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Other Review

Effect of calcium from dairy and dietary supplements on faecal fat excretion: a meta-analysis of randomized controlled trials

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Summary

Observational studies have found that dietary calcium intake is inversely related to body weight and body fat mass. One explanatory mechanism is that dietary calcium increases faecal fat excretion. To examine the effect of calcium from dietary supplements or dairy products on quantitative faecal fat excretion, we performed a systematic review with meta-analysis. We included randomized, controlled trials of calcium (supplements or dairy) in healthy subjects, where faecal fat excretion was measured. Meta-analyses used random-effects models with changes in faecal fat excreted expressed as standardized mean differences, as the studies assessed the same outcome but measured in different ways.

An increased calcium intake resulted in increased excretion of faecal fat by a standardized mean difference of 0.99 (95% confidence intervals: 0.63–1.34; $P < 0.0001$; expected to correspond to ~2g day^{-1}) with moderate heterogeneity ($I^2 = 49.5\%$) indicating some inconsistency in trial outcomes. However, the dairy trials showed homogeneous outcomes (I^2=0%) indicating consistency among these trials. We estimated that increasing the dairy calcium intake by 1241 mg day^{-1} resulted in an increase in faecal fat of 5.2 (1.6–8.8) g day^{-1}. In conclusion, dietary calcium has the potential to increase faecal fat excretion to an extent that could be relevant for prevention of weight (re-)gain. Long-term studies are required to establish its potential contribution.

Keywords: Dairy products, dietary calcium, faecal fat excretion, meta-analysis.

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Introduction

An inverse association between dietary calcium intake and body weight was first reported in the mid-1980s based on data from the first National Health and Nutritional Examination Survey in the US. (1). Since then, similar associations between calcium intake, or intake of dairy products, and body weight and body fat mass have been found in several observational studies (2–6), but not in all (7,8). A number of randomized intervention studies examining the effect of calcium supplements or dairy calcium have not produced a clear answer as to whether calcium may have a role in energy balance. In several studies, Zemel and colleagues have found that subjects randomized to a high-calcium intake achieve a significantly greater reduction in body weight and body fat during energy restriction than those with a low-calcium intake (9–11). However, a recent meta-analysis of intervention studies that examined the effect of calcium on weight loss showed that the results of the available trials are heterogeneous – with contradictory results (12).

The mechanism responsible for the potential effect of increased calcium intake on energy balance is not clear, but a number of different mechanisms have been suggested. Zemel and colleagues have advocated a hypothesis that calcium intake plays a regulatory role in lipid metabolism by influencing intracellular calcium levels via hormonal regulation (13). According to this hypothesis, an increase in dietary calcium would result in increased lipolysis and decreased *de novo* lipogenesis, thereby stimulating loss of body weight and fat (13). However, the hypothesis is mainly supported by animal studies, while recent human studies do not indicate that adipocyte and whole body fat metabolism are affected by dietary calcium intakes (14–17). Although it has been suggested that high-calcium intake may increase fat oxidation under conditions of acute energy deficit, a number of studies have failed to detect any consistent effect of calcium on energy expenditure during energy balance (14,15,18–20). However, a reduction in body fat stores cannot occur without either affecting energy intake, faecal energy loss, or increasing energy expenditure. It has recently been suggested that supplementation with a calcium plus vitamin D in subjects with a habitual low intake of calcium results in a decrease in *ad libitum* intake of energy and fat (21). More research is needed to establish if and how calcium intake affects human appetite regulation. Another mechanism, suggested by a number of studies in both humans and animals, is that dietary calcium interferes with fat absorption in the intestine by forming insoluble calcium soaps with fatty acids (FAs) and/or binding of bile acids, resulting in a decrease in the digestible energy of the diet (15,18,22–25).

We carried out a quantitative systematic review on randomized, controlled trials to determine the effectiveness of calcium supplementation – from either dairy products or dietary supplements – on changes in faecal fat excretion. A secondary aim was to explore whether an increased faecal fat loss could be explained by typical bias items, such as blinding and randomization, affecting individual trials' internal validity (26,27). Finally, we examined whether the effect could be explained by the amount of calcium administered, and whether the level of protein in the diet could modify any effect of dietary calcium on faecal fat excretion (15,18).

Methods

Study selection, assessment of eligibility criteria, data extraction and statistical analysis were performed based on a predefined protocol according to the Cochrane Collaboration guidelines (28). This article was prepared in accordance with the Quality of Reporting of Meta-analyses statement (29).

Literature search

The following databases were searched: Medline (Mid-1950s to May 2008), EMBASE (1980 to May 2008), Web of Science (1945–54 to May 2008), BiosisPreviews (1980 to May 2008), Scifinder (1907 to May 2008), Agricola (1970 to May 2008), Food Science Technical Abstracts [FSTA] (1969 to May 2008), CAB Abstracts (1973 to May 2008), Cochrane Central Register of Controlled Trials (until May 2008). The search strategy contained the following: (calcium* OR milk* OR dairy*) AND (lipid* OR fat*) AND (faeces* OR feces* OR faecal* OR fecal* OR stool*) AND human*. There were no limits on language or publication type. As a supplement to the systematic literature search, two reviewers (J. K. L. & A. A.) contacted (via email) other known experts in the field (including all first authors of the papers retrieved). Searching for potentially unpublished data was supervised by three experts in the field (E. L. M., W. H. S., A. T.). The reference lists of relevant reviews or potentially eligible papers were also checked for other possible eligible trials. Finally the Global Dairy Platform (Chicago, US) was contacted in order to reveal any extra data not already available in the public domain.

Selection criteria

All randomized and quasi-randomized controlled trials were considered eligible if they (i) Enrolled healthy participants, whether adults, adolescents or children more than 6 years of age; (ii) Examined the effect of intake of calcium from dairy products or dietary supplements and (iii) Reported changes in faecal fat – i.e. either as total fat or as FAs. There was no upper limit to the duration of trials considered eligible. In order to ensure adequate methodology, studies were eligible for inclusion if the time from starting the intervention to the measurement of the faecal fat excretion was at least 3 days. Studies with a crossover design were considered eligible for inclusion in the meta-analysis as the risk of carry-over effect was considered minimal (30). The preliminary literature search for potentially eligible studies (conducted by J. K. L. & C. R. S.) was supervised by an experienced research librarian (E. M. B.). Two investigators (J. K. L. & C. R. S.) concluded the literature search by eliminating/including studies according to the agreed eligibility criteria, and obtained approval of the result via a consensus call with the other content expert reviewers (E. L. M., W. H. S., A. T., and A. A.).

Data extraction and quality assessment

The included studies were scrutinized and reviewed (without blinding of reviewers). Data were extracted using a customized form (Microsoft Excel® spreadsheet) provided by a fourth reviewer (R. C.), including (i) Characteristics of included studies; (ii) The Cochrane Collaboration's tool for assessing risk of bias and (iii) Outcome measures applicable for the subsequent data analyses. Two

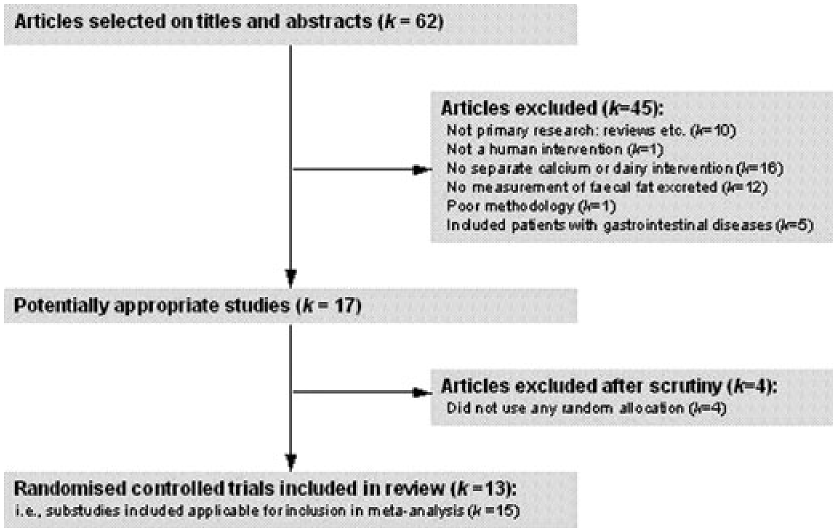

Figure 1 Quality of Reporting of Meta-analyses (QUOROM) flowchart.

investigators (R. C. & J. K. L.) were responsible for the assessment and extraction of data. The extracted outcome was the difference between the intervention and control groups (i.e. the paired mean difference in crossover studies), and the variance measure was obtained for each trial according to a standardized procedure using a data abstraction form. Data were collected on trial design (parallel vs. crossover), duration of the trial, type of calcium (dairy product, dietary supplement), calcium dosage applied compared with the control group (mg) and the estimated average level of protein intake in the study. Furthermore, data were extracted on sample size and characteristics of the study population, including the subjects' average age and sex (proportion of men).

In order to apply the Cochrane Collaboration's tool for assessing risk of bias, two reviewers (R. C., J. K. L.) independently assessed whether each of the following domains would be considered adequate – i.e. presumably with a low risk of bias (i) 'Adequate sequence generation'; (ii) 'Allocation concealment'; (iii) 'Blinding'; (iv) 'Incomplete outcome data addressed'; (v) 'Free of selective reporting' and (vi) 'Free of other biases' (published as a peer-reviewed paper). Each of these key components of methodological quality was assessed on a Yes/Unclear/No basis, handled as A, B and C, respectively (http://www.cochrane-handbook.org/). Any differences between reviewers were resolved at a subsequent consensus meeting (with A. A.).

Data synthesis and analysis

For crossover trials lacking data on standard errors (SEs) for paired differences (SE_Δ), the pooled SE was estimated assuming a correlation at a conservative level of 0 between intervention and control periods ($\rho = 0.0$) in crossover trials (30). We anticipated that the type of outcome measurement would vary across individual studies, so the standardized mean difference (SMD including Hedges's adjustment for small sample bias (31)) was used as the summary statistic in the meta-analysis – assessing the same outcome measured in a variety of ways (32). The SMD expresses the size of the treatment effect relative to the variability observed in that trial ($[\mu_{Calcium} - \mu_{Control}]/\sigma$), using slightly different calculi for parallel or crossover trial designs (31,33). To combine the individual study results, we performed meta-analyses using SAS software (PROC MIXED version 9.1.3; SAS Institute Inc., Cary, NC, USA), applying a restricted maximum likelihood (REML) method to estimate the between-study variance (i.e. τ^2) and the combined efficacy (34). We examined heterogeneity between trials with a standard Q-test statistic (35), and we present the I^2 value (36), which can be interpreted as the amount of inconsistency in the reported results between the individual studies (37). We performed a number of predefined sensitivity analyses, subgroup analyses stratifying the available trials according to calcium from dairy products vs. dietary supplements, and analyses of varying degrees of risk of bias according to the Cochrane Collaboration's tool for assessing risk of bias. REML-based (i.e. random-effects) meta-regression analysis (38) was applied in order to answer the specific question raised by the secondary hypothesis – whether the amount of extra calcium could predict the quantitative changes in faecal fat loss.

Results

Results of the search

We identified more than 300 studies in the database searches, of which 62 studies were potentially relevant and therefore read in full text (Fig. 1). Of these, a total of 45 studies were immediately excluded: 10 studies did not report original research (reviews, etc.); one was an *in vitro* study; 16 studies did not include a separate calcium or dairy intervention and the isolated contrast associated with supplementation could therefore not be evaluated; 12 had no measurement of faecal fat excretion but only measured fat in a single stool; one study only measured the fat content in faecal water; one study was judged methodologically insufficient because of problems with separation of stools from the two intervention periods; and five studies focused on patients or subjects with previous or current gastrointestinal diseases. This left 17 potentially eligible studies. However, four of these studies were not randomized in any way and were therefore excluded. The remaining 13 studies were deemed eligible for inclusion in the systematic review (15,18,39–49). Two of these studies could be handled as having a factorial design (15,40), resulting in a final total of 15 substudies included in the meta-analysis.

Description of studies

The average characteristics of the included studies are shown in Table 1. The trials were published between 1964 and 2008, and varied in participant size. The majority of trials were crossover trials. A total of 168 participants were assessed (focusing solely on the individuals included in the analyses) – receiving extra calcium, an appropriate control, or both. The length of the trials varied from 3 days to 1 month. The studies had more or less the same primary end point – either total fat excreted via faeces or a specific focus on free FAs (Table 1). In two of the included studies, the time from starting the intervention to the measurement of the faecal fat excretion was less than 3 days (45,49). In the study by Bendsen *et al.*, the intervention started 2 days before the faecal collection was started (49): in this study the mean transit time was estimated by non-absorbable faecal markers to be ~40 h and it was therefore included. In the study by Murata *et al.*, the subjects were given two different oral trace markers, one at the beginning and one at the end of the diet period (45). Excretion of these trace markers was used to determine when to begin and when to end faecal collection.

Effect of intervention

Calcium supplementation resulted in an increased excretion of faecal fat and FAs compared with control groups, with a SMD of 0.99 (95% confidence intervals [CI]: 0.63–1.34; $z = 5.44$, $P < 0.0001$; Fig. 2). There was no evidence to indicate a difference between the calcium supplements and calcium from dairy products (1.04 vs. 0.90, respectively; $z = 0.30$, $P = 0.76$). Assuming an average (±SD) fat excretion in the population of 5.4 (±2.0) g day^{-1} (43), we estimate that applying extra calcium (with a range from 800 to 6000 mg day^{-1}) would result in an increase of 2.0 (95% CI: 1.3–2.7) g faecal fat excreted (37% increase) each day. The meta-analysis was based on studies showing a moderate degree of heterogeneity ($I^2 = 49.5\%$), supporting the use of a random-effects meta-analysis. As a sensitivity analysis, the same meta-analysis based on a fixed-effects model resulted in a combined SMD of 0.80 (95% CI: 0.55–1.04, $P < 0.0001$). When the studies were divided into studies using calcium supplementation and studies using dairy calcium, a relatively high degree of heterogeneity ($I^2 = 58.5\%$) was found among the studies using calcium supplements. In contrast, the dairy calcium studies showed homogeneity ($I^2 = 0\%$). We therefore conducted a meta-analysis with the faecal fat excretion expressed as gram per day as all the dairy trials used the same outcome measure. An increased dairy calcium intake of 1241 mg day^{-1} increased faecal fat excretion by 5.2 g day^{-1} compared with low-calcium (<700 mg day^{-1}) dairy diet (95% CI: 1.6–8.8; see Fig. 3). One of the inclusion criteria was that the time from starting the intervention to measurement of faecal fat excretion was at least 3 days. However, as mentioned previously, two studies departed somewhat from this condition (44,49). In a sensitivity analysis, we therefore grouped these two studies as potentially inadequate compared with the other studies (see Table 1). There was no significant difference between the two subgroups ($z = 1.07$, $P = 0.28$), although the studies indexed as being adequate showed a less pronounced efficacy (SMD = 0.91 [SE = 0.19]) when compared with those categorized as potentially inadequate (SMD = 1.56 [SE = 0.57]).

Risk of bias in included studies

Table 2 presents results from stratified analyses. Estimates of effect sizes varied to some degree depending on the quality of the trials. When meta-analysing the trials, with explicit focus on the adequacy of random and concealed allocation, the studies indexed as adequate had the least pronounced effect size compared with those indexed as inadequate. The same pattern applied for handling of incomplete outcome data, free of selective outcome reporting, and whether or not the study had been presented in a peer-reviewed journal. In contrast, the adequately double-blinded trials seemed to have a more pronounced effect than those with an unclear risk of bias (not significant, $P = 0.78$).

Association between intake and size of faecal fat excretion

We found no relationship between amounts of extra calcium applied in the individual trials (see Fig. 4A) and the increase in faecal fat excretion expressed as SMD, as the slope was not significantly different from null ($z = 0.37$, $P = 0.71$). Accordingly the overall efficacy associated with

Table 1 Study characteristics of all participants in the eligible trials (i.e. substudies)

Study	Year	Country	Population	Intervention	Design	Calcium intake in Ca(−) (mg day^{-1})	Outcome	Faecal fat excretion (g day^{-1})		Duration		Energy intake (kJ day^{-1})	Protein intake (Energy%)	Individuals included in analysis	Men (no, %)	Age (years)
								Ca(+)	Ca(−)	Intervention	Fecal collection					
Supplements																
Lutwak *et al.*	1964	United States	Healthy girls	Controlled diet with: Ca(+): bread enriched with calcium (phosphate) Ca(−): regular bread	Parallel: two groups	1295*	Total fat (g day^{-1})	4.93 ± 2.05†	2.44 ± 0.61	24 days‡	24 days	~10 600	14	Parallel groups: $N_{Ca(+)} = 10$ $N_{Ca(-)} = 8$	0 (0%)	9 (range 8–11)
Bhattacharyya *et al.* (PUFA)	1969	United States	Healthy young men	Controlled diet with high PUFA and: Ca(+): bread enriched with calcium (carbonate and gluconate) Ca(−): regular bread	Crossover: 2 × 2 factorial design	254*	Fatty acids (g day^{-1})	1.49 ± 0.34	1.20 ± 0.39	2 weeks	7 days	~12 600§	15§	Crossover: *N* = 11	11 (100%)	Range: 21–26
Bhattacharyya *et al.* (SFA)	1969	United States	Healthy young men	Controlled diet with high SFA and: Ca(+): bread enriched with calcium (carbonate and gluconate) Ca(−): regular bread	Crossover: 2 × 2 factorial design	254*	Fatty acids (g day^{-1})	4.11 ± 1.29†	1.19 ± 0.21	2 weeks	7 days	~12 600§	15§	Crossover: *N* = 10	10 (100%)	Range: 21–26
Saunders *et al.*	1988	United States	Students/personnel in the Health Science Building	Controlled diet plus: Ca(+): calcium tablets (carbonate) Ca(−):	Crossover: two-group comparison	NA	Fatty acids (meq day^{-1})	16.8 ± 5.4†	7.9 ± 1.4	3 weeks	7 days	NA	15	Crossover: *N* = 8	5 (62.5%)	NA
Denke *et al.*	1993	United States	Men: moderate hypercholesterol aemia	Controlled diet plus: Ca(+): juice/muffin enriched with calcium and calcium tablets (mixed salts) Ca(−): regular juice/muffin and placebo tablets	Crossover: two-group comparison	410 ± 33	Fatty acids (g day^{-1})¶	5.40*,†	2.63*	10 days	3 days	~11 000§	11§	Crossover: *N* = 13	13 (100%)	43 ± 4 (Range: 38–49)
Welberg *et al.*	1994	Netherlands	Healthy volunteers	Habitual diet plus Ca(+): calcium tablets (carbonate) Ca(−): placebo tablets	Parallel group: two groups	Diet: 1450 ± 481	Total fat (g day^{-1})	7.0 ± 2.6	5.4 ± 2.0	1 week	3 days	~9 200	~16	Parallel group: $N_{Ca(+)} = 7$ $N_{Ca(-)} = 8$	NA	NA
Govers *et al.*	1996	Netherlands	Healthy men	Habitual diet plus Ca(+): regular milk products Ca(−): calcium depleted milk products	Double-blind, crossover: two-group comparison	765 ± 145	Total fat (g day^{-1})	9.3 ± 2.5†	6.7 ± 2.2	1 week	3 days	~13 000	~14	Crossover: *N* = 13	13 (100%)	38 ± 7
Murata *et al.*	1998	Japan	Healthy men	Controlled diet with: Ca(+): chocolate enriched with calcium (derived from eggshells) Ca(−): regular chocolate	Crossover: two-group comparison	504 ± 35	Total fat (g day^{-1})	7.5*,†	2.8*	3 days	~3 days**	~7 700	11	Crossover: *N* = 9	9 (100%)	NA

Table 1 *Continued*

Study	Year	Country	Population	Intervention	Design	Calcium intake in Ca(–) (mg day^{-1})	Outcome	Faecal fat excretion (g day^{-1})		Duration		Energy intake (kJ day^{-1})	Protein intake (Energy%)	Individuals included in analysis	Men (no, %)	Age (years)
Shahkhalili *et al.*	2001A	Switzerland	Healthy men	Controlled diet with: Ca(+): chocolate enriched with calcium (carbonate) Ca(–): regular chocolate	Parallel: two groups	Diet: NA	Total fat (g day^{-1})	10.4*,†	5.9*	2 weeks	7 days	NA	NA	Parallel group: $N_{Ca(+)} = 6$ $N_{Ca(-)} = 6$	NA	NA
Shahkhalili *et al.*	2001	Switzerland	Healthy men	Controlled diet with: Ca(+): chocolate enriched with calcium (carbonate) Ca(–): regular chocolate	Crossover: two-group comparison	950 ± 59	Total fat (g day^{-1})	8.40 ± 1.01†	4.36 ± 0.43	2 weeks	7 days	~13 000	14§	Crossover: *N* = 9	9 (100%)	NA
Ditscheid *et al.*	2005	Germany	Healthy men and women	Controlled diet with: Ca(+): bread enriched calcium (phosphate) Ca(–): regular bread	Crossover: two-group comparison	1193 ± 295	Total fat (g day^{-1})	4.3 ± 1.1	3.9 ± 1.4	4 weeks	5 days	~9 000	16	Crossover: *N* = 31	15 (48%)	25 (range: 21–29)
Boon *et al.* (supplement)	2007	Netherlands	Healthy men and women	Controlled diet plus: Ca(+) calcium supplement (carbonate) Ca(–) low calcium	Crossover: 2 × 1 pseudo factorial design	348 ± 28††	Total fat (g day^{-1})	6.7 ± 3.8	4.8 ± 2.5	1 week	3 days	~10 000††	20	Crossover: *N* = 10	5 (50%)	28 ± 6
Dairy products																
Jacobsen *et al.*	2005	Denmark	Healthy men and women	Controlled diet with: Ca(+) high calcium from dairy products Ca(–) low calcium	Crossover: two-group comparison	474*,††	Total fat (g day^{-1})	14.2 ± 6†	6.0 ± 2	1 week	3 days	~10 000††	15	Crossover: *N* = 8	NA	NA
Boon *et al.* (Dairy)	2007	Netherlands	Healthy men and women	Controlled diet with: Ca(+) high calcium from dairy products Ca(–) low calcium	Crossover: 2 × 1 pseudo factorial design	348 ± 28††	Total fat (g day^{-1})	7.2 ± 3.5	4.8 ± 2.5	1 week	3 days	~10 000††	20	Crossover: *N* = 10	5 (50%)	28 ± 6
Bendsen *et al.*	2008	Denmark	Healthy men and women	Controlled diet with: Ca(+) high calcium from dairy products Ca(–) low calcium	Crossover: two-group comparison	698 ± 153††	Total fat (g day^{-1})	11.5 ± 4.6†	5.4 ± 1.7	1 week	5 days	~12 500††	15	Crossover: *N* = 11	5 (45%)	33 (range 25–47)

Values are Means ± SD unless otherwise stated.
*SD not stated in the paper.
†Significant different from Ca(–) ($P < 0.05$).
‡The subjects had consumed a diet similar to the experimental diet for at least one year prior to the study.
§Standard serving of controlled diet. Intake adjusted according to individual energy requirement by removing or adding carbohydrate food.
¶Only major fatty acids (14 : 0, 16 : 0, 18 : 0 and 18 : 1) were included.
**Subjects were given two different oral trace markers, one in the beginning and one in end of the diet period. Excretion of these trace markers were used to determent when to begin and when to end faecal collection.
††Mean intake. Intake adjusted according to individual energy requirement.

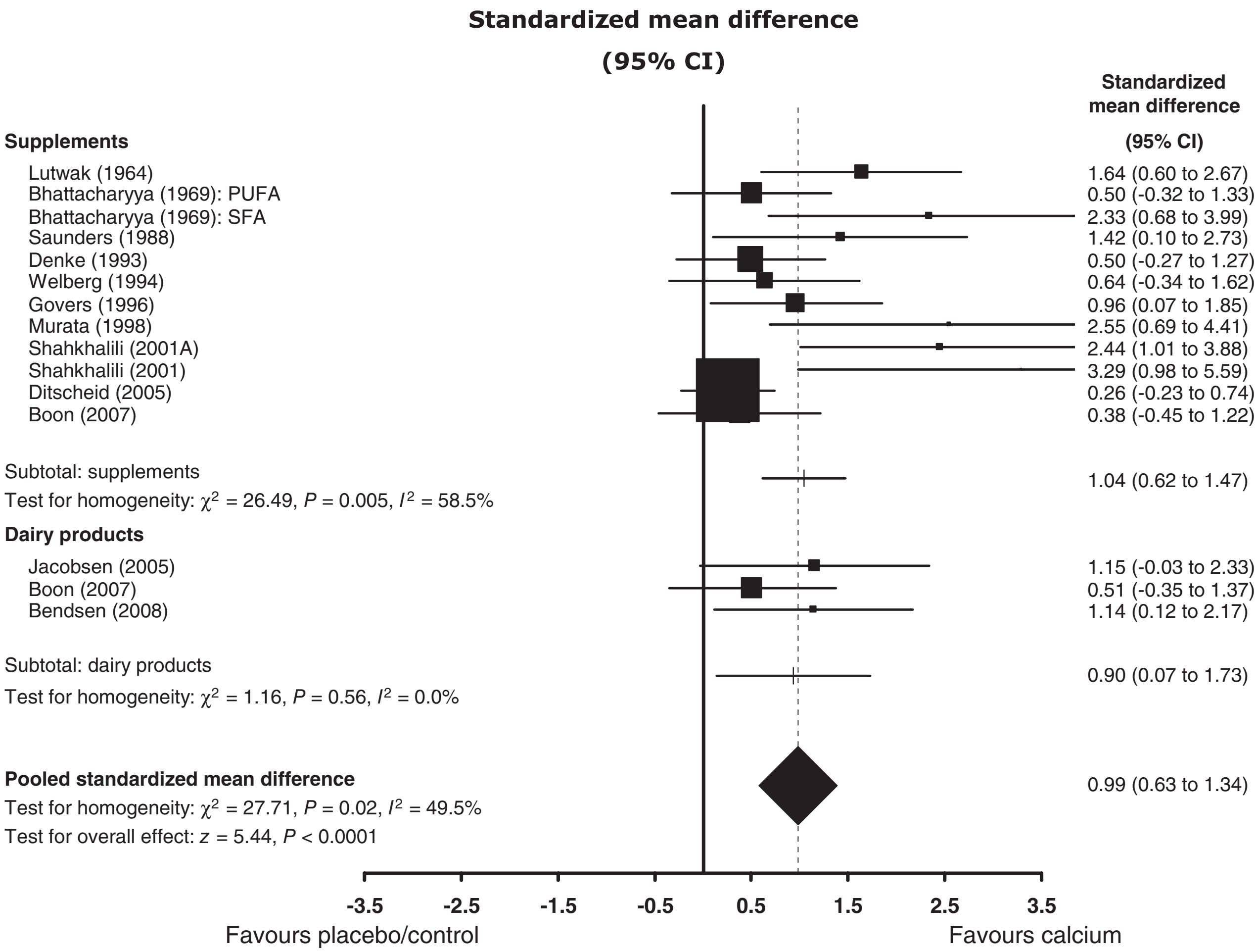

Figure 2 Effects of calcium supplementation on faecal fat excretion; presented as supplements or dairy products. Every square represents the individual study's SMD with 95% CI indicated by horizontal lines; square sizes are directly proportional to the precision of the estimate.

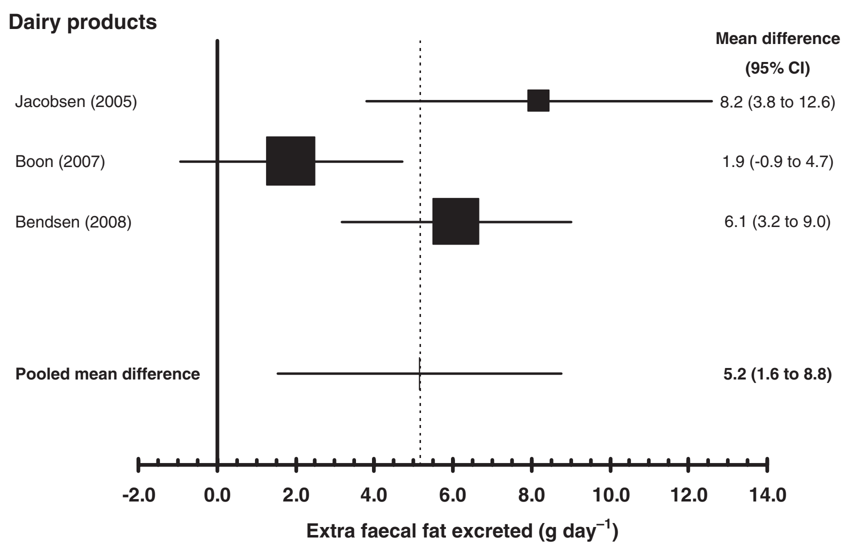

Figure 3 Amount of faecal fat excreted among the homogeneous studies following extra calcium from dairy products. Every square represents the individual study's mean difference with 95% CI indicated by horizontal lines; square sizes are directly proportional to the precision of the estimate.

Table 2 Results of the stratified meta-analyses: standardized mean difference in faecal fat excreted following extra calcium opposed to control intervention

Study	Design	Dose (Ca: mg day^{-1})	Random all[1]	Concealed all[2]	Double blinding[3]	Incomplete OD[4]	FoSOR[5]	FoB[6]
Supplements								
Lutwak *et al.* (1964)	PG	991	C	C	B	B	A	A
Bhattacharyya *et al.* (1969)	CO	2000	A	B	B	B	A	A
Bhattacharyya *et al.* (1969)	CO	2000	A	B	B	B	A	A
Saunders *et al.* (1988)	CO	6000	A	B	A	B	B	A
Denke *et al.* (1993)	CO	1800	B	B	A	B	B	A
Welberg *et al.* (1994)	PG	2000	B	B	A	B	A	A
Govers *et al.* (1996)	CO	1080	B	B	A	B	B	A
Murata *et al.* (1998)	CO	1150	B	B	A	B	B	A
Shahkhalili *et al.* (2001A)	PG	1000	B	B	A	B	B	C
Shahkhalili *et al.* (2001)	CO	900	B	B	A	B	A	A
Ditscheid *et al.* (2005)	CO	1060	B	B	A	B	A	A
Boon *et al.* (2007)	CO	800	A	A	B	A	A	A
Dairy products								
Jacobsen *et al.* (2005)	CO	1300	A	A	B	A	A	A
Boon *et al.* (2007)	CO	800	A	A	B	A	A	A
Bendsen *et al.* (2008)	CO	1600	A	A	B	A	A	C
	CO: 0.85 (0.49–1.22)	(See Figure 4)	A: 0.91 (0.36–1.45)	A: 0.75 (0.06–1.44)	A: 1.07 (0.53–1.60)	A: 0.75 (0.05–1.45)	A: 0.86 (0.44–1.29)	A: 0.87 (0.52–1.22)
	PG: 1.43 (0.65–2.21)		B: 1.01 (0.46–1.57)	B: 1.06 (0.59–1.53)	B: 0.96 (0.41–1.50)	B: 1.12 (0.67–1.57)	B: 1.28 (0.63–1.93)	B) NA
			C: 1.64 (0.21–3.06)	C: 1.64 (0.21–3.07)	C: NA	C: NA	C: NA	C: 1.64 (0.64–2.64

Values are Hedges's standardized mean differences (95% confidence intervals) stratified according potential risk of bias, and regressed vs. extra calcium dosage applied.
1: random allocation, 2: concealed allocation, 3: double blinding, 4: incomplete outcome data, 5: free of selective outcome reporting, 6: free of other bias' (i.e. published in a peer-reviewed journal)
A: adequate (i.e. low risk of bias).
B: unclear (i.e. unclear risk of bias).
C: inadequate (i.e. high risk of bias in the analysis).
Int: intercept with the y-axis (i.e. calcium dose = 0 mg); Slp: Slope (i.e. increment in effect size with 1 mg of Ca added).
CO: crossover trial; PG: parallel group design; NA, not available.

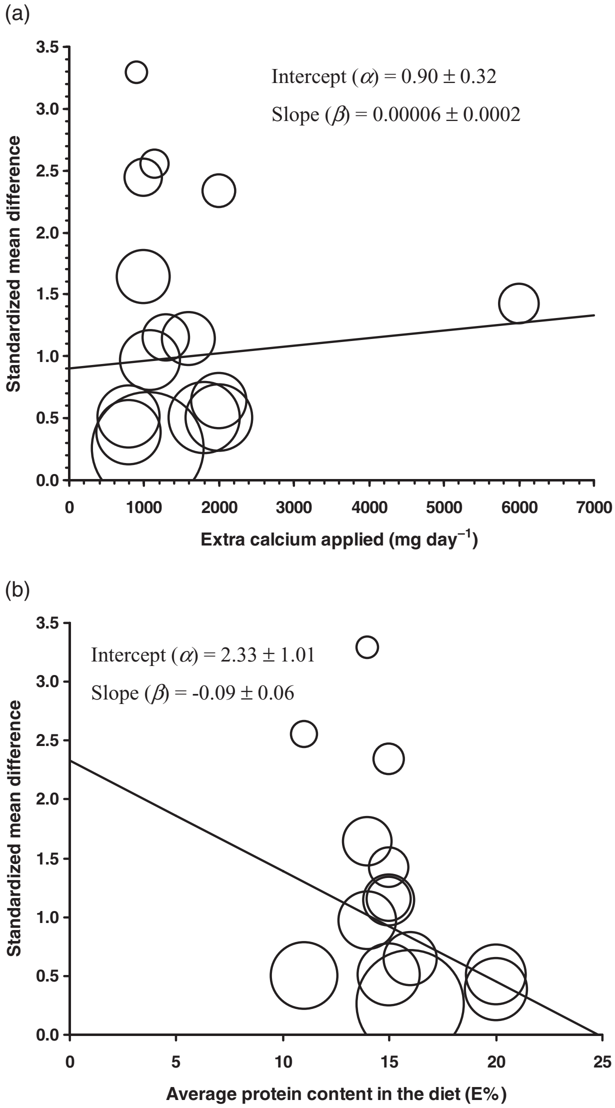

Figure 4 Meta-regression analysis: The size of the circles is proportional to the precision of the estimate used in the meta-regression. The line indicates the predicted effects (regression line). Values are given as the estimate ± SE. Effect sizes on the vertical axis are plotted against (**a**) the estimated group mean difference in calcium dose, and (**b**) the average protein content (energy%) in the diet.

use of extra calcium was best explained by applying the intercept, being a consequence of allocation to 'extra calcium' *per se* – opposed to control of 0.90 (95% CI: 0.27–1.53; $z = 2.82$, $P = 0.0048$). On a *post hoc* level, examining the impact of the concomitant level of protein intake (Table 1) showed no significant slope effect ($z = 1.46$, $P = 0.14$), although data might support a potential inverse association between faecal fat excreted and energy intake from protein in the concomitant diet (see Fig. 4B).

Discussion

The major result of this meta-analysis is that dietary calcium impairs the absorption of dietary fat and increases faecal fat excretion. Although the effect was statistically highly significant, its importance for the daily energy balance and body-weight regulation may be minor. The additional daily excretion of 2.0 g fat (~18 kcal) is equivalent to ~0.7 kg body fat or ~1 kg body weight on an annual basis, providing that no adaptation or counter regulatory mechanism offsets the effect. However, the heterogeneity of the trials ($I^2 = 49.5\%$) suggests that the outcome of the meta-analysis could be confounded by study characteristics, such as differences in study design, methods used for fat analyses, study population, calcium sources, matrix in which calcium is provided, habitual diet and interaction with other nutrients in the food matrix. In particular, high-protein intake could interfere with the calcium soap formation and consequently with fat excretion (15,18). As the dairy trials showed homogeneity ($I^2 = 0\%$) the estimate from the meta-analysis including only these studies may be more certain than the pooled estimate from the meta-analysis including all trials. The meta-analysis of these trials showed that a weighted-average increase in dairy calcium by 1241 mg day^{-1} produced an increase in faecal fat excretion of 5.2 g day^{-1}, although based on a relatively small sample ($n = 29$ participants). This is equivalent to 47 kcal day^{-1} or 1.9 kg body fat or 2.2 kg body weight over 1 year. Without further studies to produce more robust data, we estimate that increasing dietary calcium intake has the potential to increase faecal fat excretion by 2–5.2 g day^{-1}, which corresponds to a change in body weight of –1 to –2.2 kg over 1 year. In comparison, orlistat, a gastrointestinal lipase inhibitor reducing dietary fat absorption (50), has been shown to increase the amount of excreted fat by 16.13 (SD: 7.27) g day^{-1} (51). This amount of extra faecal fat corresponds to a SMD of 2.2. Orlistat therefore seems at least twice as efficacious as extra calcium (Fig. 2). Some studies have found the effect of a high-calcium diet on body-weight loss to be more pronounced than can be explained by an increase in fat excretion, indicating that there may be an additional relation between calcium and body weight (9–11). Major *et al.* found recently that supplementation with a calcium plus vitamin D supplement decreases energy and fat intake in women with a low habitual calcium intake (21). However, more research is needed to establish whether calcium affects human appetite regulation.

We failed to establish a clear dose–response relationship between intake of calcium and faecal fat excretion, which makes it difficult to make quantitative estimates of its importance for energy balance, and to translate the findings into importance for dietary guidelines. The failure to find a dose–response relationship between calcium intake and faecal fat excretion might be due to the small number of trials, few participants in each trial and the observed heterogeneity of the trials. Furthermore, scatter plots of treatment effect against the amount of extra calcium applied should be compatible with there being no effect of no extra calcium, and so a simple regression line should intercept the vertical axis at zero treatment effect (27). In this case, with the scatter plot indicating an effect independent of the calcium dose, bias could be a possible explanation. Finally, if subjects' adherence to a treatment varied across trials, a corresponding variation in treatment effects will occur. It is therefore probable that some of the studies included in this meta-analysis underestimated the true ability of calcium to impair fat absorption, as the lack of adherence to the prescribed calcium intake will tend to reduce the effect size. However, many of the studies used faecal and urinary calcium excretion as a compliance marker, and demonstrated at least some adherence.

Normally the quantitative importance for body weight would be assessed by large, controlled, randomized trials, providing high- vs. low-calcium intakes over at least 1 year in order to detect a change in body weight, but it is difficult to maintain strict adherence to specific diets over such a long period. In future trials, compliance should be monitored by measuring faecal and urinary calcium excretion, and the effect size should be adjusted to optimal compliance. Good adherence to calcium intakes with low vs. high intakes might be easier to achieve using calcium supplements, but we would still question whether this can be achieved and a meaningful efficacy assessment can be made without the use of biological adherence markers. Furthermore, if calcium is to affect fat digestibility, it is a condition that fat and calcium are present in the intestine at the same time. Therefore the time of ingestion of calcium and perhaps also the matrix in which calcium is provided (dairy products, tablet, fortified food, etc.) is crucial. In the Women's Health Initiative trial on calcium supplementation (1000 mg elementary calcium plus 400 IU of cholecalciferol [vitamin D] vs. placebo) 36 282 post-menopausal were treated for 7 years (52). Women receiving calcium vs. placebo had a consistently favourable difference in weight change of –0.13 kg (–0.21 to –0.05; $P = 0.001$) (52). After 3 years of follow-up, women with daily calcium intakes less than 1200 mg at baseline who were randomized to supplements were 11% less likely to experience small weight gains (1–3 kg) and 11% less likely to gain more moderate amounts of weight (>3 kg). However, the true effect of calcium is very likely to have been underestimated in this study because of lack of compliance (only 55–63% of the subjects consumed 80% or more of the supplements) (52). As no biological markers of calcium intake were monitored in this study, the true effect size remains an open question.

Boon *et al.* showed, in a 23-year follow-up cohort study, that longitudinal calcium intake only had a positive effect on body composition below an intake level of <800 mg day^{-1} (53). No relation was found in the group with a calcium intake of 800–1200 mg day^{-1} or in the group >1200 mg day^{-1}, suggesting a threshold of approximately 800 mg day^{-1} below which the effect of dietary calcium on body composition is most pronounced (53). It has been suggested that the effect of increased calcium intake on body weight and composition is most pronounced in subjects with a low habitual intake (54). Furthermore, the majority of the studies included in this meta-analysis, which found a significant effect of increased calcium intake on faecal fat excretion, compared a high intake of dietary calcium with a relatively low intake of dietary calcium (Table 2). Thus it is likely that subjects with a low habitual calcium intake will benefit more from an increased calcium intake than subjects with a high habitual calcium intake.

In conclusion, dietary calcium intake has the potential to increase faecal fat excretion to an extent that could be relevant for prevention of weight (re-) gain, and may potentially accentuate weight loss if no compensation occurs. The effect may be most pronounced in subjects with a low habitual dietary calcium intake. There is a need for studies of a longer duration to establish long-term effectiveness.

Conflict of Interest Statement

R. C. is a statistical editor in the *Cochrane Collaboration* (CMSG and PHRG); this is not a *Cochrane Review.*

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Contributions of authors

R. C. participated in the study conception and design, the acquisition of data, the analysis/interpretation of data, drafting and revision of the manuscript and the statistical analyses. J. K. L. participated in the study conception and design, the acquisition of data, the analysis/interpretation of data and drafting of the manuscript. C. R. S. participated in the study conception and design. E. M. B. coordinated the literature search, participated in the acquisition of data and critical revision of the manuscript. E. L. M., W. H. S. and A. T. participated in the study conception, interpretation of data, critical revision of the manuscript and supervision of the study. A. A. generated the idea and took the initiative to conduct the study – participated in the study conception and design, the acquisition of data, the interpretation of data, and drafting and revision of the manuscript. All authors have seen and approved the final version of the manuscript.

References

1. Mccarron DA, Morris CD, Henry HJ, Stanton JL. Blood pressure and nutrient intake in the United States. *Science* 1984; **224**: 1392–1398.
2. Jacqmain M, Doucet E, Despres JP, Bouchard C, Tremblay A. Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. *Am J Clin Nutr* 2003; **77**: 1448–1452.
3. Lin YC, Lyle RM, McCabe LD, McCabe GP, Weaver CM, Teegarden D. Dairy calcium is related to changes in body composition during a two-year exercise intervention in young women. *J Am Coll Nutr* 2000; **19**: 754–760.
4. Loos RJ, Rankinen T, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C. Calcium intake is associated with adiposity in Black and White men and White women of the HERITAGE Family Study. *J Nutr* 2004; **134**: 1772–1778.
5. Lorenzen JK, Molgaard C, Michaelsen KF, Astrup A. Calcium supplementation for 1 year does not reduce body weight or fat mass in young girls. *Am J Clin Nutr* 2006; **83**: 18–23.
6. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. *FASEB J* 2000; **14**: 1132–1138.
7. Kamycheva E, Joakimsen RM, Jorde R. Intakes of calcium and vitamin d predict body mass index in the population of Northern Norway. *J Nutr* 2003; **133**: 102–106.
8. Rajpathak SN, Rimm EB, Rosner B, Willett WC, Hu FB. Calcium and dairy intakes in relation to long-term weight gain in US men. *Am J Clin Nutr* 2006; **83**: 559–566.
9. Zemel MB, Thompson W, Milstead A, Morris K, Campbell P. Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. *Obes Res* 2004; **12**: 582–590.
10. Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L, Silva E. Dairy augmentation of total and central fat loss in obese subjects. *Int J Obes (Lond)* 2005; **29**: 391–397.
11. Zemel MB, Richards J, Milstead A, Campbell P. Effects of calcium and dairy on body composition and weight loss in African-American adults. *Obes Res* 2005; **13**: 1218–1225.
12. Trowman R, Dumville JC, Hahn S, Torgerson DJ. A systematic review of the effects of calcium supplementation on body weight. *Br J Nutr* 2006; **95**: 1033–1038.
13. Zemel MB. The role of dairy foods in weight management. *J Am Coll Nutr* 2005; **24**: 537S–546S.
14. Boon N, Hul GB, Viguerie N, Sicard A, Langin D, Saris WH. Effects of 3 diets with various calcium contents on 24-h energy expenditure, fat oxidation, and adipose tissue message RNA expression of lipid metabolism-related proteins. *Am J Clin Nutr* 2005; **82**: 1244–1252.
15. Boon N, Hul GB, Stegen JH, Sluijsmans WE, Valle C, Langin D, Viguerie N, Saris WH. An intervention study of the effects of calcium intake on faecal fat excretion, energy metabolism and adipose tissue mRNA expression of lipid-metabolism related proteins. *Int J Obes (Lond)* 2007; **31**: 1704–1712.
16. Bortolotti M, Rudelle S, Schneiter P, Vidal H, Loizon E, Tappy L, Acheson KJ. Dairy calcium supplementation in overweight/obese individuals; its effect on markers of fat metabolism. *Am J Clin Nutr* 2008; **88**: 877–885.
17. Astrup A. The role of calcium in energy balance and obesity: the search for mechanisms. *Am J Clin Nutr* 2008; **88**: 873–874.
18. Jacobsen R, Lorenzen JK, Toubro S, Krog-Mikkelsen I, Astrup A. Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. *Int J Obes (Lond)* 2005; **29**: 292–301.
19. Melanson EL, Donahoo WT, Dong F, Ida T, Zemel MB. Effect of low- and high-calcium dairy-based diets on macronutrient oxidation in humans. *Obes Res* 2005; **13**: 2102–2112.
20. Melanson EL, Sharp TA, Schneider J, Donahoo WT, Grunwald GK, Hill JO. Relation between calcium intake and fat oxidation in adult humans. *Int J Obes Relat Metab Disord* 2003; **27**: 196–203.
21. Major GC, Alarie FP, Doré J, Tremblay A. Calcium plus vitamin D supplementation and fat mass loss in female very low-calcium consumers: potential link with a calcium-specific appetite control. *Br J Nutr* 2008; **101**: 659–663.
22. Papakonstantinou E, Flatt WP, Huth PJ, Harris RB. High dietary calcium reduces body fat content, digestibility of fat, and serum vitamin D in rats. *Obes Res* 2003; **11**: 387–394.
23. Gacs G, Barltrop D. Significance of Ca-soap formation for calcium absorption in the rat. *Gut* 1977; **18**: 64–68.
24. Govers MJ, Termont DS, Van Aken GA, Van der MR. Characterization of the adsorption of conjugated and unconjugated bile acids to insoluble, amorphous calcium phosphate. *J Lipid Res* 1994; **35**: 741–748.
25. Govers MJ, Van der MR. Effects of dietary calcium and phosphate on the intestinal interactions between calcium, phosphate, fatty acids, and bile acids. *Gut* 1993; **34**: 365–370.
26. Juni P, Altman DG, Egger M. Systematic reviews in health care: assessing the quality of controlled clinical trials. *BMJ* 2001; **323**: 42–46.
27. Sterne JA, Egger M, Smith GD. Systematic reviews in health care: investigating and dealing with publication and other biases in meta-analysis. *BMJ* 2001; **323**: 101–105.
28. Higgins JPT, Green S. *Cochrane Handbook for Systematic Reviews of Interventions 4.2.6*. [updated September 2006]. John Wiley & Sons, Ltd: Chichester, 2006.
29. Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analyses. *Lancet* 1999; **354**: 1896–1900.
30. Elbourne DR, Altman DG, Higgins JP, Curtin F, Worthington HV, Vail A. Meta-analyses involving cross-over trials: methodological issues. *Int J Epidemiol* 2002; **31**: 140–149.
31. Hedges LV. Distribution theory for glass's estimator of effect size and related estimators. *J Educ Stat* 1981; **6**: 107–128.
32. Normand SL. Meta-analysis: formulating, evaluating, combining, and reporting. *Stat Med* 1999; **18**: 321–359.
33. Curtin F, Altman DG, Elbourne D. Meta-analysis combining parallel and cross-over clinical trials. I: continuous outcomes. *Stat Med* 2002; **21**: 2131–2144.
34. Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *Lancet* 2007; **370**: 1706–1713.
35. Cochran WG. The combination of estimates from different experiments. *Biometrics* 1954; **10**: 101–129.

36. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539–1558.
37. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557–560.
38. Thompson SG, Higgins JP. How should meta-regression analyses be undertaken and interpreted? *Stat Med* 2002; **21**: 1559–1573.
39. Lutwak L, Laster L, Gitelman HJ, Fox M, Whedon GD. Effects of high dietary calcium and phosphorus on calcium, phosphorus, nitrogen and fat metabolism in children. *Am J Clin Nutr* 1964; **14**: 76–82.
40. Bhattacharyya AK, Thera C, Anderson JT, Grande F, Keys A. Dietary calcium and fat. Effect on serum lipids and fecal excretion of cholesterol and its degradation products in man. *Am J Clin Nutr* 1969; **22**: 1161–1174.
41. Saunders D, Sillery J, Chapman R. Effect of calcium carbonate and aluminum hydroxide on human intestinal function. *Dig Dis Sci* 1988; **33**: 409–413.
42. Denke MA, Fox MM, Schulte MC. Short-term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men. *J Nutr* 1993; **123**: 1047–1053.
43. Welberg JW, Monkelbaan JF, de Vries EG, Muskiet FA, Cats A, Oremus ET, Boersma-van Ek W, van Rijsbergen H, van der Meer R, Mulder NH, Kleibeuker JH. Effects of supplemental dietary calcium on quantitative and qualitative fecal fat excretion in man. *Ann Nutr Metab* 1994; **38**: 185–191.
44. Govers MJ, Termont DS, Lapre JA, Kleibeuker JH, Vonk RJ, Van der MR. Calcium in milk products precipitates intestinal fatty acids and secondary bile acids and thus inhibits colonic cytotoxicity in humans. *Cancer Res* 1996; **56**: 3270–3275.
45. Murata T, Kuno T, Hozumi M, Tamai M, Takagi T, Kamiwaki T, Itoh Y. Inhibitory effect of calcium (derived from eggeshell)-supplemented chocolate on absorption of fat in human males. *J Jpn Soc Nutr Food Sci* 1998; **51**: 165–171.
46. Shahkhalili Y, Murset IM, Acheson K. *Calcium and magnesium supplementation of chocolate as a mean to reduce the digestible energy value of chocolate in man*. Abstract presented in the 17th International Congress of Nutrition 27–30 August 2001, Vienna, Austria 2001; (abstr).
47. Shahkhalili Y, Murset C, Meirim I, Duruz E, Guinchard S, Cavadini C, Acheson K. Calcium supplementation of chocolate: effect on cocoa butter digestibility and blood lipids in humans. *Am J Clin Nutr* 2001; **73**: 246–252.
48. Ditscheid B, Keller S, Jahreis G. Cholesterol metabolism is affected by calcium phosphate supplementation in humans. *J Nutr* 2005; **135**: 1678–1682.
49. Bendsen NT, Hother AL, Jensen SK, Lorenzen JK, Astrup A. Effect of dairy calcium quantitative and qualitative fecal fat excretion: a randomized cross-over trial. *Int J Obes (Lond)* 2008; **32**: 1816–1824.
50. Padwal RS, Majumdar SR. Drug treatments for obesity: orlistat, sibutramine, and rimonabant. *Lancet* 2007; **369**: 71–77.
51. Guerciolini R, Radu-Radulescu L, Boldrin M, Dallas J, Moore R. Comparative evaluation of fecal fat excretion induced by orlistat and chitosan. *Obes Res* 2001; **9**: 364–367.
52. Caan B, Neuhouser M, Aragaki A, Lewis CB, Jackson R, LeBoff MS, Margolis KL, Powell L, Uwaifo G, Whitlock E, Wylie-Rosett J, LaCroix A. Calcium plus vitamin D supplementation and the risk of postmenopausal weight gain. *Arch Intern Med* 2007; **167**: 893–902.
53. Boon N, Koppes LL, Saris WH, Van MW. The relation between calcium intake and body composition in a Dutch population: The Amsterdam Growth and Health Longitudinal Study. *Am J Epidemiol* 2005; **162**: 27–32.
54. Thompson WG, Rostad HN, Janzow DJ, Slezak JM, Morris KL, Zemel MB. Effect of energy-reduced diets high in dairy products and fiber on weight loss in obese adults. *Obes Res* 2005; **13**: 1344–1353.