

Recent developments in calcium-related obesity research

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Summary

The influence of calcium and dairy food intake on energy balance is the object of a growing scientific literature. This manuscript presents the information discussed by subject experts during a symposium on calcium and obesity, initially planned to document in a comprehensive manner the role of calcium and dairy food on energy balance and body composition. This manuscript is organized into 13 propositions statements which either resume the presentation of an invited speaker or integrate recent developments in calcium-related obesity research. More specifically, the effects of calcium and dairy consumption on body weight and adiposity level, appetite, weight loss intervention outcome, lipid-lipoprotein profile and the risk to develop metabolic syndrome are discussed together with the metabolic mechanisms proposed to explain these effects. Taken together, the observations presented in this manuscript suggest that calcium and dairy food intake can influence many components of energy and fat balance, indicating that inadequate calcium/dairy intake may increase the risk of positive energy balance and of other health problems.

Keywords: Calcium, dairy, obesity, symposium.

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Introduction

The identification of factors that influence energy balance is an important issue in the research field of nutrition and becomes a growing necessity in the context of obesity epidemic throughout the world. Energy intake, physical activity levels and the macronutrient composition of the diet are generally considered by health professionals and scientists as some of the major factors that explain variations in energy balance. It is generally believed that energy imbalance results from the impact of factors which directly affect caloric input or output. However, recent experimental evidence has emphasized that a positive or a negative energy balance may happen via the influence of factors that have no *a priori* caloric value. This is the case for calcium intake for which the effects have traditionally been studied in relation with bone health.

The first data that established a link between calcium intake and energy/fat balance were reported several years ago by Zemel *et al.* (1). These results, both obtained in humans and animals, favoured the initiation of numerous studies that have recently permitted description of specific roles for calcium and related nutritional factors on energy and fat balance. Furthermore, these endeavours have clinical implications which are important for the prevention and treatment of obesity.

The relevance and the motivation to better understand this issue led us to organize in December 2006 a symposium that was planned to document, in a comprehensive manner, the role of calcium and dairy food on variations in energy balance and body composition. The objective of this paper which is at the crossroads between a review and a symposium summary is to describe the main concepts derived from this meeting. Therefore, this manuscript is

organized into 13 proposition statements which either resume the presentation of an invited speaker or integrate recent developments in calcium-related obesity research. Some proposition statements in this paper were not directly addressed during the symposium but were included in order to cover all hypotheses that pertain to the effect of calcium and dairy food on body composition and energy balance. Scientific references were mainly included in this manuscript on the basis of their support to each proposition. Therefore, this paper pertains to the description of the most potential mechanisms and hypotheses that are in favour of the effect of calcium and dairy food on energy balance and body composition rather than an exhaustive literature review and critical analysis of this topic.

Unless otherwise stated, the described effects come from observations in human. The terms calcium and dairy food are used throughout the manuscript to distinguish between calcium *per se* (found in supplements, in dairy, or in non-dairy food) and calcium combined with other dietary components present in dairy food products.

Proposition 1: calcium and dairy intakes are lower than recommended for a majority of individuals

Calcium intake in the United States

The adequate intake (AI) for American and Canadian men and women 19–50 years of age is 1000 mg day⁻¹ and the Tolerable Upper Intake Level is 2500 mg day⁻¹ (2). The 1994–1996 Continuing Survey of Food Intakes by Individuals (CSFII) estimated the median intake of calcium in women 18–50 years of age to be around 610 mg day⁻¹ (2). In a more recent National Health and Nutrition Examination Survey (NHANES) (1999–2000), calcium intake was

about 770 mg day⁻¹ for women 20–59 years of age (3). A National Institutes of Health Consensus Conference in 1994 highlighted calcium as one of two nutrient deficiencies in the United States that warrant a national effort to increase average intake levels (4).

The AI for calcium is 800 mg day⁻¹ for children 4–8 years of age, and 1300 mg day⁻¹ for children and adolescents 9–18 years of age (2). Data from the 1994–1996 CSFII showed that the majority of children have usual intake of calcium that is less than the AI for calcium (5). Similar results were observed for 10-year-old children in the Bogalusa Heart Study where 69% did not meet the dietary recommendation of 1300 mg day⁻¹; the percentage was higher among women (76%) than among men (62%) ($P < 0.001$) (6). Among female and male adolescents aged 12–19 years, nearly 9 out of 10 adolescent girls and 7 out of 10 adolescent boys failed to meet the AI for calcium (5). Caucasians and Hispanics had higher calcium intake levels than African-Americans and 'others' (5,7,8). Among young adults (19–28 years of age), 77% did not meet the AI for calcium, particularly female young adults (82%) (6).

Calcium intake in Canada

The results from the Canadian Community Health Survey, cycle 2.2-Nutrition (9) suggest that 65–84% of Canadian adults (31 years and over) did not meet the milk products recommendation (9). Moreover, the nutrient intakes from food revealed that females have a lower intake of dietary calcium compared to males (10). Also, calcium intake was shown to decrease with age in both gender. The group the most at risk seems to be the men and women aged 51–70 years with less than 15% of them having a calcium intake greater than the AI (See Table 1 for more details).

Age groups (years)	Calcium AI* (mg day ⁻¹) Men and women	Percentage of Canadians with a dietary calcium intake > AI**	
		% men	% women
2–3	500		
4–8	800	77 (boys and girls)	
9–13	1300	38	17
14–18	1300	49	17
19–30	1000	53	30
31–50	1000	38	27
51–70	1200	14	8
71+	1200	Unreliable estimates due to sampling variability	

Table 1 Proportion of Canadians meeting or exceeding the adequate intake (AI) for calcium: data from the Canadian Community Health Survey cycle 2.2-Nutrition (10)

*The AI has limited uses in assessing groups (140).

If group median intake meets or exceeds the AI, prevalence of inadequacy is likely low.

**These data do not include the calcium intake from supplements.

If group median intake is below the AI, *nothing* can be concluded about inadequacy.

The percent of a group with intake below the AI cannot be assessed as *deficient*.

Milk products and soft drinks consumption

Parallel to a decrease in milk/calcium intake, there has been an increase in soft drink consumption. Indeed, children's consumption of beverages has changed dramatically during the past several decades, at least in industrialized countries (11–13). For example, milk intake has decreased and intake of carbonated soft drinks ('sodas') or sugared, fruit-flavoured drinks ('fruit drinks') has increased with time and with increasing age (13,14). Not surprisingly, children's beverage consumption has become an important focus of investigation because of its potential to contribute positively (e.g. milk) or negatively (e.g. soda, fruit drinks) to adequate nutrient intake (11,14,15).

A recent prospective study of more than 2300 9-year-old girls from the National Heart, Lung, and Blood Institute Growth and Health Study found that absolute quantities of milk consumed had decreased by 25% and sugar-sweetened beverage consumption had increased almost threefold over a 10-year period (16). Soda consumption was also associated with a statistically significant decrease in calcium intake. Other studies have indicated that in children and adolescents, sugar-sweetened beverage consumption as a percentage of total caloric intake had increased and milk consumption had decreased over the last 20 years (17,18). The same studies found that sugar-sweetened beverages accounted for 5% to 10% of caloric intake among children aged 2–16 years. Although an inverse relationship between milk and sugar-sweetened beverage intake often exists, these studies do not irrefutably demonstrate that increased sugar-sweetened beverage consumption leads to decreased milk consumption. However, one study demonstrated that when a small sample of 6- to 13-year-old children at a 4- to 8-week summer day camp was offered both milk and fruit-flavoured sugar-sweetened beverages, the children's milk consumption was significantly lower when they consumed more than 16 oz day⁻¹ of sugar-sweetened beverages compared with when they consumed no sugar-sweetened beverages (19).

In adults, a recent study that aimed to examine American beverage consumption trends and causes between 1977 and 2001 in a sample of 73 345 individuals found that sweetened beverage consumption had increased and milk consumption had decreased in all age groups (17). Overall, energy intake from sweetened beverages and from milk was increased by 135% and reduced by 38%, respectively, resulting in a 278 kcal increase in total energy intake. These trends were associated with increased proportions of Americans consuming larger portions, more servings per day of sweetened beverage and equivalent reductions in milk.

In summary, it is clear that the last several decades have shown an increase in soft drinks consumption and a decrease in milk consumption contributing to a reduction in calcium intake. Taken together, these changes in drinking

habits could contribute to a state of positive energy balance and obesity development in at-risk groups.

Proposition 2: a low-calcium/dairy intake is associated with a greater fat mass in adults

The rise of interest in calcium and dairy intake and their potential influence on energy balance has its origin in the population studies that have shown an inverse relationship between calcium and dairy consumption and adiposity levels. In the early 1980s, McCarron *et al.* observed in more than 10 000 participants aged 18–74 years from the NHANES I study that higher intakes of dietary calcium were negatively correlated with body mass (20). More recently, Zemel *et al.* demonstrated a similar relationship in more than 7000 men and women from the NHANES III study (1), and this was followed by several studies that also demonstrated that a low-calcium and/or dairy intake is associated with a greater fat mass in adults. Table 2 enumerates the main conclusions of some of these studies, classified on the basis of the relationship with fat mass being observed with calcium or dairy intake. As in adults change in body weight and body mass index (BMI) is generally due to fat mass changes, studies that reported body weight and BMI as an outcome were also included. Studies that have not shown a relationship are also mentioned in Table 2.

Based on results from these prospective population and cross-sectional studies, it seems reasonable to propose that low-calcium and/or dairy intake is associated with a greater fat mass as well as a greater risk of weight and fat mass gain over time (21). Moreover, most of the studies mentioned above reported an association with dairy and dietary calcium intake (which includes dairy food), and one study found an association with calcium supplement but not dietary calcium (22). This therefore suggests that dairy food, more than calcium *per se*, has an influence on fat mass or body weight in adults. Different components of dairy food which beside calcium could potentially account for this relationship are discussed in Propositions 5 and 13.

However, types of studies reported in Table 2 (mostly cross-sectional, retrospective and observational) make it difficult to determine a direct effect of the low-calcium and dairy products intake from the effect of a diet low in calcium and dairy, inasmuch as it could be the other components of an 'unhealthy' diet that drives up body fat. In this regard, three studies reported in Table 2 found no relationship between calcium or dairy intake and body weight. However, one could argue that the absence of a relationship between dairy intake and BMI could be explained by the fact that median consumption of total dairy products in the Snjider *et al.* study (4.1 servings per day) was well above that from other countries and also above the suggested threshold level of 500–800 mg day⁻¹ of

Table 2 List of studies and their main conclusions related to the relationship between calcium and dairy food intake and fat mass in adults

	References	Main conclusions
Low-calcium intake (from dairy and non-dairy food) is associated with greater fat mass	(1,141–143)	Lower-calcium intake increases odds ratio for being overweight or obese
	(141,144,145)	Calcium intake is negatively associated with BMI
	(146)	Calcium intake is associated with lower body fat gain
	(22)	Calcium intake is associated with lower BMI change
Low-dairy food intake is associated with greater fat mass	(45,46,147)	Calcium intake is negatively associated with body fat
	(56,146,148)	Dairy food intake is associated with lower fat mass gain
No association	(149,150)	Lower-dairy intake increases odds ratio for being overweight or obese
	(23)	Dairy food intake is not associated with BMI
	(24,151)	Calcium intake does not predict lower body weight gain

BMI, body mass index.

habitual calcium intake above which the effect on body weight and composition are less marked (23), as discussed in proposition 4. Also, the outcome measured in these studies (23,24) was body weight, not body fat, and previous work failed to detect dairy-induced differences in body weight in the absence of energy restriction, although body fat was reduced (25). Therefore, whether the relationship between higher dairy intakes and lower body fat is the reflection of more healthy diets and behaviours could be considered still questionable, whereas most studies have adjusted for obvious confounding factors such as energy and composition of the diet, evidence suggesting a direct relationship could also be regarded as quite large.

Proposition 3: low-calcium intake predicts excess body weight and fat in children and adolescents

As in adults, some studies conducted in children and adolescents have shown that a low-calcium intake predicts excess body weight and fat mass. Table 3 presents the main conclusions of some of these studies, classified once again on the basis of their independent variable being calcium or dairy food intake, with a distinction between dependent variables body weight and fat mass. As can also be seen from Table 3, there are inconsistencies between studies. Although a reasonable amount of observational studies found a negative relationship between *habitual* calcium/dairy intake and body weight or fat mass (see Table 3), the same relationship was not observed with long-term calcium intake *supplementation*. A meta-analysis on 17 randomized control trials involving 2088 participants (1005 calcium supplemented and 1083 placebo) found no statistically significant effect of calcium supplementation on weight or fat (26). However, it has to be pointed out that the reported

associations between calcium supplementation and body weight come from studies that had originally been designed to assess the effect of a supplementation on bone mass with no original intent to assess body weight and fat mass, which complicates the interpretation of the results (Table 3). On the other hand, trying to quantify the effect of a supplementation of calcium on body weight and fat mass gain in children and adolescents is a delicate task as the metabolic and hormonal changes that happen during growth and puberty are likely to interfere with any type of treatment tested, and complicate or even compromise interpretation of the results.

As part of this issue, it is still unclear if recommendations towards increasing dairy food intake in children and adolescents would prevent or not excess adiposity (27). Berkey *et al.* observed an increase in energy intake and body weight gain in adolescents who reported the higher milk intake, while other studies where the instruction had been given to increase dairy products consumption (28–31) reported no greater increase in body weight compared with no change in the diet. In fact, Lappe *et al.* observed that despite an average excess of 150 kcal day⁻¹ in the calcium supplemented compared with the control group, no greater body weight or fat mass gain was observed after a 2-year supplementation in dairy and calcium-rich foods (31). Another point that needs to be clarified is the influence of habitual calcium intake in children on the outcome of the calcium/dairy supplementation, as it is discussed for adults in Proposition 4. On one hand, results from DeJongh *et al.* suggest an influence of habitual calcium intake as these authors observed that in the context of a 1-year randomized calcium and activity trial in 178 children aged 3–5 years, those in the lowest tertile of dietary calcium intake who were randomly assigned to receive supplemental calcium (1000 mg day⁻¹) had lower gains in fat mass

Table 3 List of studies and their main conclusions related to the relationship between calcium and dairy food intake and body weight and fat mass in children and adolescents

	References	Main conclusions
Low-calcium intake (from dairy and non-dairy food) predicts excess body weight and fat in children and adolescents	(152–154) (33,153,155–157) (153,156,158*)	Children with lower-calcium intake have higher body weight and fat Calcium intake is negatively associated with body fat Calcium intake is negatively associated with body weight or BMI
Low-dairy food intake predicts excess body weight and fat in children and adolescents	(27) (155) (154)	Low-dairy food intake in early childhood increases the risk for excess body fat in adolescence Higher-dairy food intake is associated with lower body fat Dairy food intake is negatively associated with child obesity
Opposite or no effect	(159) (160,161)	Milk intake is associated with greater body weight gain Calcium intake is not associated with body weight or body fat
Relationship between calcium intake and body weight in supplementation trials	(32) [†] (28–31,31,36,162–165) [‡]	A calcium supplementation lowers gain in fat mass No effect of a calcium/dairy supplementation (≥ 1 year) on BMI and fat mass gain

*In girls only.

[†]In children in the lowest tertile of dietary calcium intake only.

[‡]Trials with bone mass issue.

BMI, body mass index.

than did children on placebo (32). However, another study also reported no interaction effect of habitual calcium intake (median- vs. low-calcium intake, 1000–1304 vs. <713 mg day⁻¹ respectively) on the outcome of a calcium supplementation (33). Therefore, as suggested by a recent review on the subject (34) and despite several observational studies having demonstrated that low-calcium intake correlates with excess body weight and fat in children and adolescents, more studies are warranted to better identify associations between calcium/dairy intake and precise measurements of growth-related changes in body composition and metabolism in children and adolescents before important recommendations can be made.

Proposition 4: calcium/dairy food supplementation accentuates body weight and fat loss in low-calcium consumers

The suggestion that calcium/dairy supplementation are factors potentially enhancing the success of obesity treatment naturally followed the hypothesis exposed above. A recent systematic review and meta-analysis that included 13 randomized controlled trials in which calcium supplementation (calcium supplements or dairy products) was given as treatment and body weight reported as a final outcome measure found no evidence of a benefit from these supplementations on body weight (35). Similarly, among

the 26 randomized controlled trials on calcium supplementation (9 of increased dairy food intake and 17 of calcium supplement intake) included in another systematic review, only one obtained results showing greater weight loss in the calcium supplemented group (36). However, among the randomized control trials considered in both these systematic reviews only one (37), reviewed by Trowman *et al.* was specifically designed and powered to examine whether or not calcium supplementation led to a change in body weight at follow-up (35). In this specific study, Zemel *et al.* observed a 26% and a 70% increase in body weight loss in the high-calcium (800 mg day⁻¹ of calcium carbonate) and high-dairy (1200–1300 mg day⁻¹ of dietary calcium) supplemented groups, respectively, compared with the placebo group in which habitual calcium intake (400–500 mg day⁻¹) was maintained constant during the 24-week weight loss intervention (–500 kcal day⁻¹) (37).

More recently, two other studies by the Zemel group showed concordant results. In the first one, a group of obese low-calcium consumers (initial calcium intake between 500 and 600 mg day⁻¹) were randomized for a 12-week yogurt-supplemented diet (1100 mg of calcium per day) and experienced a 22% and 61% significantly greater body weight (6.6 vs. 5 kg) and fat mass loss (4.4 vs. 2.8 kg) loss compared with the control group in the context of a weight reducing programme inducing an energy deficit of 500 kcal day⁻¹ (38). The second study, conducted

in obese African-American low-calcium consumers (<600 mg day⁻¹), consisted into a weight maintenance period followed by a second phase during which in addition to a 500 kcal day⁻¹ energy deficit, participants were randomized either to a control diet providing a 0–1 serving of low-fat dairy product per day (~500 mg of calcium) or to a high-dairy diet providing three servings of dairy per day (~1000 mg of calcium per day). They found that the high-dairy diet induced a twofold higher weight and fat mass loss compared with the low-dairy diet (25). Another recent randomized controlled trial on the effect of a calcium+vitamin D supplementation on body weight and fat mass loss during a weight reduction programme has been conducted in overweight and obese women (39). Results showed that among very low-calcium consumers (initial calcium intake ≤600 mg day⁻¹), those who received the calcium+vitamin D supplement lost significantly more body weight and fat mass (5.8 vs. 1.4 kg and 4.7 vs. 1.2 kg respectively) compared with the placebo during the 15-week programme (39).

Results from these three recent randomized clinical trials showed an effect of calcium, dairy, or calcium+vitamin D supplementation on weight and fat mass loss in circumstances of energy restriction in overweight/obese men and women low-calcium consumers. On the basis of these studies, it is possible to add to the conclusions formulated in the systematic reviews on the topic (35,36), and to suggest that the initial (low) calcium intake of participants might predict the outcome of a calcium-supplemented weight loss programme. Considering this hypothesis, it could be interesting to better define what can be considered a low-calcium intake in order to determine the threshold below which low-calcium consumers might benefit from a supplementation in calcium to achieve significant fat mass loss. Indeed, intervention studies that have observed no significant effect of a calcium or dairy supplemented hypocaloric diets on body weight and fat mass loss (thus similar changes between the treatment and placebo groups) were performed in subjects whose usual calcium intake could be qualified as moderate, i.e. >600 mg day⁻¹ (40–42). A recent study that also showed no effect of a 800 mg calcium lactate, calcium phosphate, or milk supplementation during an hypocaloric diet (–500 kcal) on fat mass loss did not assess the habitual calcium intake of participants prior to intervention (43). As for the absence of significant difference in body weight between control (<800 mg day⁻¹), medium-dairy (1000–1100 mg day⁻¹) and high-dairy groups (1300–1400 mg day⁻¹) after 1 year reported by Gunther *et al.*, it was in isocaloric diet conditions and therefore cannot be compared with the energy restriction trials reported above (44). On the basis of results obtained in the Zemel *et al.* and Major *et al.* studies, one could suggest 500–600 mg day⁻¹ as a cut-off value. Whereas results based on the Québec Family Study (45)

and the AGAHL study (46,47) in which body weight or/and fat mass, rather than decrease in fat mass, was the measured outcome, one would suggest a threshold of 600 and 800 mg day⁻¹ respectively. More recently, the current recommended daily intake (RDI) for calcium in menopausal women was found to be a discriminatory cut-off value to distinguish between women who received a calcium (1000 mg)+vitamin D (400 IU) supplementation during ~7 years and gained significantly less weight compared with placebo (mean habitual Ca²⁺ intake <1200 mg day⁻¹) than those for whom the same supplementation did not result in lower weight gain (mean habitual Ca²⁺ intake >1200 mg day⁻¹) (48). Therefore, although nothing can be concluded from the large range of habitual calcium intake values actually proposed to characterize low- or very low-calcium consumers, intervention studies reported above suggest nevertheless that calcium+vitamin D or dairy supplementation might enhance fat mass loss compared with a controlled condition, in overweight or obese individuals that have calcium intake well below the RDI for their age group.

Proposition 5: high-dairy diet preserves fat-free mass during energy restriction

In addition to its potential impact on increasing fat mass loss as discussed in Proposition 4, dairy food supplementation has been shown to protect muscle mass during energy restriction and to increase muscle mass on eucaloric diets (25,49,50). These effects have been observed in two randomized trials from the Zemel group which are also described in the previous proposition (25). In the first trial, 34 African-American obese adults were randomized in a 26-week weight maintenance isocaloric low-calcium (500 mg day⁻¹)/low-dairy (<1 serving per day) or high-dairy (1200 mg of calcium per day, including three servings of dairy with at least one in the form of fluid milk) diets. It was observed that although body weight remained stable in both groups, participants in the high-dairy diet group experienced significant decrease in total body fat and in trunk fat as well as significant increase in lean mass compared with participants in the low-dairy diet group (25). In the second trial, 29 African-American obese adults were randomized into a control diet providing 0–1 serving of low-fat dairy product per day (~500 mg of calcium) or into a high-dairy diet providing three servings of dairy per day (~1000 mg of calcium per day). Although both diets provided a 500 kcal day⁻¹ energy deficit and a similar protein content (17 and 18% of energy respectively), weight and fat loss were significantly increased and lean body mass loss significantly reduced in subjects who received the high-dairy diet compared with those who received the low-dairy diet (25).

It has been well demonstrated that milk proteins promote a more sustained net positive protein balance after

resistance exercise in human (51,52). This seems to be due to the amino acid content of the whey and casein proteins contained in milk as well as their digestion rate which stimulates postexercise protein anabolism (52). The branch chain amino acids which are increased along with the increase in dairy intake could also explain the effect of dairy supplementation on fat-free mass. In this regard, it has been suggested that the increase in lean mass could be largely mediated by leucine modulation of protein synthesis. As protein synthesis is energetically expensive, an increase in fatty acid oxidation which is fuelled, in part, by adipose tissue stores (53) would be necessary in order to cover the energetic 'cost' associated with increase dairy intake. In support of this hypothesis, leucine was found to significantly stimulate fatty acid oxidation in muscle cells and to decrease fatty acid synthase in adipocytes *in vitro* (53). These results also represent another mechanism explaining the effect of high-dairy diets on fat oxidation as discussed in Proposition 10.

Proposition 6: calcium deficiency influences the risk to develop metabolic syndrome

Beyond the effect of calcium and dairy consumption on body weight, the interest in calcium intake from an obesity treatment perspective has come from epidemiological studies that have shown inverse relationship between dietary calcium intake and hypertension (20,54), plasma cholesterol concentrations (discussed in Proposition 7), glucose intolerance, type 2 diabetes (55) and finally the simultaneous presence of these symptoms, establishing an inverse correlation with the metabolic syndrome (56–59). McCarron *et al.* in their analyses of the data from 10 372 individuals aged 18–74 years from the Health and Nutrition Examination Survey I (HANES I) found that dietary calcium intake was most predictive of hypertension (systolic blood pressure ≥ 160 mmHg), after controlling for age, race and sex (20). Analyses of data from 3157 participants (923 overweight or obese) in the Coronary Artery Risk Development in Young Adults (CARDIA) study (60) revealed that the 10-year adjusted cumulative incidence of the insulin resistance syndrome's components assessed (abnormal glucose homeostasis, obesity, elevated blood pressure and dyslipidemia) was reduced with increasing categories of dairy food intake for overweight individuals (56). The 10-year incidence was 72% lower among overweight individuals consuming ≥ 5 portions compared with those consuming < 1.5 portion of dairy/day (56). In the Tehran Lipid and Glucose Study cohort ($n = 827$ men and women aged 18–74 years), Azadbakht *et al.* found an inverse relation between dairy food consumption and the metabolic syndrome as defined in the third report of the National Cholesterol Education Program, Adult Treatment Panel NCEP-ATP III report (57,61). Subjects in the highest

quartile of dairy food consumption had significantly lower odds ratio of having the metabolic syndrome than those in the lowest quartile. This relationship was partly explained by calcium intake as adjustment for the effect of calcium weakened the probability. Using the same metabolic syndrome definition, Liu *et al.* found that higher intakes of total, dietary and supplemental calcium were significantly and inversely associated with the prevalence of the metabolic syndrome in 10 066 women aged 45 years and older from the Women's Health Study (58,62). Also using the ATP-III definition, Snijder *et al.* recently published results from the Hoorn Study showing inverse associations adjusted for age and sex between total dairy consumption and systolic and diastolic blood pressure and with triacylglycerol concentration. After adjusting for several confounding variables, no component of the metabolic syndrome, except a lower diastolic blood pressure, was found to be significantly associated with total dairy food consumption (23). Whether the older age or the greater daily mean dairy food intake of the population studied could explain the absence of relationship between other metabolic variables will need to be confirmed by further research.

Among these studies, an inverse association was demonstrated between the prevalence of the metabolic syndrome and calcium intake *per se* (57,58) as well as intakes of dairy (independently of calcium) (56). This is similar to what has been shown regarding the beneficial effect on blood pressure of dietary as well as non-dietary calcium supplementation (63). Therefore, although there is a need to understand more precisely by what mechanisms calcium and dairy intake independently modulate metabolic deteriorations, this does not undermine the results suggesting that lower intake of calcium and dairy food influences the risk of developing the metabolic syndrome and that an adequate calcium and dairy food intake might be a protective factor for numerous metabolic diseases. A recent study found that a 500 mg calcium and 700 IU vitamin D supplementation consumed over 3 years prevented the increases in plasma glucose and insulin resistance in participants with impaired fasting glucose (55). Therefore, randomized clinical trials will help clarifying the impact of calcium and dairy food substances on the metabolic syndrome risk factors and their potential in clinical practice.

Proposition 7: calcium supplementation improves the lipid-lipoprotein profile and decreases visceral adipose tissue accumulation

Lipid-lipoprotein profile

High plasma low-density lipoprotein cholesterol (LDL-C) and triglyceride concentrations and low-plasma high-density lipoprotein cholesterol (HDL-C) concentrations are

recognized as a major cause of cardiovascular diseases (61). For this reason, any variation in these concentrations (a decrease in LDL-C and triglycerides and an increase in HDL-C) is considered an improvement in the lipid-lipoprotein profile. Diet, and more precisely type of fat and alcohol consumption, is known to influence the lipid-lipoprotein profile (64–66). However, recent results from population studies have suggested that calcium/dairy consumption could also affect plasma cholesterol concentration and be associated with a more favourable lipid-lipoprotein profile (45,56–58).

Results from recent randomized controlled trials on calcium supplementation support the existence of a causal relationship between calcium/dairy consumption and improvement in the lipid-lipoprotein profile. Major *et al.* reported a significant decrease in total : HDL-C and LDL : HDL-C ratios after 15 weeks of daily supplementation with 1200 mg calcium and 400 UI vitamin D in women characterized by a low ($<800 \text{ mg day}^{-1}$) calcium intake (67). In this study, a tendency was also observed for an increase in HDL-C and for a decrease in triacylglycerol and total cholesterol. Groot *et al.* found that oral calcium carbonate supplementation significantly increased the serum apolipoprotein A-I (the main protein of HDL) and decreased the LDL-C in 50 children with familial hypercholesterolemia (type II-A) during consumption of a low-cholesterol high-polyunsaturated fat diet (68). In 13 men with moderate hypercholesterolemia, a 10-day high-calcium diet containing 2200 mg of calcium citrate malate-enriched food and 34% of energy from fat resulted into a 11% lower LDL-C, 7% lower apo lipoprotein-B (the main protein of LDL) and 6% lower total cholesterol concentration compared with a low-calcium diet (410 mg day^{-1}) (69). A 2-week calcium supplementation (0.9 g day^{-1}) administered through consumption of calcium-enriched chocolate induced a 15% decrease in LDL-C and did not affect HDL cholesterol concentration compared with the consumption of control chocolate (70). Participants in this study were 10 healthy men fed with 39% fat control diets. Bhattacharyya *et al.* studied the effect of a low- (250 mg day^{-1}) or a high- (2000 mg day^{-1} calcium supplement) calcium diet with a predominantly saturated or polyunsaturated fat content. After 2 weeks of the diet with high-saturated fat content, total cholesterol concentrations were significantly decreased by 5% in the high-calcium compared with the low-calcium diet group (71). In a study assessing the effects of calcium supplementation on fractures, Reid *et al.* supplemented a group of postmenopausal women with 1 g day^{-1} of calcium citrate (72). They found that after 12 months, the supplemented group had a greater increase in HDL-C as well as in the ratio HDL-C : LDL-C compared with the placebo group (72). Bostick *et al.* studied the impact of a 4-month supplementation with 1.0 and 2.0 g day^{-1} of elemental calcium in patients with

sporadic adenoma. They observed a non-significant decrease (2–4%) in total cholesterol and total cholesterol : HDL-C ratio (2–3%) (73). However, as this study was primarily designed to assess the impact of calcium on colon cell proliferation, blood samples were not taken during fasting state and no indication as regard to the change in the use of lipid-lowering medications in participants was mentioned, which are factors that might have attenuated the observed effect of calcium supplementation on plasma lipoprotein concentration (73,74).

Visceral adipose tissue

In a study on the effect of calcium/dairy on body weight and fat mass loss, Zemel *et al.* found no significant effect of a high-calcium (calcium carbonate) or high-dairy supplemented low-energy diets on HDL-C, LDL-C and triglyceride plasma concentrations compared with a low-calcium low-energy diet (37). However, in this study, a significant effect of the high-calcium and high-dairy low-energy diets was seen on the distribution of body fat loss. The participants on the low-calcium diet lost $5.3 \pm 2.3\%$ of abdominal fat (assessed with dual energy x-ray absorptiometry) compared with a loss of $12.9 \pm 2.2\%$ and $14.0 \pm 2.3\%$ in the high-calcium and high-dairy diet respectively (37). These significant differences between low- and high-calcium/dairy diets were also reflected in waist circumference changes. Abdominal and more precisely abdominal-visceral fat accumulation is a strong predictor of a deteriorated lipid-lipoprotein profile (75,76). Whether the effect of calcium/dairy consumption on lipid-lipoprotein concentration could be partly explained by a decrease in visceral fat is not known and is not supported by results from the last study (37). Moreover, the study by Major *et al.* showed a significant improvement in total : HDL-C and LDL : HDL-C ratios which was independent from change in fat mass and decrease in waist circumference (67) which is a strong indicator of visceral adipose tissue accumulation (77). Alternative explanations for the effect of calcium on lipid-lipoprotein concentrations could be related, as discussed in Propositions 10 and 11, to the capacity of calcium to increase fecal fat excretion, decrease intestinal fat absorption and postprandial plasma lipid concentrations (69,74,78) and to promote lipolysis and lipid oxidation (79–81).

Proposition 8: a low-calcium plasma concentration is paradoxically related to an increase in the calcium content of soft tissues

The adult human body contains roughly 1000–1500 g of calcium, of which 99% is located in bones (82). Intracellular calcium ($[\text{Ca}^{2+}]_i$) concentrations of any cell is extremely low ($\leq 10^{-7} \text{ M}$), whereas the concentration of

free Ca^{2+} in the extracellular fluid is high ($\sim 10^{-3}$ M) (83). The concentration of free cytosolic calcium and the maintenance of a steep Ca^{2+} gradient is critical in controlling cell membrane permeability, a large variety of enzymatic reactions, endocrine and exocrine hormones secretions, and in regulating cardiac and skeletal muscle contractions. The lower $[\text{Ca}^{2+}]_i$ concentration is maintained by the constant extrusion of calcium through calcium–magnesium ATPase and sodium–calcium exchange mechanisms (83,84).

Intracellular and extracellular calcium concentrations are tightly regulated by the parathyroid hormone (PTH), calcitriol (1,25-dihydroxyvitamin D) and calcitonin (85). A slight decrease in extracellular calcium concentrations stimulates PTH secretion, whose main effects are the synthesis of calcitriol and the reabsorption of calcium in the kidney and the mobilization of calcium from the bone. A small component of bone calcium (approximately 1%) is rapidly exchangeable with extracellular fluids, contributing to the rapidly responsive regulation of extracellular calcium concentration (84). PTH-induced stimulation of calcitriol synthesis in the kidney also leads to the production of calbindin which increases calcium absorption in the intestine. Calcitriol is also involved in the PTH mediated calcium resorption by bone. In contrast, calcitonin is involved in the inhibition of mobilization of calcium from the bone, in order to lower calcium concentration in plasma (85). In addition to their role in calcium mobilization, PTH and calcitriol stimulate by sodium–calcium exchange calcium transport from extracellular fluid into the intracellular compartment of soft tissues. In addition, hormones can activate calcium direct entry in the cell from extracellular sites by transmembrane diffusion or by the voltage-dependent slow- or agonist-dependent channels (85). The calcitriol-mediated stimulation of calcium influx is a rapid event which is not mediated by the classical nuclear vitamin D receptor, but is instead mediated by a rapidly acting non-genomic membrane receptor, the membrane-associated rapid response to steroid, or MARRS protein (86–89).

The increase in $[\text{Ca}^{2+}]_i$ in presence of a low-plasma calcium concentration is referred by some as the ‘calcium paradox’ and is at the basis of several pathologies associated with calcium deficiency and also with low-calcium intake (90). In this regard, the calcitriol-stimulated increase in $[\text{Ca}^{2+}]_i$ in primary culture of human adipocytes has been demonstrated by the Zemel *et al.* group and proposed as one of the main mechanism explaining the relationship between low-calcium intake and body fat accumulation (1). As explained in the next sentences, these authors have demonstrated that an increase in $[\text{Ca}^{2+}]_i$ promotes energy storage in human adipocytes by stimulating the expression and activity of fatty acid synthase and by inhibiting lipolysis (91,92). Ca^{2+} response sequence has been mapped to the fatty acid synthase promoter region (93,94), and increasing

adipocyte $[\text{Ca}^{2+}]_i$ via either voltage- or receptor-mediated Ca^{2+} channel activation was shown to result in stimulation of fatty acid synthase expression and activity (94,95). This increase in lipogenic gene expression is coordinated with suppression of lipolysis. Increasing Ca^{2+} influx dose dependently inhibits lipolysis in both rat (96) and human (97,98) adipocytes. This anti-lipolytic effect of $[\text{Ca}^{2+}]_i$ is due to direct activation of phosphodiesterase 3B, resulting in a decrease in cAMP and, consequently, reduced ability of agonists to stimulate phosphorylation and activation of hormone sensitive lipase (98). Thus, increases in $[\text{Ca}^{2+}]_i$ promote adipocyte triglyceride storage by exerting a coordinated control of lipogenesis and lipolysis, serving to stimulate the former and inhibit the latter.

Proposition 9: calcium intake promotes fat cell apoptosis

In addition to regulating adipocyte metabolism via the MARRS protein, calcitriol also acts via the ‘classical’ nuclear vitamin D receptor in adipocytes to inhibit the expression of uncoupling protein 2 (UCP2) (99). Consistent with this, suppression of calcitriol levels by feeding high-calcium diets to mice results in increased adipose tissue UCP2 expression and attenuates the decline in thermogenesis which otherwise occurs with energy restriction (100). These observations suggest that high-calcium diets may also affect energy partitioning by suppressing calcitriol-mediated inhibition of adipocyte UCP2 expression, as discussed in the next section.

Recent data demonstrate that calcitriol modulation of UCP2 also results in corresponding modulation of adipocyte apoptosis, possibly further contributing to the anti-obesity effect of dietary calcium (101). The apoptotic effect of dietary calcium appears to be mediated, in large part, via inhibition of UCP2 expression and a consequent increase in mitochondrial potential, a key regulator of apoptosis; an additional role is played by calcitriol regulation of cytosolic calcium and of calcium flux between endoplasmic reticulum and mitochondria [(101) and Zemel *et al.* (unpublished data)]. Consequently, adipocyte apoptosis is significantly impaired in association with increased calcitriol levels in mice fed low-calcium diets, while there is a marked increase in adipocyte apoptosis in mice fed high-calcium and/or high-dairy diets (101). Although this appears contrary to multiple published reports which indicate a pro-apoptotic effect of calcitriol in other tissues, this apparent discrepancy is the result of dosing differences. The literature which supports a pro-apoptotic effect of calcitriol utilizes supra-physiological concentrations (≥ 100 nM); similar pro-apoptotic responses (resulting from mitochondrial calcium overload) to such pharmacological doses of calcitriol have also been observed in human adipocytes. In contrast, physiological concentrations exerted a dose

responsive anti-apoptotic effect which was reversed by pharmacological concentrations of the hormone in human adipocytes (101). The physiological anti-apoptotic effect appears to primarily be due to suppression of UCP2 expression with minimal changes or reductions in mitochondrial calcium levels [(101) and Zemel *et al.* (unpublished data)].

Proposition 10: high-calcium intake increases UCP2 expression in mice and increase fat oxidation in low-calcium consumers

Together with its capacity to stimulate lipolysis, inhibit fatty acid synthase and *de novo* lipogenesis, it is suggested that calcium supplementation could modulate metabolic efficiency through an increase in UCP2 expression via suppression of $1\alpha,25\text{-(OH)}_2\text{-D}_3$ -induced calcium influx in mice adipocytes. In a first study in aP2-agouti transgenic mice made obese on a low-calcium high-sucrose diet, Shi *et al.* observed that administration of calcium or dairy-rich low-energy diets caused a significantly greater decrease in body weight compared with a low-calcium low-energy diet (100). Interestingly, this study also revealed a significant increase in core temperature in mice fed the calcium or dairy-rich low-energy diets, together with a 80% increase in white adipose tissue UCP2 expression (100). Further results from the same authors showed that treating human adipocytes with $1\alpha,25\text{-(OH)}_2\text{-D}_3$ exerts an inhibitory effect on adipocytes UCP2 expression (99). This suggests that calcium supplementation could have the opposite effect and could, in accordance with what was observed in mice, upregulate UCP2 expression through a decrease in $1\alpha,25\text{-(OH)}_2\text{-D}_3$ and $[\text{Ca}^{2+}]_i$. As UCP2 is implicated in thermogenesis (102,103), these results suggest, at least in mice, a role for calcium in increasing energy expenditure through a decrease in energy metabolism efficiency.

However, although this is an attractive hypothesis, the role of UCP2 in thermogenesis is not clear, and the observed thermogenic effect may be mediated by other, as of yet unidentified mechanisms. Moreover, thermogenic effects of dietary calcium and/or dairy food have not yet been demonstrated in humans. Indeed, intervention studies conducted in human have not replicated as clearly the animals and *in vitro* results as in the majority no effect of calcium supplementation on 24-h energy expenditure (80,104,105) and on diet-induced thermogenesis (106) was reported. In exception to these results, Gunter *et al.* observed that consumption of a low-calcium meal (<100 mg) induced a significantly greater increase in diet-induced thermogenesis in women formerly low-calcium consumers after 1 year on a diet containing 1000–1400 mg day⁻¹ calcium mainly from dairy food compared with those on a control diet with a daily calcium content <800 mg (107).

In addition to inducing a mitochondrial proton leak, UCP2 serves to mediate mitochondrial fatty acid transport and oxidation (108), suggesting that calcitriol suppression of UCP2 expression may contribute to decrease fat oxidation and increase lipid accumulation on low-calcium diets. In this regard, human studies support a role for calcium supplementation on energy balance via alteration in nutrient partitioning and increased fat oxidation. Melanson *et al.* analysed 24-h whole-room calorimeter data from 35 healthy normal-weight individuals and found that acute calcium intake (mg kcal⁻¹) was positively correlated with 24-h and sleeping fat oxidation (79). In this study, acute calcium intake explained 10% of the variance in 24-h fat oxidation between individuals. Another study by the same group compared the effect of two diets containing either three to four servings per day of dairy product (~1400 mg of calcium) or about one serving per day of dairy (~500 mg of calcium) (80). Diets were administered in a crossover design to 10 healthy individuals during a stay in a whole-room calorimeter, and subjects were tested under conditions of energy balance and acute energy deficit (–600 kcal 24 h⁻¹). Results showed that 24-h fat oxidation was significantly increased by 28% with the high-calcium compared with the low-calcium diet under energy deficit conditions (80). Cummings *et al.* compared the acute effect of three meals with different calcium source on lipid oxidation: a low-dairy (low calcium and low vitamin D), a high-calcium (calcium citrate supplement) and a high-dairy (high calcium and high vitamin D) meal. They observed in response to both the high-calcium and high-dairy meals a lesser suppression of fat oxidation rate and of non-esterified fatty acid level compared with the low-dairy meal, indicating an increase in fat oxidation (106). Concordant results were obtained by Gunther *et al.* who assessed the acute and chronic effect of a high-calcium intake on fat oxidation before and after a 1-year diet containing either 1000–1400 or less than 800 mg day⁻¹ of calcium (107), mainly from dairy food. Participants in this study were women low-calcium consumers (mean habitual calcium intake <800 mg day⁻¹). At baseline, increasing the calcium content of a meal with dairy food had no effect on postprandial fat oxidation. However, after 1 year, the low- and high-calcium meals induced a significantly greater increase in fat oxidation in the calcium supplemented group compared with the low-calcium control group (107). These results thus suggest that a long-term calcium supplementation in low-calcium consumers may enhance their ability to oxidize fat from a meal.

It is important to note that the ability of calcium to increase fat oxidation has not always been proven to be significant, and this may be related to the level of habitual calcium intake. Indeed, Jacobsen *et al.* observed no difference between 7-day isocaloric low- or high-calcium diets from mainly low-fat dairy food with different protein

content on fat oxidation in subjects tested under energy balance conditions (105). Neither did Boon *et al.* who showed that 7-day consumption of three types of diet (high-calcium/high-dairy, high-calcium/low-dairy, low-calcium/low-dairy) adjusted to maintain subjects in energy balance had no effect on 24-h energy expenditure, sleeping fat oxidation and respiratory quotient (104). However, these last two studies were conducted in individuals with an habitual diet containing 1214 or 1027 mg day⁻¹ of calcium, respectively (104,105), which is close to the recommended daily calcium intake for adults. Whether habitual calcium intake and energy balance states (see [86]) might modulate the effect of calcium supplementation on fat oxidation will need to be further investigated.

Proposition 11: high-dietary calcium intake increases fecal fat excretion and attenuates postprandial lipidemia

Among the different mechanisms that have been suggested to be responsible for the effect of a high-calcium intake on energy balance figures the capability of calcium to impair absorption of fat in the gut presumably via the formation of insoluble calcium fatty acid soaps or by binding of bile acids. This mechanism was proposed following animal and human studies that have demonstrated a dairy food- and a calcium-induced increase in fecal excretion of fat (69,70,105,109–114). Indeed, Papakonstantinou *et al.* observed in their study a substantial increase in fecal fat and energy excretion in rats fed a high-calcium diet based on dairy products (111). Jacobsen *et al.* (105) also observed in their short-term intervention study in human that increasing daily calcium intake increased fecal fat and energy excretion. Specifically, they observed that an ~1300 mg day⁻¹ increase in calcium intake, mainly from low-fat dairy products, increased daily fecal fat excretion by 8.2 g in subjects consuming a diet with 30% of energy derived from fat. This corresponds to an increase in energy loss of 312 kJ day⁻¹ or 113.9 MJ year⁻¹. As for calcium *per se*, Denke *et al.* observed that a 10-day diet (34% energy from fat) containing calcium citrate malate-enriched food for a total calcium intake of 2200 mg day⁻¹ induced a 6–13% increase in dietary saturated fat excretion compared with a diet containing 410 mg day⁻¹ of calcium (69). In another study, Welberg *et al.* observed that a 0, 2 and 4 g calcium carbonate supplement added to a diet significantly increased total fat excretion by 6.8%, to 7.4%, and 10.2% of total fat intake respectively (113). Finally, Shakhhalili *et al.* observed that a 2-week diet (34% of energy from fat) supplemented with 101 g day⁻¹ of calcium-enriched chocolate (for a total calcium intake of 0.9 g day⁻¹) resulted into a twofold increase in fecal fat excretion (4.4–8.4 g day⁻¹) compared with a 98 g day⁻¹ control dark chocolate (70). Moreover, the calcium enrichment reduced the absorbable

energy value of the chocolate by ~9%, decreasing the absorbable energy from 2170 to 2021 kJ 100 g⁻¹ of calcium-enriched chocolate (70).

Recently, the effect of calcium intake on postprandial fat metabolism has been examined by Lorenzen *et al.* (78) in an attempt to bring forward our understanding of the calcium-induced decrease in fat absorption consequences. They used a randomized crossover design where four isocaloric meals differing in amounts of calcium or in calcium source were tested in 18 subjects. The major finding was that a high-calcium intake (350 and 793 mg MJ⁻¹) from dairy products decreased postprandial lipidemia, calculated as the area under the curve for chylomicron triacylglycerol plasma concentration, significantly more than a low-calcium intake (68 mg MJ⁻¹) from dairy products or than calcium carbonate (850 mg MJ⁻¹) from supplement. The authors concluded that the decreased lipid response in chylomicron triacylglycerols observed in their study reflected a decrease in fat absorption rather than an increase in chylomicron clearance (which would also result in a decrease lipid response), as total cholesterol and HDL cholesterol concentrations were unaffected by the calcium supplementation (78). It is, however, not clear why in this study calcium from dairy products, but not free calcium from supplement, was found to inhibit fat absorption and decrease postprandial lipidemia, specifically as a decreased fat absorption from calcium supplement was reported in another study (70). Differences in pH, in chemical form of calcium or in the solubility of calcium from milk and calcium carbonate, or even the presence of other bioactive components in dairy products have been proposed to explain this (78). Nevertheless, the exact mechanisms beyond the difference in effects of calcium carbonate and calcium from dairy on postprandial lipidemia remain to be determined.

Although significant, the increase in fecal fat and in energy loss reported in the above-mentioned studies is modest and insufficient to explain the magnitude of the effects of calcium on energy balance and decreased body weight observed in clinical trials (as discussed in previous propositions). Above-mentioned studies reported an increase in fecal fat excretion of 8.2 g, 6–13%, 10.2% and 4% with a 1300 mg, 2200 mg, 4 g and 0.9 g day⁻¹ of calcium intake (69,70,105,113). In comparison, in order to achieve a clinically meaningful (albeit modest) contribution to weight loss, the pancreatic lipase inhibitor Orlistat must produce approximately a 30% inhibition of total dietary fat absorption (115). However, it was shown in obese mice that a high-calcium high-fat diet did exert a substantial greater anti-obesity effect compared with high-calcium low-fat diet, largely due to increased fecal energy loss in the mice on the high-fat diets (89). For this reason, the increase in fecal fat and in energy loss resulting from dairy/calcium intake should not be disregarded as it could help maintain

body weight and promote weight loss over an extended period of time.

Proposition 12: an inadequate calcium intake negatively influences appetite control

As briefly described in the eighth proposition of this manuscript, intracellular and plasma calcium concentrations as well as bone calcium content are under tight hormonal regulation. From another perspective, Tordoff described in a recent paper several arguments in favour of the existence of a sensory and behavioural regulation of body calcium which underlies the existence of a taste and an appetite for calcium (116). The calcium appetite, defined by Tordoff as the motivation to seek out or choose calcium-containing items (116), is a phenomenon that has been observed for other minerals in vertebrates. Several studies have shown that rats fed with a diet with a low content of a specific mineral developed a preference for substances with higher concentration in the minerals they had been deprived of. This is the case for calcium, sodium and magnesium (117–122). The existence of a phosphorus, iron, zinc and copper-specific appetite in response to their specific deficient state in rats has also been suggested (118,123–126). In a recent paper, Paradis and Cabanac showed that rats fed for 6 weeks with a low-calcium purified diet (calcium concentration <0.02%) did not differ from rats on a regular diet (calcium concentration = 0.97%) or from rats on a diet supplemented with chicken bones for BMI or fat mass (119). However, when the three groups of rats were offered four solutions of different CaCl₂ concentrations, the calcium-deprived group preferred and consumed significantly more of the high CaCl₂ solution. This preference disappeared in this group after 6 weeks on a normal diet (119).

Whether a calcium specific appetite exists in humans has never been directly assessed. The only indication for a behavioural control for calcium homeostasis in humans is anecdotal observations that have reported preference for calcium-rich edible substances (water with high millimolar quantities of calcium), or craving for non-edible substances with a high-calcium content (animal bones, powdered rock, ashes stalks, clay, chalk, plaster) under different calcium deficiency circumstances (116). However, it has been suggested that if an innate behavioural mechanism to respond to calcium deficiency exists in humans, it would be associated with the complex flavour profile that characterizes the most appreciable source of calcium (116). This not only makes even more complex the identification of a calcium appetite behaviour in human but also implies that in calcium deficient individuals such a behaviour could result in an increased energy intake despite being incidental to the need for calcium as observed in animals.

In order to better investigate this issue in the context of a double-blind placebo-controlled study, energy and macronutrient intakes during an *ad libitum* buffet-type meal was measured before and at the end of a 15-week calcium+vitamin D or placebo supplemented weight loss intervention in overweight and obese women very low-calcium consumers (39). It was found that among women characterized by the lowest initial calcium intake (≤ 600 mg day⁻¹), those who were allocated to the calcium+vitamin D supplement had a significant decrease in fat intake (g and percentage kcal), which was significantly different from the increased fat intake observed in very low-calcium consumers women allocated to the placebo (39). Another double-blind randomized placebo-controlled study in non-menopausal women showed that a 3-month calcium supplement (1200 mg day⁻¹) significantly reduced food cravings in those usually suffering from premenstrual symptoms such as cravings for sweets or salts (127). Based on these results, one could infer that the co-occurrence of fats, sugar and calcium in an abundance of food, especially dairy food, might orientate calcium deficient individuals towards a selection of high-fat food or cravings for sugar as a result of the learnt association between these nutrients, as suggested (116). As for salt craving, it has been proposed that one response to calcium deficiency is to consume sodium because this temporarily alleviates calcium deficiency based on data obtained in rats showing that sodium liberates calcium from plasma proteins (128,129).

These results therefore suggest an effect of calcium homeostasis on variables influenced by appetite control. However, it cannot be excluded that in the above-mentioned studies, low-calcium intakes were not isolated from other possible deficiencies in the diet, or that a calcium deficiency necessarily leads to overeating. Therefore, whether or not an increase in food intake as a result of low-calcium intake could be considered a potential mechanism that links calcium and dairy food intake to variations in energy balance and body composition remains to be determined. Furthermore, at present, there is no mechanism that can be proposed for the possibility that low-calcium intake increases hunger in addition to the specific appetite for calcium.

Proposition 13: milk is more than calcium regarding the impact on energy balance

The positive association between increased dairy product consumption and healthier body weights has been attributed to several milk components (130,131) and particularly calcium as discussed in this review. However, of the macronutrients protein is more satiating than fat and carbohydrate and is often found to suppress appetite and intake more than can be accounted for by its caloric content alone

(132,133). Dairy products and dairy proteins also suppress short-term food intake, increase subjective satiety and stimulate food intake regulatory mechanisms known to signal satiation and satiety (130–133). Thus, milk proteins may be the best explanation for the association between dairy consumption and healthier body weights.

Of the milk proteins, the physiological functionality of whey protein has been studied the most, probably because it is readily available as a by-product of cheese making (131). It is clear, however, that both casein and whey have the following physiological actions:

1. Reduce food intake.
2. Affect satiation and satiety by the actions of the protein *per se*, bioactive peptides and amino acids released during digestion, and the combined action of the milk proteins or (and) peptides or (and) amino acids with other milk constituents.
3. Stimulate well-established biomarkers of satiety including gastro-intestinal hormones, insulin and amino acids.
4. Are insulinotropic and peptides derived from them affect the renin-angiotensin system. Therefore, milk proteins have potential as a physiologically functional food component for persons with obesity and its comorbidities (hypertension, type II diabetes, hyperlipidemia).

Whey and casein are present in cow milk in proportions of approximately 20% and 80% respectively. Whey has been found in some studies to suppress food intake more than other proteins. In a comparison with soy protein and egg albumin, a 50 g preload of whey in flavoured water resulted in the strongest suppression of food intake at a pizza meal consumed 60 min later (134). Whey has also been reported to suppress food intake more than casein, but both proteins suppress food intake, and their comparative effects depend on the time of measurement of food intake after their consumption and whether or not they are consumed in the presence of other macronutrients. Moreover, some results suggest that the combination of whey and casein such as in complete milk protein (which contains 80% and 20% casein and whey respectively) might accentuate the effect on food intake suppression (135) (SE Moore and GH Anderson – unpublished data).

Among proteins, casein and whey have unique inherent properties that lead to the generation of many preabsorptive (e.g. gut hormones) and postabsorptive satiety signals that may account for the reduction in food intake and appetite after milk and dairy consumption (130,131,133). However, their physiologic and metabolic actions are different over time due to their rates of digestion and effects on satiety biomarkers. Based on their rates of digestion, casein and whey have been defined as slow and fast proteins respectively (136). An increase in plasma amino acid concentrations after protein consumption is a well-

described cause of satiety (130,132). Whey protein is digested quickly, resulting in peak plasma amino acid concentrations by 30–40 min that is sustained for more than 2 h. In contrast, casein is digested more slowly and results in a lower rise, but a more sustained increase in plasma amino acid concentrations (SE Moore and GH Anderson – unpublished data). Whey preloads also result in higher plasma concentrations of cholecystokinin, glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide compared with the casein. This suggests that the ‘slow’ casein protein would be expected to be more satiating than the ‘fast’ whey protein over a longer period of time while complete milk proteins would be expected to be intermediate to the two milk proteins, once again highlighting the fact that the food intake suppression induced by proteins is source-dependent (SE Moore and GH Anderson – unpublished data).

Milk proteins are insulinotropic, which may contribute to the association between dairy consumption and reduced prevalence of the metabolic syndrome through mechanisms other than their effect on satiety. Of the milk proteins, whey leads to higher pre-meal insulin concentrations than casein (137) and many other proteins and contains the predominant insulin secretagogue (138). Milk proteins are also high in leucine, valine and isoleucine, amino acids known to stimulate insulin secretion (138). When dairy products (with or without fat) are consumed with carbohydrates, the glycemic effect of the carbohydrate is markedly reduced (132,133). Furthermore, milk protein digestion releases small peptides that are absorbed and are angiotensin converting enzyme inhibitors (131,133) which may also be an explanation for the inverse associations found between blood pressure and consumption of dairy products (139). Thus, dairy products, because of their protein, in addition to their calcium content, may also have potential in the management of glycemic control and the metabolic syndrome.

In summary, in addition to supporting the maintenance of lean body mass under circumstances of energy restriction as discussed in the previous proposition, milk proteins and peptides are bioactive components whose effects on satiety and energy intake are relevant in the context of weight management. Thus, the epidemiologic associations found suggesting that increased dairy consumption contributes to healthier body weights are plausible. Nonetheless, the favourable effects of dairy components on food intake, subjective satiety and intake regulatory mechanisms have been usually observed in short-term experiments where the components were consumed in amounts higher than that found in usual serving sizes of dairy products. Therefore, it remains unclear if usual consumption of dairy products has any direct effect on satiety beyond the energy that they contain. However, the current data point towards many opportunities to consider further the exploration of roles of

dairy products and their functional components in dietary plans and in functional foods aimed at controlling appetite and metabolism.

Conclusion

The happening of a symposium that was planned to document in a comprehensive manner the role of calcium and dairy food on variations in energy balance and body composition led to the redaction of this manuscript which regroups all hypotheses related to this issue into 13 propositions. Taken as a whole, this paper suggests that calcium metabolism and intake influence many components of energy and fat balance. In some studies, the effects of dairy products on energy/fat balance are more pronounced than those of calcium supplementation alone, suggesting that other nutrients, e.g. milk peptides and proteins, may also be involved.

On the basis of the studies reported throughout the propositions, it goes without saying that calcium and dairy food are far from simply being magic ingredients that can induce a negative energy balance. The integration of hypotheses and mechanisms rather suggest that calcium and dairy food intake, while being associated with healthier food choice potentially substituting for other calorie and fat-rich food more susceptible to induce a positive energy balance, have the potential to increase fat oxidation, decrease fat absorption, promote fat cells apoptosis and increase satiety and decrease food intake thus favouring a healthier metabolic profile, a stable or negative energy balance and ultimately a decrease or maintenance of fat mass over time. This is supported by results from clinical trials having shown that in condition of energy restriction, a calcium+vitamin D or dairy food supplementation is essential to achieve or accentuate fat mass loss in individuals with habitual low-calcium intake.

Certainly, many questions and confounding factors have not been directly addressed and underlying mechanisms are still missing to properly understand the complex influence of calcium and dairy food on energy balance. Nevertheless, actual evidence supports an influence of calcium and dairy food on energy balance and point towards the recognition that adequate calcium and dairy food intake is an important determinant in the relationship with energy balance and other health problems.

Conflict of Interest Statement

No conflict of interest was declared.

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