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

Taru K. Pilvi¹,², Riitta Korpela¹,², Minna Huttunen³, Heikki Vapaatalo¹ and Eero M. Mervaala¹,⁴*¹

Institute 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 syndrome. The prevalence of obesity is increasing dramatically both in Western countries and in the developing world. 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 and since then the association has been further confirmed in several epidemiological studies. 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. 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.

One possible factor explaining the inverse relationship between Ca intake and BMI is the fatty acid-binding capacity of Ca. Some studies have found it to be a significant factor explaining the anti-obesity effect of dietary Ca, while other researchers argue that the effect that Ca has on fat absorption is too small to explain the influence Ca has on body weight.

The alternative hypothesis explaining the anti-obesity effect of Ca links dietary Ca intake, serum 1,25-dihydroxyvitamin D₃ (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. 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 lipolysis. These actions altogether lead to increased fat accumulation in a situation of low Ca intake, when serum ...
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 metabolism. 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 diet.

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. 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.

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. 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.

The major protein classes of milk are whey (20%) and casein (80%) proteins. 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. 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 ± 0.7, 25.6 ± 0.6 and 25.5 ± 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

<table>
<thead>
<tr>
<th>Group and product code...</th>
<th>High-Ca whey (D05031104M)</th>
<th>High-Ca casein (D05031102M)</th>
<th>Low-Ca casein (D05031101M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (Alacid 714) (g)</td>
<td>0</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Whey protein isolate (Alacen™ 895) (g)</td>
<td>186</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maltodextrin 10 (g)</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>66.8</td>
<td>68.8</td>
<td>68.8</td>
</tr>
<tr>
<td>Cellulose (BW200) (g)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Soyabean oil (g)</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Lard (g)</td>
<td>245</td>
<td>245</td>
<td>245</td>
</tr>
<tr>
<td>CaCO3 (g)</td>
<td>34.3</td>
<td>35.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Potassium phosphate (monobasic) (g)</td>
<td>19.4</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Potassium carbonate, 1 H2O (g)</td>
<td>0</td>
<td>6.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Energy (KJ/g dry weight)</td>
<td>21.4</td>
<td>21.0</td>
<td>21.9</td>
</tr>
<tr>
<td>Protein (% energy)</td>
<td>18.0</td>
<td>18.1</td>
<td>18.1</td>
</tr>
<tr>
<td>Carbohydrate (% energy)</td>
<td>20.5</td>
<td>20.6</td>
<td>20.6</td>
</tr>
<tr>
<td>Fat (% energy)</td>
<td>61.5</td>
<td>61.3</td>
<td>61.3</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>1.80</td>
<td>1.80</td>
<td>0.40</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.59</td>
<td>0.58</td>
<td>0.60</td>
</tr>
<tr>
<td>K (%)</td>
<td>0.79</td>
<td>0.75</td>
<td>0.78</td>
</tr>
</tbody>
</table>

* 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 PRO 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 method

Ca content was determined by the Schmid–Bondzynski–Ratzlaff method

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 \left( \frac{\text{fat intake} - \text{faecal fat}}{\text{fat intake}} \right)

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 % × cumulative energy intake from fat) as described previously.

**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; Arkay 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 CO₂–O₂ (95:5, v/v) (AGA, Riihimäki, Finland) and decapitated. Blood samples were taken in plastic tubes, and serum was separated by centrifugation at +4°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
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)$_2$D$_3$ 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)$_2$D$_3$ 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
more weight than what is normally seen in the same mice fed a low-fat diet. In contrast with previous experimental studies investigating the effect of Ca supplementation on body-weight gain, 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 casein, 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 intake.

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 Ca. 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. 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. 3](https://www.cambridge.org/core/core/fig/3_5.0.0.0.png)

**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).

![Fig. 4](https://www.cambridge.org/core/core/fig/4_5.0.0.0.png)

**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.
there is accumulating evidence supporting the role of 1,25(OH)2-D3 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(OH)2-D3-levels in obese than in non-obese subjects, which is in contrast with the theory that decreasing the amount of 1,25(OH)2-D3 by dietary Ca would lead to a decreased accumulation of fat. In addition to 1,25(OH)2-D3, PTH has also been shown to regulate adipocyte intracellular Ca and it has been suggested to be a potential mediator of the anti-obesity effect of dietary Ca. 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. 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(OH)2-D3 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 diet. 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). 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 diet. 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.

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 meat. 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 mice. 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 synthesis and modulating insulin–PI3 kinase signalling. 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...
restriction in Wistar rats\textsuperscript{56}, 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 \textit{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\textsuperscript{72,58}. 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


Milk proteins, calcium and body weight


