

High-calcium diet with whey protein attenuates body-weight gain in high-fat-fed C57Bl/6J mice

Taru K. Pilvi^{1,2}, Riitta Korpela^{1,2}, Minna Huttunen³, Heikki Vapaatalo¹ and Eero M. Mervaala^{1,4*}

¹Institute of Biomedicine, Pharmacology, Biomedicum Helsinki, PO Box 63, FIN-00014 University of Helsinki, Finland

²Foundation for Nutrition Research, PO Box 30, FIN-00390 Helsinki, Finland

³Division of Nutrition, Department of Applied Chemistry and Microbiology, PO Box 66, FIN-00014 University of Helsinki, Finland

⁴Department of Pharmacology and Toxicology, PO Box 1627, University of Kuopio, 70211 Kuopio, Finland

(Received 16 December 2006 – Revised 20 April 2007 – Accepted 24 April 2007)

An inverse relationship between Ca intake and BMI has been found in several studies. It has been suggested that Ca affects adipocyte metabolism via suppressing 1,25-dihydroxycholecalciferol (1,25(OH)₂-D₃) and decreases fat absorption. We studied the effect of Ca and milk proteins (whey and casein) on body weight in C57Bl/6J mice. Male mice, age 9 weeks, were divided into three groups (ten mice per group) receiving modified high-fat (60% of energy) diets. Two groups received a high-Ca diet (1.8% calcium carbonate (CaCO₃)), with casein or whey protein (18% of energy), and one group received a low-Ca diet (0.4% CaCO₃) with casein for 21 weeks. Food intake was measured daily and body weight twice per week. Body fat content (by dual-energy X-ray absorptiometry) of all mice and faecal Ca and fat excretion of seven mice/group were measured twice during the study. Final body weight (44.1 (SEM 1.1) g) and body fat content (41.6 (SEM 0.6) %) were significantly lower ($P < 0.05$) in the high-Ca whey group than in the low-Ca casein group (48.1 (SEM 0.8) g and 44.9 (SEM 0.8) %). Body weight and body fat content of the high-Ca casein group did not differ significantly from the low-Ca casein group even though serum 1,25(OH)₂-D₃ levels were significantly lower ($P < 0.001$) in both high-Ca groups than in the low-Ca casein group. Thus changes in serum 1,25(OH)₂-D₃ do not seem to affect body weight in this animal model. There was a significant difference in fat excretion between the high-Ca whey and low-Ca casein groups (3.9 (SEM 0.9) % in the high-Ca whey v. 1.4 (SEM 0.2) % in the low-Ca casein group; $P < 0.05$), which may partly explain the effect on body weight.

Calcium: Obesity: Milk proteins: Body weight

Obesity is associated with several co-morbidities such as cardiovascular complications, type 2 diabetes and certain types of cancers and it is considered to be the main factor leading to the development of the metabolic syndrome¹. The prevalence of obesity is increasing dramatically both in Western countries and in the developing world². Since nutrition is one of the most important factors affecting the development of obesity, nutritional approaches to prevent and control this growing epidemic are crucial.

The inverse relationship between dietary Ca intake and BMI was first observed in the late 1980s^{3,4} and since then the association has been further confirmed in several epidemiological studies^{5–8}. In addition to the epidemiological findings, the inverse relationship between dietary Ca intake and body weight is supported by data from both human and animal studies^{5,9–12}. However, the mechanisms by which Ca affects body weight are still not fully understood, and studies clarifying the mechanisms are continuously demanded in the literature^{13,14}.

One possible factor explaining the inverse relationship between Ca intake and BMI is the fatty acid-binding capacity of Ca^{15,16}. Some studies have found it to be a significant factor

explaining the anti-obesity effect of dietary Ca^{17,18}, while other researchers argue that the effect that Ca has on fat absorption is too small to explain the influence Ca has on body weight¹⁹.

The alternative hypothesis explaining the anti-obesity effect of Ca links dietary Ca intake, serum 1,25-dihydroxyvitamin D₃ (1,25-dihydroxycholecalciferol; 1,25(OH)₂-D₃) concentration and adipocyte intracellular Ca concentration. This theory is based on the finding that adipocyte intracellular Ca is a regulator of both lipogenesis and lipolysis^{20–22}. The hypothesis is further supported by *in vitro* data which show that one of the factors regulating the intracellular Ca concentration of adipocytes is 1,25(OH)₂-D₃, which is affected by changes in Ca intake. Low Ca intake increases 1,25(OH)₂-D₃ concentration, whereas high Ca intake suppresses 1,25(OH)₂-D₃ levels. 1,25(OH)₂-D₃ has been found to increase the influx of Ca into the adipocytes and thereby increase the adipocyte intracellular Ca concentration which in turn stimulates lipogenesis by increasing the activity and mRNA expression of fatty acid synthase (FAS) and inhibits lipolysis²³. These actions altogether lead to increased fat accumulation in a situation of low Ca intake, when serum

Abbreviations: FAS, fatty acid synthase; 1,25(OH)₂-D₃, 1,25-dihydroxycholecalciferol; PTH, parathyroid hormone.

* **Corresponding author:** Professor Eero Mervaala, fax +358 9 191 25364, email eero.mervaala@helsinki.fi

1,25(OH)₂-D₃ concentration is high. In addition to that, 1,25(OH)₂-D₃ has been shown to inhibit uncoupling protein 2 expression in human adipocytes via the nuclear vitamin D receptor and thereby affect the regulation of thermogenesis and energy metabolism²⁴. Furthermore, 1,25(OH)₂-D₃ is found to be a regulator of adipocyte apoptosis, which may contribute to the effects of a high-Ca diet²⁵.

Experimental studies investigating the mechanisms of dietary Ca and body-weight control have mainly been performed using the aP2-agouti transgenic mouse. This particular mouse expresses the *agouti* gene in adipose tissue under the control of the aP2 promoter. aP2-agouti transgenic mice are not obese when fed a standard laboratory diet, but are susceptible to diet-induced obesity and develop marked adipose tissue accumulation on a high-fat–high-sucrose diet. aP2-agouti transgenic mice respond dramatically to changes in Ca content in the diet and the effectiveness of high-Ca diets in reducing adiposity has been shown repeatedly in this model^{5,9,10,26}. However, these results have not been confirmed in other obese animal models or human subjects, and some human and animal studies have failed to show an inverse relationship between Ca intake and body weight^{27–30}.

In most of the studies reporting the anti-obesity effects of dietary Ca, the effects of dairy products have been comparable or superior to the effects of Ca alone^{5,9–11}. These results point out that there are factors in milk that either potentiate the effects of Ca or have independent health effects. So far the specific components or molecules of dairy products responsible for these effects are not known, but the protein fraction has been suggested to be the source of these bioactive substances^{31–33}.

The major protein classes of milk are whey (20%) and casein (80%) proteins³⁴. The whey protein fraction, especially, has been suggested to contain bioactive substances responsible for the pronounced anti-obesity effect of dairy products, but this has not been demonstrated so far. In the present study we compared the effect of two high-Ca diets with different protein sources (whey and casein) in inhibiting weight and fat-tissue gain in C57Bl/6J mice during a high-fat diet. The C57Bl/6J mouse is a well-established model of

diet-induced obesity^{35–37}. This inbred mouse strain develops obesity and insulin resistance when fed a high-fat diet and thus serves as a very human-like experimental model of obesity research.

Methods

Animals and diets

Male C57Bl/6J mice, age 8 weeks, were purchased from Harlan (Horst, The Netherlands). The mice were housed five in a cage in a standard experimental animal laboratory, illuminated from 06.30 to 18.30 hours, temperature 22 ± 1°C. The protocols were approved by the Animal Experimentation Committee of the University of Helsinki (Finland). The mice had free access to feed and tap water during the experiment. After a 1-week acclimatisation period on a low-fat diet (D12450B; protein 20%, carbohydrate 70% and fat 10% of energy; Research Diets Inc., New Brunswick, NJ, USA) the body weight-matched mice (25.6 ± 0.1 g) were divided into three groups receiving modified high-fat diets (60% of energy from fat) for 21 weeks. The mice were divided into two high-Ca groups (1.8% calcium carbonate (CaCO₃); whey protein group and casein group) and a low-Ca casein group (0.4% CaCO₃) (Table 1). In the beginning of the study the body weights were 25.6 (SEM 0.7), 25.6 (SEM 0.6) and 25.5 (SEM 0.4) g in the high-Ca whey, high-Ca casein and low-Ca casein groups, respectively. All the protein (18% of energy) in the diet of the high-Ca whey group was of whey protein isolate (Alacen™ 895; NZMP, Auckland, New Zealand) and all the protein (18% of energy) in the casein groups was of casein isolate (Alacid 714; New Zealand Milk Products Inc., Santa Rosa, CA, USA). Research Diets Inc. manufactured the diets. The powdered diets were moistened with tap water (200 ml/kg in the high-Ca whey, 100 ml/kg in the high-Ca casein and 110 ml/kg in the low-Ca casein diet) using an industrial dough mixer, packed in 1 d portions and stored at –20°C.

Body weight was monitored twice per week and the consumption of feed was monitored daily using a standard table

Table 1. Diet composition*

Group and product code...	High-Ca whey (D05031104M)	High-Ca casein (D05031102M)	Low-Ca casein (D05031101M)
Casein (Alacid 714) (g)	0	200	200
Whey protein isolate (Alacen™ 895) (g)	186	0	0
Maltodextrin 10 (g)	125	125	125
Sucrose (g)	66.8	68.8	68.8
Cellulose (BW200) (g)	50	50	50
Soyabean oil (g)	25	25	25
Lard (g)	245	245	245
CaCO ₃ (g)	34.3	35.6	7.6
Potassium phosphate (monobasic) (g)	19.4	13	13
Potassium citrate, 1 H ₂ O (g)	0	6.2	6.2
Energy (kJ/g dry weight)	21.4	21.0	21.9
Protein (% energy)	18.0	18.1	18.1
Carbohydrate (% energy)	20.5	20.6	20.6
Fat (% energy)	61.5	61.3	61.3
Ca (%)	1.80	1.80	0.40
P (%)	0.59	0.58	0.60
K (%)	0.79	0.75	0.78

* Diets were formulated and produced by Research Diets Inc. (New Brunswick, NJ, USA).

scale (Ohaus Scout™ Pro SP4001; Ohaus Scout, Nänikon, Switzerland). Mean and cumulative energy intake was calculated from the food intake data. Body fat content was analysed by dual-energy X-ray absorptiometry (Lunar PIXImus; GE Healthcare, Chalfont St Giles, Bucks, UK) on weeks 14 and 20 of the treatment period.

Faecal fat and calcium excretion

For the collection of faeces, the mice (seven per group) were housed individually in metabolism cages for 72 h during weeks 3 and 16 of the treatment period. The intake of feed and drink was monitored daily. All faeces excreted during the 72 h period were collected at the end of the 72 h period. The faeces were weighed and stored at -70°C until assayed. The fat content of the faecal samples was determined by the Schmid–Bondzynski–Ratzlaff method³⁸ and Ca content was determined using inductively coupled plasma MS (Elan 6100; Perkin Elmer, Boston, MA, USA). Apparent fat absorption was calculated from the amount of feed consumed and the amount of fat excreted during the housing in metabolism cages. Apparent fat absorption (%) was determined as $100 \times ((\text{fat intake} - \text{faecal fat})/(\text{fat intake}))$. To estimate how much the increased fat excretion actually decreased the amount of absorbed energy from fat during the whole study period, we calculated ‘the apparent cumulative energy absorption from fat’. For this we used the cumulative energy intake data and apparent fat absorption percentage (apparent fat absorption % \times cumulative energy intake from fat) as described previously¹⁷.

Blood glucose and serum lipids

Blood glucose and serum lipids were analysed from the blood samples taken at the termination of the animals. Blood glucose was determined by glucometer (Super Glucocard™ II, GT-1630; Arkray Factory Inc., Shiga, Japan). Serum lipids were analysed by an accredited laboratory (HUSLAB, Helsinki University Central Hospital, Helsinki, Finland) using a Hitachi 912 Automatic Analyser (Hitachi Ltd, Tokyo, Japan).

Core temperature

Core temperature was used as an indirect indicator of body thermogenesis. Temperature was measured using a thermocouple (BAT-12; Physitemp, Clifton, NJ, USA), immediately before the termination of the animals. The probe was inserted into the rectum of each mouse at a constant distance (1.5 cm) and the temperature was recorded after stabilisation. All the measurements were performed between 08.00 and 11.00 hours.

Sample preparation

At the end of the treatment period the mice were rendered unconscious with $\text{CO}_2\text{-O}_2$ (95:5, v/v) (AGA, Riihimäki, Finland) and decapitated. Blood samples were taken in plastic tubes, and serum was separated by centrifugation at $+4^{\circ}\text{C}$ for 15 min.

1,25-Dihydroxycholecalciferol and parathyroid hormone

Serum 1,25(OH)₂-D₃ was determined by a RIA-kit (IDS Ltd, Boldon, Tyne & Wear, UK) and serum intact parathyroid

hormone (PTH) was determined by ELISA (Immutopics Inc., San Clemente, CA, USA) according to the instructions of the manufacturer.

Statistical analysis

Data are presented as mean values with their standard errors. Statistically significant difference in mean values were tested by ANOVA followed by Tukey’s test. ANOVA for repeated measurements was applied for data consisting of repeated observations at successive time points. The difference was considered significant when $P < 0.05$. The data were analysed using GraphPad Prism (version 4.02; GraphPad Software, Inc., San Diego, CA, USA) and SPSS (version 10.1; SPSS Inc., Chicago, IL, USA).

Results

Body-weight gain

Body-weight gain in the high-Ca casein group did not differ from the low-Ca casein group ($P > 0.05$) (Fig. 1(a)). However, the mice in the high-Ca whey group gained less body weight than the mice in the high- or low-Ca casein groups. The dips in the body-weight curves at weeks 3, 14 and 20 represent a normal response to housing in metabolism cages and dual-energy X-ray absorptiometry measurements, which were performed at those time points. The final body weight in the high-Ca whey group (44.1 (SEM 1.1) g) was 7.9% lower than in the high-Ca casein (47.9 (SEM 1.0) g) and 8.3% lower than in the low-Ca casein group (48.1 (SEM 0.8) g) (Fig. 1(b)).

Body fat content

The body fat content of the mice increased significantly during the high-fat diet. The whey protein and Ca significantly attenuated the increase in body fat content ($P < 0.05$) when compared with the high-Ca casein and low-Ca casein groups (Fig. 2). There was no difference in the body fat gained during the treatment period between the two casein groups.

Food and energy intake

Mean daily food intake was greatest in the high-Ca whey group (3.3 (SEM 0.05) g/mouse per d) in comparison with the high-Ca casein (3.0 (SEM 0.12) g/mouse per d; $P > 0.05$) and the low-Ca casein group (2.7 (SEM 0.002) g/mouse per d; $P < 0.05$). Cumulative food intake was also greatest in the high-Ca whey group (Fig. 3). However, there were no differences in mean daily energy intakes (56.3 (SEM 0.8) kJ/mouse per d in the high-Ca whey, 55.9 (SEM 2.1) kJ/mouse per d in the high-Ca casein and 52.5 (SEM 0.04) kJ/mouse per d in the low-Ca casein group) or in the cumulative energy intake between the groups.

Faecal fat and calcium excretion

Faecal fat content was significantly greater in the high-Ca whey group when compared with both casein groups ($P < 0.05$), but there was no statistical difference in the

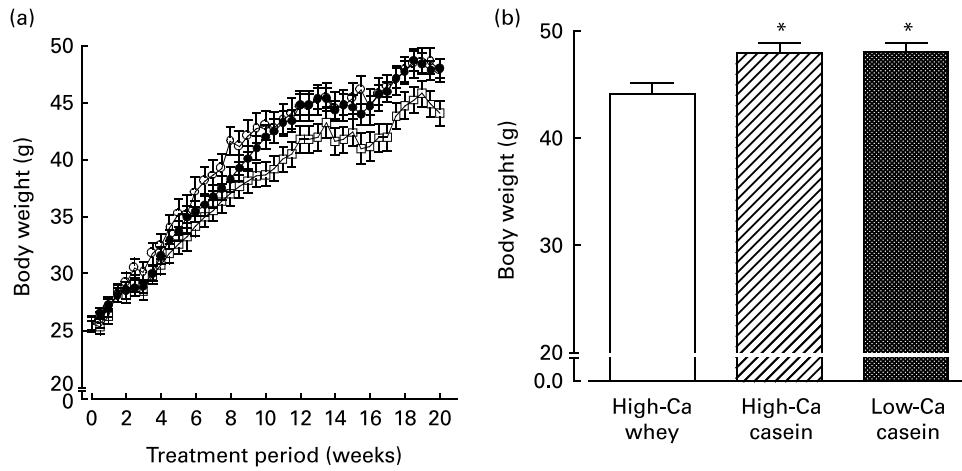


Fig. 1. (a) Body-weight gain of C57Bl/6J mice fed a high-fat diet with high-Ca whey (□), high-Ca casein (○) and low-Ca casein (●). (b) Final body weight after 21 weeks feeding of a high-fat diet with high-Ca whey, high-Ca casein and low-Ca casein. Values are means for ten mice per group, with their standard error of the means represented by vertical bars. *Mean value was significantly different from that of the high-Ca whey group ($P < 0.05$).

faecal fat content between the casein groups (Fig. 4(b)). Fat excretion did not change significantly between the first and second collection (data not shown). There was no statistical difference between the high-Ca or casein groups in apparent fat absorption (%), but apparent fat absorption in the high-Ca whey group was significantly smaller than in the low-Ca casein group ($P < 0.05$) (Fig. 4(c)). However, the apparent cumulative energy absorption from fat did not differ between the groups (4916.4 (SEM 84.9) kJ/mouse per 21 weeks in the high-Ca whey, 4891.2 (SEM 202.5) kJ/mouse per 21 weeks in the high-Ca casein and 4602.9 (SEM 8.4) kJ/mouse per 21 weeks in the low-Ca casein group; ANOVA; $P = 0.295$). There was a significant difference in Ca excretion between the low-Ca casein group and high-Ca groups (whey and casein) in line with the Ca content of the diets (Table 2).

1,25-Dihydroxycholecalciferol and parathyroid hormone

Serum concentrations of 1,25(OH)₂-D₃ and intact PTH were significantly lower in the high-Ca (whey and casein) groups than in the low-Ca casein group (Fig. 5). There were no significant differences in either 1,25(OH)₂-D₃ or intact PTH concentrations between the high-Ca groups.

Core temperature

There were no statistically significant differences in the core temperature between the groups (Table 2).

Blood glucose and serum lipids

There was no statistically significant difference in blood glucose levels between the groups, although blood glucose level was higher in the low-Ca casein group than in the Ca-treated groups (Table 2). Serum HDL-cholesterol concentration was significantly lower in the high-Ca casein group than in the high-Ca whey and low-Ca casein groups ($P < 0.05$) and so was the concentration of total cholesterol. There were no differences in serum LDL-cholesterol or TAG concentrations between the groups.

Discussion

In the present study we have shown for the first time that a high-Ca diet containing whey protein attenuates body-weight and adipose-tissue gain in a model of diet-induced obesity. In accordance with the literature, the mice gained significantly

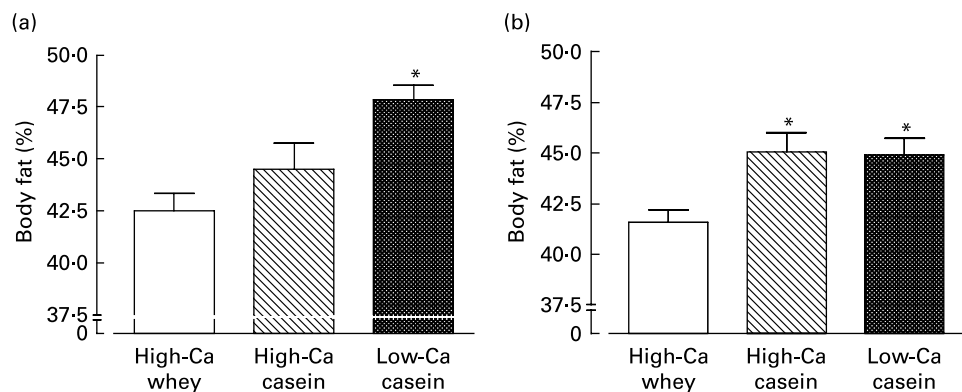


Fig. 2. (a) Body fat content of high-fat-fed C57Bl/6J mice after 14 weeks of treatment in high-Ca whey, high-Ca casein and low-Ca casein groups. (b) Body fat content of high-fat-fed C57Bl/6J mice after 20 weeks of treatment. Values are means for ten mice per group, with their standard error of the means represented by vertical bars. *Mean value was significantly different from that of the high-Ca whey group ($P < 0.05$).

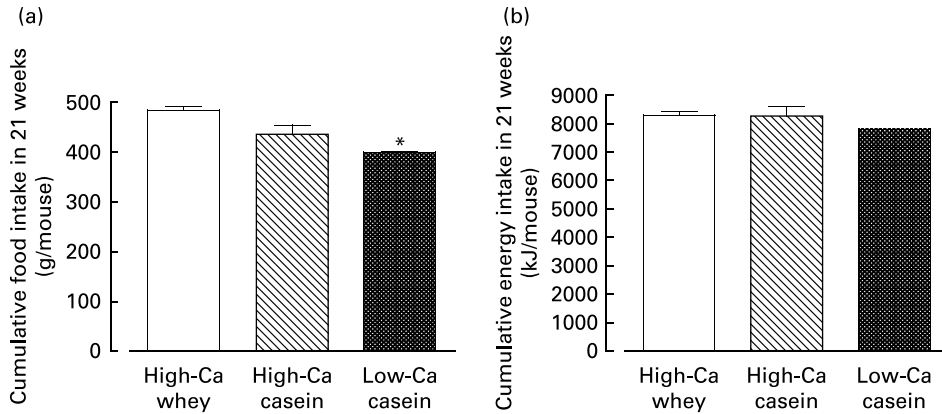


Fig. 3. (a) Cumulative food intake during the study for the high-Ca whey, high-Ca casein and low-Ca casein groups. (b) Cumulative energy intake during the study. Values are means for ten mice per group, with their standard error of the means represented by vertical bars. *Mean value was significantly different from that of the high-Ca whey group ($P < 0.05$).

more weight than what is normally seen in the same mice fed a low-fat diet^{35,39}. In contrast with previous experimental studies investigating the effect of Ca supplementation on body-weight gain^{5,17}, we found no benefit from CaCO₃ supplementation in combination with casein protein in attenuating weight and fat-tissue gain.

One possible explanation for reduced fat tissue content in the high-Ca whey group could be energy intake. However, neither the cumulative nor mean daily food intake was smaller in the high-Ca whey group than in the low-Ca casein group. If anything, the high-Ca whey group seemed to have greater food intake than the other groups during the study. Although it has previously been shown that whey protein load acutely increases the plasma levels of cholecystokinin and glucagon-like peptide-1 and thereby increases satiety in comparison with casein⁴⁰, cumulative energy intake was not affected by the whey protein diet in the present study. This is in accordance with recent clinical data on the effect of whey protein on control of energy intake⁴¹.

The formation of insoluble fat–Ca salts has been claimed to explain some of the anti-obesity effects of Ca. In the present study, Ca supplementation indeed increased the excretion of fat and the effect was pronounced in the high-Ca whey group. The difference between the high-Ca groups in fat

excretion could be explained by the fact that the phosphate groups in casein are known to also bind Ca⁴². Hence there could be less free Ca to bind fatty acids in the casein diet. Since there was more added phosphate in the whey protein diet to balance the P levels to the same level as in the casein-based diets, it can also be argued that the supplemental phosphate in the whey diet could be responsible for more amorphous calcium phosphate complexes and thus more fat excretion. However, when the apparent fat absorption percentage was used to estimate the cumulative amount of fat absorbed during the 21-week study period, there was no difference in the amount of absorbed fat between the treatment groups. Although the cumulative amount of absorbed fat is based on calculations, it indicates that the increased fat excretion does not seem to fully explain the differences in body weight in the present study.

Previous studies on the anti-obesity effect of Ca, both in aP2-agouti transgenic mice and adipocyte cultures, have supported the theoretical framework where 1,25(OH)₂-D₃ plays a central role in the regulation of adipose tissue metabolism^{5,9,10,23,24}. However, in the present study we were not able to see the effect on the level of fat tissue accumulation in the high-Ca casein group in spite of a significant reduction in the serum concentration of 1,25(OH)₂-D₃. Even though

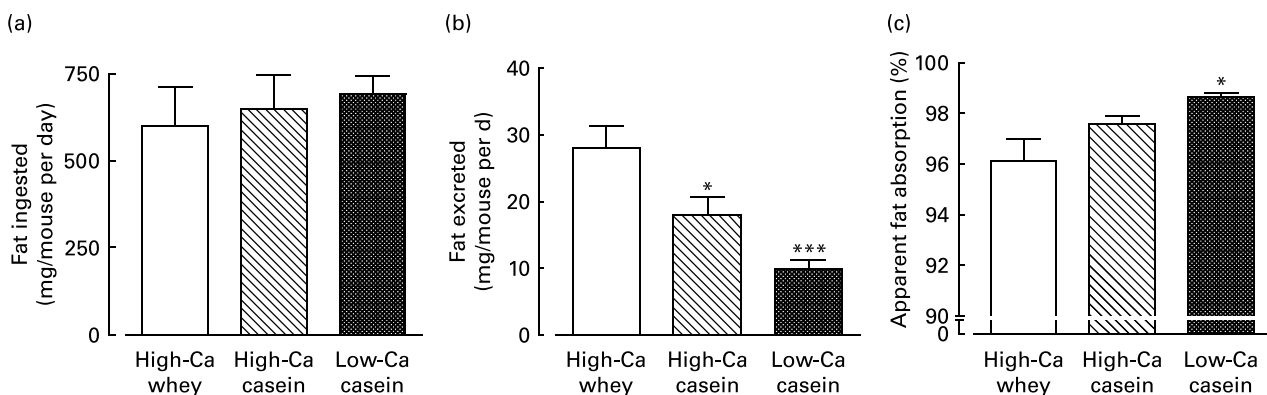


Fig. 4. (a) Fat ingested during the housing in metabolism cages on week 20 of the study for the high-Ca whey, high-Ca casein and low-Ca casein groups. (b) Faecal fat excretion. (c) Calculated fat absorption percentage. Values are means for seven mice per group, with their standard error of the means represented by vertical bars. Mean value was significantly different from that of the high-Ca whey group: * $P < 0.05$, *** $P < 0.001$.

Table 2. Faecal calcium excretion, blood glucose, serum lipids and core temperature at the end of the study (Mean values with their standard error of the means; ten mice per group)

Group...	High-Ca whey		High-Ca casein		Low-Ca casein		P (ANOVA)
	Mean	SEM	Mean	SEM	Mean	SEM	
Ca excretion in 24 h (mg/mouse)	18.8*	3.1	26.0**	4.6	6.6	0.3	0.002
Blood glucose (mmol/l)	8.8	0.6	8.8	0.4	9.6	0.4	0.420
Serum total cholesterol (mmol/l)	6.4†	0.4	7.4	0.2	6.2†	0.3	0.017
Serum HDL-cholesterol (mmol/l)	5.4†	0.4	6.5	0.2	5.3†	0.3	0.012
Serum LDL-cholesterol (mmol/l)	0.47	0.04	0.41	0.08	0.46	0.05	0.708
Serum TAG (mmol/l)	1.1	0.1	1.1	0.04	1.0	0.1	0.968
Core temperature (°C)	36.9	0.2	37.0	0.1	37.5	0.2	0.046

Mean value was significantly different from that of the low-Ca casein group: * $P < 0.05$, ** $P < 0.01$.

† Mean value was significantly different from that of the high-Ca casein group ($P < 0.05$).

there is accumulating evidence supporting the role of $1,25(\text{OH})_2\text{-D}_3$ in adipose tissue metabolism, its significance in obesity is still not clear. For instance, in a study on 302 healthy volunteers, Parikh *et al.* found significantly lower plasma $1,25(\text{OH})_2\text{-D}_3$ -levels in obese than in non-obese subjects⁴³, which is in contrast with the theory that decreasing the amount of $1,25(\text{OH})_2\text{-D}_3$ by dietary Ca would lead to a decreased accumulation of fat. In addition to $1,25(\text{OH})_2\text{-D}_3$, PTH has also been shown to regulate adipocyte intracellular Ca^{5,44} and it has been suggested to be a potential mediator of the anti-obesity effect of dietary Ca^{28,45}. To support the role of PTH in obesity, a positive correlation between serum intact PTH and both BMI and fat mass has been reported in several studies^{43,46,47}. In the present study, however, the changes in PTH levels did not reflect the differences in body weight or fat mass.

According to the current hypothesis explaining the anti-obesity effect of Ca, $1,25(\text{OH})_2\text{-D}_3$ regulates FAS, the key enzyme of lipogenesis via adipocyte intracellular Ca. It has been suggested that *de novo* lipogenesis contributes to the development of obesity, particularly during a high-carbohydrate diet⁴⁸. This might explain the controversy between the present results and earlier studies investigating the anti-obesity effect of Ca. The diets used by Zemel *et al.* have been rich in carbohydrate (sucrose, 64% of energy) and fat, whereas the main energy source in the present study was fat (lard and soyabean oil, 60% of energy)^{5,9,10}. However, at

least rat adipose tissue FAS mRNA expression has been shown to be unaffected by changes in the fat and carbohydrate content of the diet⁴⁹. In addition, the significance of adipose tissue *de novo* lipogenesis in obesity is still controversial and the contribution of adipose tissue FAS activity to obesity is not clear^{50,51}.

Various health effects of whey protein have been described in the literature, but studies investigating the possible mechanisms are scarce. Previously, whey has been reported to inhibit body-weight gain in Wistar rats in comparison with red meat⁵². On the other hand, whey protein isolate and its hydrolysate were proven to be less effective than soya protein in a weight-reduction study in genetically obese yellow KK mice⁵³. However, in both of these studies whey was used as a part of a high-protein diet, in contrast to the present study, in which the protein content of the diet was in the normal range.

One possible factor contributing to the effect of whey protein is the amino acid content of whey. Whey contains substantial amounts of branched-chain amino acids; leucine, isoleucine and valine. Leucine, especially, is known to have various metabolic functions including the initiation of muscle protein synthesis⁵⁴ and modulating insulin-PI3 kinase signalling⁵⁵. This might direct the consumption of energy to muscle protein synthesis instead of energy storage in adipose tissue. Long-term leucine supplementation has indeed been shown to increase body-fat loss during food

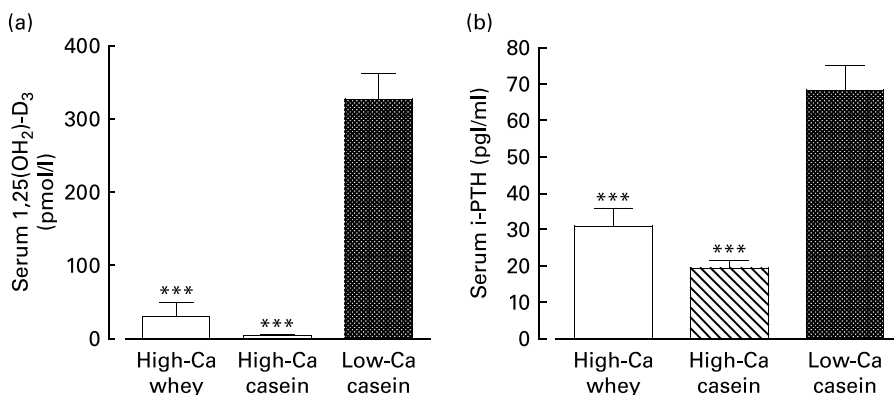


Fig. 5. (a) Serum $1,25$ -dihydroxycholecalciferol ($1,25(\text{OH})_2\text{-D}_3$) concentration at the end of the study for the high-Ca whey, high-Ca casein and low-Ca casein groups. (b) Serum intact parathyroid hormone (i-PTH) concentration at the end of the study. Values are means for ten mice per group, with their standard errors represented by vertical bars. ***Mean value was significantly different from that of the low-Ca casein group ($P < 0.001$).

restriction in Wistar rats⁵⁶, but it is not known whether leucine supplementation is able to inhibit weight gain during a high-fat diet by promoting muscle protein synthesis.

A whey protein-rich diet has also been shown to have an effect on liver and muscle lipogenic enzyme activities. Morifuji *et al.* have shown in non-obese Sprague–Dawley rats that a whey protein diet decreases the activity of various hepatic lipogenic enzymes including glucose-6-phosphate dehydrogenase, acetyl-CoA carboxylase and FAS and increases glucokinase activity in comparison with casein^{57,58}. Additionally, the skeletal muscle FAS and glycogen synthase activity were significantly increased and 6-phosphofructokinase decreased in their studies. This shows that there are factors in whey which may modulate the activity of different key enzymes of energy metabolism and thereby affect energy homeostasis in the body.

In conclusion, we found that a high-Ca diet with whey protein isolate inhibits body-weight gain during a high-fat diet in C57Bl/6J mice. These results indicate that whey protein contributes to the previously described anti-obesity effect of dairy products. The mechanisms of weight-gain attenuation and possible functional compounds in whey protein remain to be studied further.

Acknowledgements

The present study was supported by the Foundation for Nutrition Research, Academy of Finland, Sigrid Juselius Foundation and Valio Ltd, Helsinki, Finland. We are grateful to Ms Sari Laakkonen, Mrs Anneli von Behr, Mr Berndt Köhler, Ms Sonja Latvakoski and Ms Anne-Maria Riihimäki for expert technical assistance.

References

- Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH, American Heart Association & Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism (2006) Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* **113**, 898–918.
- York DA, Rossner S, Caterson I, Chen CM, James WP, Kumanyika S, Martorell R, Vorster HH & American Heart Association (2004) Prevention Conference VII: Obesity, a worldwide epidemic related to heart disease and stroke: Group I: worldwide demographics of obesity. *Circulation* **110**, e463–e470.
- McCarron DA, Morris CD, Henry HJ & Stanton JL (1984) Blood pressure and nutrient intake in the United States. *Science* **224**, 1392–1398.
- Metz JA, Karanja N, Torok J & McCarron DA (1988) Modification of total body fat in spontaneously hypertensive rats and Wistar-Kyoto rats by dietary calcium and sodium. *Am J Hypertens* **1**, 58–60.
- Zemel MB, Shi H, Greer B, Dirienzo D & Zemel PC (2000) Regulation of adiposity by dietary calcium. *FASEB J* **14**, 1132–1138.
- Davies KM, Heaney RP, Recker RR, Lappe JM, Barger-Lux MJ, Rafferty K & Hinders S (2000) Calcium intake and body weight. *J Clin Endocrinol Metab* **85**, 4635–4638.
- Loos RJ, Rankinen T, Leon AS, Skinner JS, Wilmore JH, Rao DC & Bouchard C (2004) Calcium intake is associated with adiposity in black and white men and white women of the HERITAGE Family Study. *J Nutr* **134**, 1772–1778.
- Skinner JD, Bounds W, Carruth BR & Ziegler P (2003) Longitudinal calcium intake is negatively related to children's body fat indexes. *J Am Diet Assoc* **103**, 1626–1631.
- Shi H, Dirienzo D & Zemel MB (2001) Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice. *FASEB J* **15**, 291–293.
- Sun X & Zemel MB (2004) Calcium and dairy products inhibit weight and fat regain during *ad libitum* consumption following energy restriction in Ap2-agouti transgenic mice. *J Nutr* **134**, 3054–3060.
- Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L & Silva E (2005) Dairy augmentation of total and central fat loss in obese subjects. *Int J Obes (Lond)* **29**, 391–397.
- Zemel MB, Richards J, Milstead A & Campbell P (2005) Effects of calcium and dairy on body composition and weight loss in African-American adults. *Obes Res* **13**, 1218–1225.
- Harris RB (2005) Dairy protein, calcium and body weight – the need for a mechanism. *Int J Obes (Lond)* **29**, 388–390.
- Sakhaee K & Maalouf NM (2005) Dietary calcium, obesity and hypertension – the end of the road? *J Clin Endocrinol Metab* **90**, 4411–4413.
- Denke MA, Fox MM & Schulte MC (1993) Short-term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men. *J Nutr* **123**, 1047–1053.
- Tadayyon B & Lutwak L (1969) Interrelationship of triglycerides with calcium, magnesium and phosphorus in the rat. *J Nutr* **97**, 246–254.
- Papakonstantinou E, Flatt WP, Huth PJ & Harris RB (2003) High dietary calcium reduces body fat content, digestibility of fat, and serum vitamin D in rats. *Obes Res* **11**, 387–394.
- Jacobsen R, Lorenzen JK, Toubro S, Krog-Mikkelsen I & Astrup A (2005) Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. *Int J Obes (Lond)* **29**, 292–301.
- Zemel MB (2002) Regulation of adiposity and obesity risk by dietary calcium: mechanisms and implications. *J Am Coll Nutr* **21**, 146S–151S.
- Xue B, Greenberg AG, Kraemer FB & Zemel MB (2001) Mechanism of intracellular calcium ([Ca²⁺ + i]) inhibition of lipolysis in human adipocytes. *FASEB J* **15**, 2527–2529.
- Jones BH, Kim JH, Zemel MB, Woychik RP, Michaud EJ, Wilkison WO & Moustaid N (1996) Upregulation of adipocyte metabolism by agouti protein: possible paracrine actions in yellow mouse obesity. *Am J Physiol* **270**, E192–E196.
- Kim JH, Mynatt RL, Moore JW, Woychik RP, Moustaid N & Zemel MB (1996) The effects of calcium channel blockade on agouti-induced obesity. *FASEB J* **10**, 1646–1652.
- Shi H, Norman AW, Okamura WH, Sen A & Zemel MB (2001) 1 α ,25-Dihydroxyvitamin D₃ modulates human adipocyte metabolism via nongenomic action. *FASEB J* **15**, 2751–2753.
- Shi H, Norman AW, Okamura WH, Sen A & Zemel MB (2002) 1 α ,25-Dihydroxyvitamin D₃ inhibits uncoupling protein 2 expression in human adipocytes. *FASEB J* **16**, 1808–1810.
- Sun X & Zemel MB (2004) Role of uncoupling protein 2 (UCP2) expression and 1 α , 25-dihydroxyvitamin D₃ in modulating adipocyte apoptosis. *FASEB J* **18**, 1430–1432.
- Sun X & Zemel MB (2006) Dietary calcium regulates ROS production in aP2-agouti transgenic mice on high-fat/high-sucrose diets. *Int J Obes (Lond)* **30**, 1341–1346.
- Harvey-Berino J, Gold BC, Lauber R & Starinski A (2005) The impact of calcium and dairy product consumption on weight loss. *Obes Res* **13**, 1720–1726.

28. Gunther CW, Legowski PA, Lyle RM, McCabe GP, Eagan MS, Peacock M & Teegarden D (2005) Dairy products do not lead to alterations in body weight or fat mass in young women in a 1-y intervention. *Am J Clin Nutr* **81**, 751–756.
29. Paradis S & Cabanac M (2005) Calcium deficiency cannot induce obesity in rats. *Physiol Behav* **85**, 259–264.
30. Zhang Q & Tordoff MG (2004) No effect of dietary calcium on body weight of lean and obese mice and rats. *Am J Physiol Regul Integr Comp Physiol* **286**, R669–R677.
31. Zemel MB (2005) The role of dairy foods in weight management. *J Am Coll Nutr* **24**, 537S–546S.
32. Zemel MB (2004) Role of calcium and dairy products in energy partitioning and weight management. *Am J Clin Nutr* **79**, 907S–912S.
33. Zemel MB (2003) Mechanisms of dairy modulation of adiposity. *J Nutr* **133**, 252S–256S.
34. Huffman LM & Harper WJ (1999) Maximizing the value of milk through separation technologies. *J Dairy Sci* **82**, 2238–2244.
35. Collins S, Martin TL, Surwit RS & Robidoux J (2004) Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. *Physiol Behav* **81**, 243–248.
36. Koza RA, Nikonova L, Hogan J, Rim JS, Mendoza T, Faulk C, Skaf J & Kozak LP (2006) Changes in gene expression foreshadow diet-induced obesity in genetically identical mice. *PLoS Genet* **2**, e81.
37. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA & Feinglos MN (1988) Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* **37**, 1163–1167.
38. Nordic Committee on Food Analysis (NMKL) (1989) *Fett. Bestämning enligt SBR (Schmid–Bondzynski–Ratslaff) i Kött och Köttvaror (Fat. Determination according to SBR in Meat and Meat Products), Method of NMKL no. 131* Espoo, Finland: Technical Research Centre of Finland (VTT).
39. Lin S, Thomas TC, Storlien LH & Huang XF (2000) Development of high fat diet-induced obesity and leptin resistance in C57BL/6J mice. *Int J Obes Relat Metab Disord* **24**, 639–646.
40. Hall WL, Millward DJ, Long SJ & Morgan LM (2003) Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *Br J Nutr* **89**, 239–248.
41. Bowen J, Noakes M, Trenerry C & Clifton PM (2006) Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. *J Clin Endocrinol Metab* **91**, 1477–1483.
42. Fox PF & McSweeney PLH (1998) *Dairy Chemistry and Biochemistry*, 1st ed. Weinheim, Germany: Blackie Academic & Professional.
43. Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J & Yanovski JA (2004) The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab* **89**, 1196–1199.
44. Ni Z, Smogorzewski M & Massry SG (1994) Effects of parathyroid hormone on cytosolic calcium of rat adipocytes. *Endocrinology* **135**, 1837–1844.
45. Teegarden D (2005) The influence of dairy product consumption on body composition. *J Nutr* **135**, 2749–2752.
46. Kamycheva E, Sundsfjord J & Jorde R (2004) Serum parathyroid hormone level is associated with body mass index. The 5th Tromso study. *Eur J Endocrinol* **151**, 167–172.
47. Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, Seidell JC & Lips P (2005) Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab* **90**, 4119–4123.
48. Kersten S (2001) Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Rep* **2**, 282–286.
49. Letexier D, Pinteur C, Large V, Frering V & Beylot M (2003) Comparison of the expression and activity of the lipogenic pathway in human and rat adipose tissue. *J Lipid Res* **44**, 2127–2134.
50. Schutz Y (2004) Concept of fat balance in human obesity revisited with particular reference to *de novo* lipogenesis. *Int J Obes Relat Metab Disord* **28**, Suppl. 4, S3–S11.
51. Schutz Y (2004) Dietary fat, lipogenesis and energy balance. *Physiol Behav* **83**, 557–564.
52. Belobrajdic DP, McIntosh GH & Owens JA (2004) A high-whey-protein diet reduces body weight gain and alters insulin sensitivity relative to red meat in Wistar rats. *J Nutr* **134**, 1454–1458.
53. Aoyama T, Fukui K, Nakamori T, Hashimoto Y, Yamamoto T, Takamatsu K & Sugano M (2000) Effect of soy and milk whey protein isolates and their hydrolysates on weight reduction in genetically obese mice. *Biosci Biotechnol Biochem* **64**, 2594–2600.
54. Anthony JC, Anthony TG, Kimball SR & Jefferson LS (2001) Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. *J Nutr* **131**, 856S–860S.
55. Patti ME, Brambilla E, Luzi L, Landaker EJ & Kahn CR (1998) Bidirectional modulation of insulin action by amino acids. *J Clin Invest* **101**, 1519–1529.
56. Donato J Jr, Pedrosa RG, Cruzat VF, Pires IS & Tirapegui J (2006) Effects of leucine supplementation on the body composition and protein status of rats submitted to food restriction. *Nutrition* **22**, 520–527.
57. Morifuji M, Sakai K, Sanbongi C & Sugiura K (2005) Dietary whey protein downregulates fatty acid synthesis in the liver, but upregulates it in skeletal muscle of exercise-trained rats. *Nutrition* **21**, 1052–1058.
58. Morifuji M, Sakai K, Sanbongi C & Sugiura K (2005) Dietary whey protein increases liver and skeletal muscle glycogen levels in exercise-trained rats. *Br J Nutr* **93**, 439–445.