casual way; for example, low 25(OH)D levels in obesity could be observed because of its increased sequestration in the obese adipose tissue or because higher levels of physical outdoor activities could increase sun exposure and 25(OH)D levels or decrease adiposity directly.

Mechanisms of action of calcium and vitamin D in obesity

The research employing cellular and animal models identified several mechanisms of action of Ca and vitamin D in adipose tissue supporting their possible involvement in the regulation of body weight.

Calcium intake and faecal fat excretion

Ca can impair the absorption of fat in the intestine via formation of insoluble Ca fatty acid soaps or by binding of bile acids. This mechanism was demonstrated in animal and human studies, indicating that supplemental Ca and dairy foods increase faecal excretion of fat. deWit *et al.*⁽³⁵⁾ observed a significant increase in the faecal excretion of fatty acids and bile acids in mice fed a high-Ca diet. Buchowski *et al.*⁽¹¹⁰⁾ also found that a high-Ca diet causes an increase in faecal fat excretion independent of the Ca source. Specifically, they showed that, compared with a low-Ca diet, a high-Ca diet increased the faecal fat excretion in human subjects by 1.8 g/d, or about 3% of daily fat intake. This increase in fat excretion could cause 0.4–0.7 kg of body-fat loss over a 1-year period. Christensen *et al.*⁽¹¹¹⁾ examined the effect of Ca on fat excretion by performing a systematic meta-analysis review and estimated that increasing daily Ca intake by 1241 mg/d resulted in an increase in faecal fat of 5.2 (1.6–8.8) g/d. This effect was most pronounced in subjects with a low habitual dietary Ca intake. However, the effect of Ca on faecal fat excretion has not been investigated in long-term studies.

Intracellular calcium, 1,25-dihydroxyvitamin D₃ and apoptosis

Apoptosis, a highly regulated form of cell death, plays an important role during development and adult life via intimate involvement in cellular and tissue homeostasis.

Table 2. Intervention studies examining the effects of vitamin D on body weight*

Reference	Population	Design	Intervention	Results
Sneve <i>et al.</i> ⁽¹⁰⁵⁾	<i>n</i> 445; 83% female; age 47.6 (SD 11.4) years; BMI 28.0–47.0 kg/m ² ; serum 25(OH)D at baseline 53.1 (SD 16.9) nmol/l	Length 12 months; latitude 70°N	Ca 500 mg/d + 40 000 IU vitamin D ₃ per week (<i>n</i> 153); Ca 500 mg/d + 20 000 IU vitamin D ₃ per week (<i>n</i> 143); Ca 500 mg/d + placebo twice per week (<i>n</i> 149)	No significant difference in changes in body weight, body fat and BMI was observed between groups
Zhou <i>et al.</i> ⁽¹⁰⁸⁾	<i>n</i> 1179; 100% female; age 66.0 (SD 6.9) years; BMI 28.8 (SD 5.3) kg/m ² ; serum 25(OH)D at baseline 73.2 (SD 19.9) nmol/l	Length 4 years; latitude 41°N	Ca (1400 or 1500 mg/d) + placebo (<i>n</i> 445); Ca (1400 or 1500 mg/d) + vitamin D ₃ (1100 IU/d) (<i>n</i> 446); placebo daily (<i>n</i> 288)	Ca had no effect on BMI but significantly lowered trunk fat, compared with the placebo group. Vitamin D may have no additional effect on body composition
Major <i>et al.</i> ⁽¹⁰⁶⁾	<i>n</i> 63; 100% female; age 43.6 (SD 5.0) years; BMI 27.0–40.0 kg/m ² ; serum 25(OH)D at baseline N/A	Length 15 weeks, 700 kcal/d (2929 kJ/d) energy restriction; latitude 46°N	Ca (600 mg/d) + vitamin D (200 IU/d) (<i>n</i> 30); placebo daily (<i>n</i> 33)	Vitamin D plus Ca has no significant effect on body weight, body fat and BMI
Caan <i>et al.</i> ⁽¹⁰⁹⁾	<i>n</i> 36 282; 100% female; age 50–79 years; BMI < 25 to > 35 kg/m ² ; serum 25(OH)D at baseline N/A	Length 7 years; latitude N/A	Ca (1000 mg/d) + vitamin D ₃ (400 IU/d) (<i>n</i> 18 176); placebo daily (<i>n</i> 18 106)	Vitamin D plus Ca has a small effect on body weight with inadequate Ca intakes
Zittermann <i>et al.</i> ⁽¹⁰⁴⁾	<i>n</i> 165; 69% female; age 18–70 years; BMI < 25 to > 35 kg/m ² ; mean serum 25(OH)D baseline 30 nmol/l	Length 12 months, weight-reduction programme; latitude 48°N	Vitamin D ₃ (3332 IU/d) (<i>n</i> 82); placebo daily (<i>n</i> 83)	No significant difference in changes of body weight, body fat and BMI was observed between groups
Holecki <i>et al.</i> ⁽¹⁰⁷⁾	<i>n</i> 40; 100% female; age 45–55 years; BMI > 30 kg/m ² ; serum 25(OH)D at baseline N/A	Length 3 months, 1000–1200 kcal/d (4184–5021 kJ/d) energy restriction; latitude 52°N	Control diet with Ca (2000 mg/d) + vitamin D ₃ (250 IU/d) (<i>n</i> 20); control diet (500 mg Ca/d; <i>n</i> 20)	No significant difference in changes in body weight, body fat and BMI was observed between groups

25(OH)D, 25-hydroxyvitamin D; N/A, not applicable.

* 1 IU vitamin D₃ = 0.025 µg vitamin D₃.

Apoptosis is the main mechanism for controlling cell number in most tissues⁽¹¹²⁾. Obesity is the result of an increase in the adipose tissue mass, which can result from both hypertrophy, an increase in adipocyte size, and hyperplasia, an increase in adipocyte number^(9,113). Weight loss can be caused not only by a decrease in adipocyte size (i.e. increasing lipolysis with a potential for lipotoxic effects), but also in adipocyte number (for example, by stimulating apoptosis). An increase in the rate of adipocyte apoptosis will prevent excessive accumulation of adipose tissue and may result in a significant loss of adipose tissue mass over time, in contrast to that which occurs after energy restriction. However, studies on the role of apoptosis in fat tissue have been limited by the fact that mature, differentiated adipocytes are extremely stable and not thought to be capable of undergoing apoptosis. Induction of death of adipocytes through apoptosis may emerge as a promising strategy for the prevention and treatment of obesity because removal of adipocytes via this mechanism will result in reducing body fat and a long-lasting maintenance of weight loss.

Cellular Ca^{2+} has been implicated in the induction of apoptosis and regulation of the apoptotic signalling pathways^(20–22,65,114,115); however, mechanisms of Ca^{2+} signalling in apoptosis remain obscure. We^(22,55,56,65,68,116–118) and others^(20,114,115,119) have shown that increases in the concentration of intracellular Ca^{2+} occur in the early and late stages of apoptosis. It appears that the critical characteristic of the apoptotic Ca^{2+} signal is a sustained increase in intracellular Ca^{2+} concentration, reaching elevated, but not cytotoxic levels. Although there is little doubt that such an increase in intracellular Ca^{2+} concentration triggers cell death via apoptosis, the mechanisms of action of intracellular Ca^{2+} in apoptotic pathways are not known and, particularly, interactions of the cellular Ca^{2+} signal with molecular Ca^{2+} targets in cells undergoing apoptosis have not been identified. A family of intracellular cysteine proteases, the caspases, is responsible for most biochemical and morphological alterations during apoptosis, although additional or alternative apoptosis initiation and execution pathways have been demonstrated^(20,22,120). Ca^{2+} -dependent caspases and Ca^{2+} -dependent neutral proteases, the calpains, are considered as the primary Ca^{2+} -activated apoptotic targets^(117,121–125).

Interaction of the Ca^{2+} signal with intracellular Ca^{2+} buffers plays a particularly important role in the apoptotic process. A key element of the cytosolic Ca^{2+} -buffering system is the vitamin D-dependent Ca^{2+} -binding proteins, calbindins. Elevated levels of calbindins dramatically increase the cytosolic Ca^{2+} -buffering capacity and an increase in Ca^{2+} buffering via forced expression of calbindin- $\text{D}_{28\text{k}}$ protects cells against Ca^{2+} -mediated apoptosis^(65,67,69,117).

We have shown^(21,22,65) that a sustained (not reaching cytotoxic levels) increase in intracellular Ca^{2+} concentration signals the cell to enter the apoptotic pathway via activation of the Ca^{2+} -dependent protease μ -calpain followed by activation of the Ca^{2+} /calpain-dependent

caspase-12 and other executor caspases (for example, caspase-3). A lack of expression or low levels of the cytosolic Ca^{2+} -binding proteins (for example, calbindin-D) diminish the ability of the cell to buffer intracellular Ca^{2+} concentration increases and, thus, facilitate induction of apoptosis. On the other hand, agents that induce expression of the intracellular Ca^{2+} buffers or suppress pathways for the generation of the apoptotic Ca^{2+} signal may protect against Ca^{2+} -mediated apoptosis.

It is well established that $1,25(\text{OH})_2\text{D}_3$ can induce Ca^{2+} signals in different cell types, including adipocytes. $1,25(\text{OH})_2\text{D}_3$ activates the voltage-dependent and voltage-insensitive Ca^{2+} entry pathways and triggers Ca^{2+} release from the endoplasmic reticulum stores through the inositol 1,4,5-trisphosphate and ryanodine receptors^(21,22,56,65,117). Importantly, we have also shown that $1,25(\text{OH})_2\text{D}_3$ induces apoptosis in adipocytes^(23,58) and that apoptosis induced by $1,25(\text{OH})_2\text{D}_3$ in these cells depends on Ca^{2+} signalling^(22,24,117).

Below we summarise the present results regarding the role of $1,25(\text{OH})_2\text{D}_3$ in generating Ca^{2+} signals in adipocytes and provide evidence that $1,25(\text{OH})_2\text{D}_3$ -induced Ca^{2+} signals can determine adipocyte fate by apoptosis (Fig. 1). These findings may help in the rational search for therapeutic and preventive agents for obesity that act via Ca^{2+} -dependent molecular targets in apoptotic pathways.

1,25-Dihydroxyvitamin D₃ induces Ca²⁺-mediated apoptosis in adipocytes

The mechanism controlling adipocyte apoptosis is unknown and even the ability of adipocytes to undergo apoptosis has not been conclusively demonstrated. We have recently shown⁽²³⁾ that $1,25(\text{OH})_2\text{D}_3$ induces apoptosis in mature mouse 3T3-L1 adipocytes via activation of the Ca^{2+} -dependent calpain and Ca^{2+} /calpain-dependent caspase-12. Treatment of adipocytes with $1,25(\text{OH})_2\text{D}_3$ induced, in a concentration- and time-dependent fashion, a sustained increase in the basal level of intracellular Ca^{2+} . The increase in intracellular Ca^{2+} concentration was associated with induction of apoptosis and activation of μ -calpain and caspase-12. Importantly, susceptibility of mature, differentiated adipocytes to the Ca^{2+} -elevating effect of $1,25(\text{OH})_2\text{D}_3$ appears to be dependent on the reduced Ca^{2+} -buffering capacity of these cells. The results demonstrated that Ca^{2+} -mediated apoptosis can be induced in mature, differentiated adipocytes and that the apoptotic molecular targets activated by $1,25(\text{OH})_2\text{D}_3$ in these cells are Ca^{2+} -dependent calpains and caspases. It is interesting to note that a different class of compounds, flavonoids, which are present in the common human diet, also induces Ca^{2+} -mediated apoptosis in adipocytes^(126–128). These findings provide a strong rationale for evaluating the role of vitamin D in the prevention and treatment of obesity.

$1,25(\text{OH})_2\text{D}_3$ is not only an important determinant of adipocyte apoptosis, but also appears to regulate adipocyte

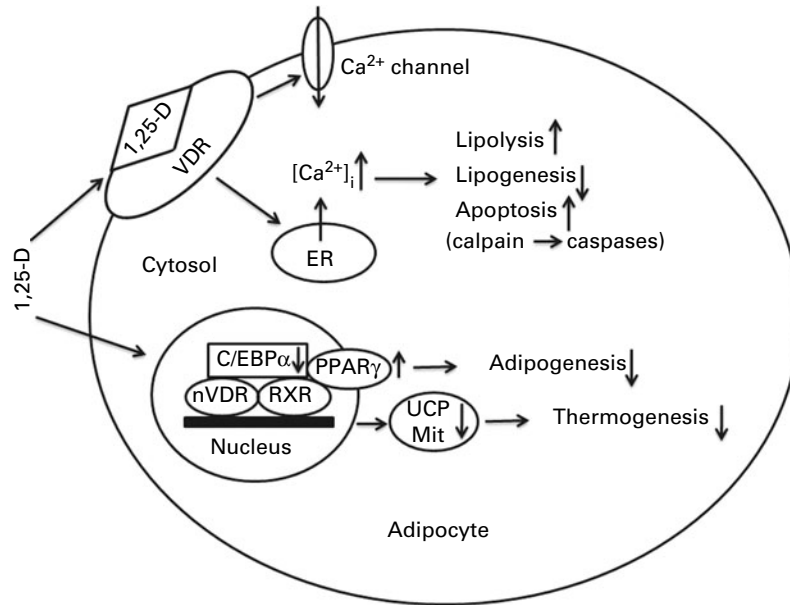


Fig. 1. Role of cellular Ca^{2+} and 1,25-dihydroxyvitamin D_3 ($1,25(\text{OH})_2\text{D}_3$; 1,25-D) in the regulation of adipocyte functions. 1,25(OH) $_2\text{D}_3$ generates the cellular Ca^{2+} signal via the regulation of Ca^{2+} entry from the extracellular space, Ca^{2+} mobilisation from the intracellular stores and intracellular Ca^{2+} buffering. The mechanism includes interactions of 1,25(OH) $_2\text{D}_3$ with the membrane vitamin D receptor (VDR) and Ca^{2+} channels/receptors in the plasma and endoplasmic reticulum (ER) membrane. The 1,25(OH) $_2\text{D}_3$ -induced cellular Ca^{2+} signal (a sustained increase in concentration of cytosolic Ca^{2+}) inhibits lipogenesis, stimulates lipolysis and induces apoptosis (via activation of the Ca^{2+} -dependent apoptotic pathway) in mature adipocytes. 1,25(OH) $_2\text{D}_3$ also inhibits adipogenesis by suppressing adipocyte differentiation. The process involves down-regulation of a transcriptional factor CCAAT enhancer-binding protein- α (C/EBP α) and up-regulation of the nuclear PPAR γ . 1,25(OH) $_2\text{D}_3$ appears to inhibit expression of the mitochondrial uncoupling proteins (UCP) in adipocytes, which may counteract body-fat loss (a high UCP expression may promote the shift toward thermogenesis and, thus, a loss of body fat). [Ca^{2+}] $_i$, intracellular Ca^{2+} concentration; nVDR, nuclear VDR; RXR, retinoid X receptor; Mit, mitochondria.

differentiation. It has been shown that 1,25(OH) $_2\text{D}_3$ inhibits adipogenesis in 3T3-L1 cells by blocking their differentiation to mature adipocytes⁽¹²⁹⁾. The process involves suppression of a transcriptional regulator CCAAT-enhancer-binding protein (C/EBP α) and up-regulation of PPAR γ . PPAR γ is a primary regulator of fatty acid storage and glucose metabolism, and the genes activated by PPAR γ are linked to stimulation of lipid uptake by adipocytes and adipogenesis⁽¹³⁰⁾. C/EBP α promotes adipogenesis by inducing the expression of PPAR γ ⁽¹³¹⁾. The nuclear VDR appears to play an essential role in adipogenesis via C/EBP α and PPAR γ (for example, in the absence of 1,25(OH) $_2\text{D}_3$, 'knock-down' of the VDR using siRNA delays the formation of adipocytes⁽¹³²⁾).

It is worth mentioning that 'pharmacological' concentrations of 1,25(OH) $_2\text{D}_3$ (10–100 nmol/l) are used in *in vitro* studies. Under normal physiological conditions, the serum circulating concentration of 1,25(OH) $_2\text{D}_3$ is tightly regulated at the level of 100–125 pmol/l, and normal levels of 1,25(OH) $_2\text{D}_3$ in blood can be maintained within a wide range of 25(OH) D_3 concentrations (10–250 nmol/l)⁽²¹⁾. On the other hand, concentrations of 1,25(OH) $_2\text{D}_3$ in different tissues can approach a nanomolar range due to *in situ* biosynthesis of the hormone^(21,74). Therefore, it is difficult to make a direct comparison of *in vitro* studies using 1,25(OH) $_2\text{D}_3$ and clinical studies using either

supplementation with vitamin D or measuring the circulating concentration of 25(OH) D_3 .

Calcium and lipid metabolism

Ca^{2+} may play a role in the regulation of lipid metabolism and TAG storage. The hypothesis advocated by Zemel & Sun⁽¹³³⁾ suggests that depressed levels of 1,25(OH) $_2\text{D}_3$ (due to a high dietary Ca intake) cause a decrease in intracellular Ca^{2+} concentration, and, thus, stimulate lipolysis, and inhibit fatty acid synthase and *de novo* lipogenesis. However, a basal, steady-state intracellular Ca^{2+} concentration is maintained by mechanisms (pumps and buffers) independent of Ca intake, and a decrease in circulating 1,25(OH) $_2\text{D}_3$ will not directly affect basal intracellular Ca^{2+} concentration. Interestingly, Sampath *et al.*⁽¹³⁴⁾ administered 1500 mg supplemental Ca per d for 3 months to overweight or obese subjects and measured the rates of lipolysis in adipose tissue, whole-body lipid oxidation and circulating concentrations of several hormones. They failed to demonstrate the effect of Ca supplementation on the rate of lipid oxidation or lipolysis. This was confirmed in the study by Bortolotti *et al.*⁽¹³⁵⁾, where subjects receiving 800 mg dairy Ca per d for 5 weeks demonstrated no effects of Ca supplementation on markers of lipid metabolism.

Vitamin D and mitochondrial uncoupling proteins

An increase in the expression of mitochondrial uncoupling proteins (UCP) promotes a shift toward thermogenesis and away from ATP synthesis. As ATP production diminishes, the dynamics of the catabolic breakdown and biosynthesis of stored nutrients shifts toward catabolism in an effort to replenish the ATP stores⁽¹³⁶⁾. Theoretically, therefore, a higher expression of UCP could be beneficial for body-fat loss. The study conducted by Sun & Zemel⁽¹³⁷⁾ found that treatment of human adipocytes with 1,25(OH)₂D₃ inhibits UCP-2 mRNA and the protein level via a mechanism linked to the nuclear VDR. The potential role of UCP was also demonstrated in the normocalcemic, VDR knockout mouse⁽¹⁷⁾. The lean phenotype of VDR knockout mice was characterised by a reduced serum leptin concentration, a compensatory increase of food intake and was associated with elevated levels of UCP-1 in adipose tissue. These studies show that 1,25(OH)₂D₃ may suppress UCP expression in adipocytes. However, such an effect would not necessarily contribute to fat accumulation in normal adipose tissue because ATP synthesis by mitochondria is tightly regulated, and physiological thermogenesis occurs mainly in brown adipose tissue.

Conclusion

The reviewed observational studies indicate that higher Ca intake and increased vitamin D status are inversely associated with body weight and fat in humans. However, intervention studies examining the effect of dietary Ca and vitamin D status on body-fat mass and body weight are inconclusive. Emerging data provide a mechanistic framework for evaluating the role of Ca and vitamin D in adiposity and energy balance. These mechanisms include the modulation of faecal fat excretion, adipocyte apoptosis, adipogenesis and lipid metabolism, and mitochondrial UCP (see Fig. 1). Although precise molecular pathways linking vitamin D, Ca and energy balance are not identified, the evidence discussed in the present review reinforces the importance of unravelling these mechanisms to better understand the role of vitamin D and Ca in the prevention of obesity and overweight. Conflicting results on the role of Ca and vitamin D in adipose tissue suggest that multiple factors such as Ca intake, vitamin D status, and the interactions of Ca–vitamin D cellular signalling pathways may act synergistically or antagonistically to regulate energy balance and body-fat gain. Clearly, investigations on the role of Ca and vitamin D and obesity are urgently needed.

Acknowledgements

The studies by I. N. S. reviewed in this article were supported by the United States Department of Agriculture (no. SD00294-H, SD00H167-061HG and 2009-35200-05008). Q. S. and I. N. S. contributed equally in the writing

and revising of the manuscript. The authors declare no competing interests.

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