Dairy calcium intake, serum vitamin D, and successful weight loss

Danit R Shahar, Dan Schwarzfuchs, Drora Fraser, Hillel Vardi, Joachim Thiery, Georg Martin Fiedler, Matthias Blüher, Michael Stumvoll, Meir J Stampfer, and Iris Shai for the DIRECT Group

ABSTRACT
Background: The role of dairy calcium intake and serum vitamin D concentrations in weight loss is controversial.
Objective: The objective was to assess the association of dairy calcium intake and serum vitamin D with weight loss.
Design: We analyzed data from participants in the 2-y Dietary Intervention Randomized Controlled Trial (DIRECT) [n = 322; mean body mass index (BMI; in kg/m²): 31; mean age: 52 y]. A representative sample (n = 126) was followed for 6 mo for serum vitamin D changes.
Results: Baseline serum 25-hydroxyvitamin D [25(OH)D] concentrations decreased significantly across the tertiles of baseline BMI (25.6 ± 8.0, 24.1 ± 8.9, and 22.9 ± 6.8 ng/mL, respectively; P for trend = 0.02). Baseline concentrations of vitamin D and dairy calcium intake were not associated with subsequent weight loss. However, in repeated-measures models adjusted for age, sex, baseline BMI, total fat intake, and diet group assignment, higher 6-mo tertile concentrations decreased significantly across the tertiles of baseline BMI (P = 0.043; −3.1, −3.8, and −5.6 kg, respectively, for vitamin D; P = 0.013). In a multivariate logistic regression adjusted simultaneously for age, sex, baseline BMI, total fat intake, diet group, vitamin D concentration, and dairy calcium, an increase of 1 SD in dairy calcium intake increased the likelihood of weight loss of 4.5 kg in the preceding 6 mo [odds ratio (OR): 1.45; 95% confidence interval (CI): 1.27, 1.66; P = 0.009]. A similar increase was seen for serum 25(OH)D at the 6-mo point (OR: 1.7; P = 0.009).
Conclusion: Our study suggests that both higher dairy calcium intake and increased serum vitamin D are related to greater diet-induced weight loss. This trial was registered at clinicaltrials.gov as NCT00160108.

INTRODUCTION
The association of dairy products with body weight is controversial. Several studies (1–3) suggested inverse relations, in which increased dairy intake is associated with lower weight, whereas others showed no association (4, 5). In the few randomized intervention trials of weight loss, increased intake of dairy products led to increased weight loss and decreased fat mass and waist circumference (6, 7). These results were confirmed in our previous 6-mo dietary intervention study in overweight diabetic patients (8), in which increased low-fat dairy intake was associated with enhanced weight loss. A protective association of dairy intake with cardiometabolic measurements such as trunk fat, blood pressure, serum triglycerides, and insulin was previously suggested in some (9–11) but not all studies (8, 12, 13). The effect of calcium supplementation on weight loss is also not clear (14). Vitamin D increases calcium absorption into the bloodstream. It is obtained by sun exposure, from food (mainly fish liver oils, fatty fish, and eggs), fortified foods (such as milk, yogurt, margarine, oil spreads, and breakfast cereal), and supplements. Calcitriol [1,25(OH)₂D₃] is the active form of vitamin D found in the body and calcidiol [25(OH)D₃] is the form measured in the blood to assess vitamin D status (15). Vitamin D status is consistently inversely related to body mass index (BMI) (16, 17), but the direction and causality of this association is uncertain.

We therefore addressed the association between dairy calcium intake and serum vitamin D (25-hydroxyvitamin D [25(OH)D]) with weight loss during the 2-y Dietary Intervention Randomized Controlled Trial (DIRECT) (18).

SUBJECTS AND METHODS
Study population and dietary intervention
The 2-y DIRECT study (18) was conducted between July 2005 and June 2007 in a research center workplace with an active onsite medical clinic. Eligible participants were men and women aged 40–65 y, with a BMI (in kg/m²) >27 or presence of type 2 diabetes or coronary heart disease regardless of age or BMI.

1 From the S Daniel Abraham Center for Health and Nutrition and the Department of Epidemiology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel (DRS, DF, HV, and IS); the Nuclear Research Center Negev, Dimona, Israel (DS); the Department of Medicine and the Institute of Laboratory Medicine, University of Leipzig, Leipzig, Germany (JT, GMF, MB, and MS); the Channing Laboratory, Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA (MJS); and the Departments of Epidemiology and Nutrition, Harvard School of Public Health, Boston, MA (MJS).

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3 Address correspondence to DR Shahar, The S Daniel Abraham International Center for Health and Nutrition, Department of Epidemiology and Health Services Evaluation, Ben-Gurion University of the Negev, PO Box 653, Beer-Sheva 84105, Israel. E-mail: dshahar@bgu.ac.il.
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Participants were randomly assigned to receive 1 of 3 diets: a low-fat diet (19), a Mediterranean diet (20), or a low-carbohydrate (low-carb) diet (21). Each food item available in the self-service cafeteria in the workplace was labeled, showing the number of calories and the number of grams of carbohydrates, fat, and saturated fat. The labels were also color-coded according to diet group and updated daily.

Dairy consumption was not specifically predetermined by any of the diet plans and varied within and across the groups by personal choice. For the low-fat and Mediterranean diets, low-fat dairy products (0–5% fat) were included in the protein group and thus could be consumed in lieu of other sources of protein such as meat. For the low-carb group, all low-carb dairy products could be consumed, regardless of fat content.

The study was approved and monitored by the Human Subjects Committee of the Soroka University Medical Center and Ben-Gurion University (Israel). Each participant provided written informed consent at the beginning of the trial.

Dietary intake

Dietary intakes, including dairy products, were evaluated by a validated food-frequency questionnaire (FFQ) (12, 22) that included 127 food items and 3 portion-size pictures for 17 items (23). The questionnaires were self-administered electronically via the workplace intranet. The electronic questionnaires enabled completeness of data through prompts to avoid missing lines and permitted rapid automated reporting for the group dietitians. Participants completed the questionnaires at baseline, 6 mo (reflecting the dietary intake of months 0–6), 12 mo (reflecting months 6–12), and 24 mo (reflecting months 12–24). Dietary calcium and vitamin D intakes were calculated from the FFQ, stratified by dairy and nondairy sources. We aggregated milk and dairy products from the FFQ into a “dairy” group, and the nutrient (macro- and micronutrients) contribution by these products was calculated. The dairy group included 12 items, low- and regular-fat milk, chocolate milk, low- and regular-fat yogurts with and without fruit and sugar, all sorts of low-fat and regular-fat cream, and yellow and white hard cheeses. Some of these items are fortified with either calcium or vitamin D, and this was taken into account in our calculations. We used dairy calcium as an indicator for dairy intake (7, 24). We analyzed our data with the Israeli food database (25).

Serum vitamin D

A blood sample was drawn by venipuncture at 0800, after a 12-h fast, and stored at –80°C until assay for serum 25(OH)D and 1,25-dihydroxyvitamin D [1,25(OH)2D] at the University of Leipzig, Leipzig, Germany. These concentrations were determined in samples drawn at baseline and after 6 mo in random subsamples of 42 participants drawn equally from each of the 3 diet types (total of 126 participants). The random sampling was obtained independently for each diet group by using identification numbers of the participants and a random-digit table. Determinations of 25(OH)D were made in serum by a chemiluminescence immunoassay by using the LIAISON platform (DiaSorin, Stillwater, MN). The functional sensitivity of this test was <4.0 ng/mL. Intra- and interassay CVs were between 4.5% and 7.1% for the range of 19–57 ng 25(OH)D/mL. Measurements of 1,25(OH)2D were accomplished by a radioimmunoassay with 125I-labeled antigen after an immunoextraction procedure for serum matrices (IDS, Boldon, United Kingdom). The sensitivity of this test was <3.4 pg/mL. Intra- and interassay CVs were between 6.3% and 10.2% for the range of 13.6–54.5 pg 1,25 (OH)2D/mL.

Outcomes

Body weight was measured without shoes to 0.1 kg every month. Height was measured to the nearest millimeter by using a wall-mounted stadiometer at baseline for BMI determination. Waist circumference was measured halfway between the last rib and the iliac crest.

Statistical analysis

Statistical analysis was performed by using SPSS PASW, version 17 (SPSS Inc, Chicago, IL). We evaluated means and the linear trends of baseline characteristics across tertiles of BMI and used Spearman’s correlation coefficients to assess the correlations between dairy and nondairy dietary calcium and serum 25(OH)D and 1,25(OH)2D at baseline. We assessed the associations between dietary dairy intake and serum vitamin D at baseline with weight loss in the first 6 mo of the study.

Our main aim was to address the association of tertiles of dairy calcium and serum vitamin D status assessed after 6 mo of the dietary intervention with weight loss during the preceding 6 mo and subsequent weight loss during the remaining 18 mo of the intervention. We used 2 strategies: the first used the actual status at 6 mo, reflecting the dairy calcium intake in the first 6 mo of the intervention and serum 25(OH)D at 6 mo; the second used the change of dairy intake and vitamin D concentrations from baseline to 6 mo. We performed repeated-measures (24 time points) general linear models (GLM) analysis, adjusted for the potential confounders of age, sex, baseline BMI, total fat intake, and assigned diet group to assess the relation of 6-mo levels for dairy calcium intake, serum 25(OH)D, and 1,25(OH)2D with dynamic of weight change during 24 mo of the weight-loss intervention. We repeated these analyses to evaluate associations of changes between baseline and the 6-mo levels for dairy calcium intake per 1000 kcal and serum 25(OH)D with weight changes during the 24 mo of intervention. Goodness-of-fit measures were used to select the best-fitting model. Models with or without diet group did not change the independent contribution of dairy calcium and 25(OH)D to weight loss.

Finally, we performed a multivariable logistic regression model to determine the independent contribution of dairy calcium intake and serum 25(OH)D on the odds for successful weight loss (above the median weight loss of 4.5 kg) by using increments of SD to the mean at 6 mo, when both are considered within one model after adjustment for the possible confounders listed above.

RESULTS

Serum baseline 25(OH)D concentrations (Table 1) were significantly lower within the higher tertiles of mean (±SD) baseline BMI (25.6 ± 8.0, 24.1 ± 8.9, and 22.9 ± 6.8 ng/mL respectively; P for trend = 0.02). The proportion of participants with suboptimal serum 25(OH)D (<30 ng/mL) (27) ranged from 72.6%, 78.0%, and 86.9% across tertiles of BMI (P = 0.06).
There was a positive significant association between serum 25(OH)D and dairy calcium intake at baseline ($r = 0.14, P = 0.025$). Nondairy calcium intake was not correlated with serum 25(OH)D. Dairy products were the main contributors of dietary vitamin D. Serum 1,25(OH)₂D was not significantly correlated with either baseline BMI, dairy or nondairy calcium intake, or serum 25(OH)D. Dairy calcium intake and serum 25(OH)D concentrations at baseline were not associated with weight loss in the first 6 mo.

In a GLM repeated-measures analysis, adjusted for age, sex, baseline BMI, total fat intake, and assigned diet group (Figure 1), we observed an independent association of higher tertiles levels and of dairy calcium intake (reflecting the dietary intake within the previous 6 mo) and serum vitamin D at 6 mo with successful weight loss during 24 mo of intervention. Across higher tertiles of dairy calcium assessed at 6 mo (median for tertiles: 156.5, 358.0, and 582.9 mg/d, respectively), the 24-mo weight losses were $-3.3 \pm 5.6, -3.5 \pm 6.1,$ and $-5.3 \pm 5.4$ kg, respectively ($P$ value for the between-group effect = 0.043) (Figure 1A). Across higher tertiles of serum 25(OH)D assessed at 6 mo (median for tertiles = 14.5, 21.2 ± 1.9, and 30.2 mg/mL, respectively), the 24-mo weight losses were $-3.1 \pm 5.7, -3.8 \pm 4.4,$ and $-5.6 \pm 6.6$ kg, respectively ($P$ value for the between-group effect = 0.013) (Figure 1B). 1,25(OH)₂D was not related to 24-mo weight loss (data not shown).

In a secondary analysis, we performed similar multivariate GLM repeated-measures models by using tertiles of changes from baseline to 6 mo of dairy calcium intake per 1000 kcal and changes of serum 25(OH)D from baseline (Figure 2). Changes in dairy calcium intake per 1000 kcal (median for tertiles = $-62.4$ mg/1000 kcal, $+16.4$ mg/1000 kcal, and $+129.4$ mg/kcal, respectively) were not significantly related to successful weight loss during the 24 mo of intervention; weight loss across tertiles was $-3.7 \pm 5.6, -4.2 \pm 6.1,$ and $-4.2 \pm 5.4$ kg, respectively ($P$ value for the between-group effect =0.19) (Figure 2A). Between baseline and 6 mo, the average concentrations of serum 25(OH)D decreased ($-3.0 \pm 7.0$ ng/mL), corresponding to the seasonal changes from summer to winter (28). Tertiles of change in serum 25(OH)D (median for tertiles = $-9.2, -2.6,$ and $+2.5$ ng/mL, respectively) were significantly associated with greater 24-mo weight loss ($-2.5 \pm 4.9, -4.0 \pm 5.3,$ and $-5.8 \pm 7.0$ kg, respectively; $P$ value for the between-group effect = 0.009) (Figure 2B).

In a multivariate logistic regression model (Table 2), we simultaneously included the 6-mo concentrations of 25(OH)D and dietary dairy calcium intake, reflecting diet in the previous 6 mo since the start of the intervention. An increment of 1 SD in dairy calcium (SD = 240 mg/d) was associated with an odds ratio (OR) of 1.45 (95% CI: 1.006, 2.08; $P = 0.046$) for 6-mo weight loss above the median. For serum 25(OH)D (SD = 8 ng/mL), the corresponding OR was 1.7 (95% CI: 1.14, 2.55; $P = 0.009$). Thus, both factors were independently and significantly associated with successful weight loss (above median = 4.5-kg weight loss) at 6 mo, the “weight-loss phase.” When using the same model to predict 24-mo weight loss (above median = 3.2 kg weight loss), only dairy calcium remained close to significance (OR: 1.42; 95% CI: 0.998, 2.01; $P = 0.051$).

### DISCUSSION

In this 2-y dietary intervention trial, we addressed the association of dairy calcium intake and serum 25(OH)D with weight loss. Concentrations of serum 25(OH)D at baseline were inversely associated with BMI and were directly associated with consumption of dairy calcium. Neither dairy calcium intake nor serum 25(OH)D at baseline was associated with weight loss.
during the intervention. However, both higher dietary dairy calcium and increased serum 25(OH)D at the 6-mo point were significantly associated with successful weight loss over the 24-mo intervention.

Our study merits discussion of some limitations. Participants were not randomly assigned to dietary groups that varied by dairy calcium intake, so we cannot directly show a causal effect. Results from the subsample of vitamin D measurements were more robust, but we recognize the limited sample size. Finally, the dietary assessment was based on a FFQ, which is better for ranking, rather than by evaluating absolute values. Nevertheless, the correlations between the FFQ and 24-h recall questionnaires were examined in a subgroup of the DIRECT (29) and were >0.8 for most nutrients, suggesting that the FFQ estimation of dietary intake is fairly reliable. The strengths of our study include the long-term, one-phase trial; the careful dietary assessment; and the high retention rate.

Our results regarding the contribution of dairy calcium to weight loss confirm those of previous studies (2, 8, 10, 26). In addition, in obesity-prone transgenic mice (30), low-calcium diets impeded body fat loss, whereas high-calcium diets suppressed fat accretion and weight gain on an obesity-promoting diet and markedly accelerated weight and fat loss during caloric restriction regimen (9, 30, 31). In humans, dairy calcium exerted significantly greater antiobesity effects than did supplemental calcium, probably due to its rich content of bioactive compounds (31, 35, 36). For example, milk proteins contain significant angiotensin-converting enzyme (ACE) inhibitory activity (32, 33). Data indicate that adipocyte lipogenesis is regulated, in part, by angiotensin II and that adipocytes have an intact paracrine/autocrine rennin-angiotensin system (32). Moreover, ACE inhibition mildly attenuates obesity in rodents. Thus, dairy-based ACE inhibition may explain, at least in part, the significantly greater effect exerted by dairy calcium than by nondairy calcium on the percentage of weight loss in our participants.

It is also possible that a portion of the antiobesity effects of dietary calcium may be due to an increase in fecal fatty acid excretion, because clinical studies show that substantial (2–4 g) increases in dietary calcium result in statistically significant, but modest, increases in fecal fat losses (34, 35). A supplement of 2 g calcium (as calcium carbonate) has been shown to result in an

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**FIGURE 1.** Weight change across 6-mo tertiles of dairy calcium intake (reflecting dairy intake in the previous 6 mo) and serum 25-hydroxyvitamin D [25(OH)D] at 6 mo by using repeated-measures models adjusted for age, sex, baseline BMI, total fat intake, and diet group assignment. A: Weight change by tertiles of dairy calcium intake during the first 6 mo (n = 322) (P value for the between-group effect = 0.043). B: Weight change by tertiles of serum 25(OH)D at 6 mo (n = 126) (P value for the between-group effect = 0.013).

**FIGURE 2.** Adjusted weight change by tertiles of change (from baseline to 6 mo) in dairy calcium intake per 1000 kcal (mg/1000 kcal) and serum 25-hydroxyvitamin D [25(OH)D] (ng/mL) by using repeated-measures models adjusted for age, sex, baseline BMI, total fat intake, and diet group assignment. A: Weight change by tertiles of change (from baseline to 6 mo) in dairy calcium intake per 1000 kcal (n = 322) (P value for the between-group effect = 0.19). B: Weight change by tertiles of change in serum 25(OH)D from baseline to 6 mo (n = 126) (P value for the between-group effect = 0.009).
increase in fecal fat excretion from 6.8% to 7.4% of total fat intake (35, 7). Although this will contribute to a net negative energy balance, this effect is too small to explain the large effect size shown in our present study. Thus, it seems that the primary effects are likely to result from the contributions deriving from dairy-derived bioactive compounds discussed above and from inhibition of fat absorption.

We observed that both higher dietary dairy calcium intake and increased serum vitamin D were independently associated with weight loss. Supplements-related studies are more common than dietary studies on the topic of calcium and vitamin D intake and weight loss. In the Women’s Health Initiative Study (36), women randomly assigned to the calcium and vitamin D supplementation arm had significantly less weight gain; although the absolute effect was modest, they 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) (P for interaction for baseline calcium intake = 0.008). The effect was observed primarily in women who reported inadequate calcium intakes. In this case, it was not possible to distinguish between the effect of calcium and vitamin D supplementation, and the distinction of the intervention and treatment groups was obscured due to substantially attenuated adherence. An intervention trial of supplementation with calcium and vitamin D in 63 women showed more profound results among women with calcium intake <800 mg/d. In this study, the supplements enhanced the beneficial effect of body weight loss on the lipid and lipoprotein profile in overweight or obese women (17).

Despite the natural decrease in sun exposure from baseline (summer) to the 6-mo visit (winter) (39), we witnessed an increase in serum 25(OH)D among participants who lost more weight. Baseline concentrations of serum 25(OH)D were inversely associated with BMI, as shown in previous studies (37, 38). One possible explanation of the association is a causal relation by which higher vitamin D concentrations promote metabolic pathways favoring loss of weight or enhanced lean body mass (2). Teegarden et al (31) showed an increase in the thermic effect of food and a tendency toward increased fat oxidation in overweight women with higher 25(OH)D concentrations. Alternatively, obesity may reduce vitamin D concentrations, potentially by fat sequestering vitamin D. Wortsman et al (37) suggested that obesity did not affect the capacity of the skin to produce vitamin D but may alter the release of vitamin D from the skin into the circulation. Their studies showed that an identical amount of ultraviolet-B irradiation resulted in a smaller increase in blood vitamin D concentrations by 57% compared with nonobese subjects, 24 h after the exposure, despite larger body surface area of exposure. The authors suggested that the expanded subcutaneous fat in obese persons, which is known to store vitamin D, sequestered more of the cutaneous, synthesized vitamin D (37).

The 2005 Dietary Guidelines for Americans include a recommendation for 3 cups (720 mL) of milk per day, an amount that was shown to be beneficial for weight loss in our study. However, before recommending an increase in dairy consumption, it is important to take into consideration the possible negative effect of dairy products on prostate and breast cancer. Although some epidemiologic studies have shown a relation between dairy consumption and prostate cancer risk (40), more recent studies have shown no relation or a reduction of risk with dairy consumption (41, 42), which may vary with fat content (43). Moreover, a review of the data on the association between dairy foods and breast cancer (44) did not reveal any association. These data were confirmed in a recent review by Weaver (45).

In summary, our findings suggest that both higher consumption of dairy calcium and increased serum vitamin D are independently associated with successful weight loss. The causal relation between these factors needs further clarification.

The authors’ responsibilities were as follows—DRS: conceptualized the idea, analyzed the data, and wrote the first draft of the manuscript; DS: contributed to the conceptualization of the idea, interpreted the data, and contributed to drafts of the manuscript; DF and HV: were responsible for the statistical analyses and interpretation of the results and reviewed drafts of the manuscript; JT, GMF, MB, and MS: were in charge of all the laboratory work of the current study and reviewed drafts of the manuscript; and MJS and IS: are the principal investigators of the DIRECT study and were involved in data analyses and writing and reviewing the drafts of the manuscript. All authors approved the last version of the manuscript. The German Research Foundation was not involved in any stage of the design, conduct, or analysis of the study and had no access to the study results before publication. None of the authors had any financial or any other kind of personal conflicts with this article.

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