

## Short Communication

# Calcium plus vitamin D supplementation and fat mass loss in female very low-calcium consumers: potential link with a calcium-specific appetite control

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This randomized, double-blind, placebo-controlled study was conducted to compare the effect of a 15-week weight-reducing programme (–2900 kJ/d) coupled with a calcium plus vitamin D (calcium+D) supplementation (600 mg elemental calcium and 5 µg vitamin D, consumed twice a day) or with a placebo, on body fat and on spontaneous energy/macronutrient intake. Sixty-three overweight or obese women (mean age 43 years, mean BMI 32 kg/m<sup>2</sup>) reporting a daily calcium intake <800 mg participated in present study. Anthropometric variables, resting energy expenditure and spontaneous energy intake were measured before and after the 15-week programme. The calcium+D supplementation induced no statistically significant increase in fat mass loss in response to the programme. However, when analyses were limited to very low-calcium consumers only (initial calcium intake ≤600 mg/d, *n* 7 for calcium+D, *n* 6 for placebo), a significant decrease in body weight and fat mass ( $P<0.01$ ) and in spontaneous dietary lipid intake ( $P<0.05$ ) was observed in the calcium+D but not in the placebo group. In very low-calcium consumers, change in fat mass was positively correlated with change in lipid intake. During the weight-reducing programme, a calcium+D supplementation was necessary in female overweight/obese very low-calcium consumers to reach significant fat mass loss that seemed to be partly explained by a decrease in lipid intake. We propose that this change in lipid intake could be influenced by a calcium-specific appetite control.

**Calcium: Vitamin D: Body fat: Lipids: Appetite**

The hypothesis that calcium/dairy supplementation might accentuate fat mass loss in the context of a weight-reducing programme in obese individuals compared to a non-supplemented control condition has been demonstrated in some<sup>(1,2)</sup>, but not all<sup>(3)</sup> studies. One explanation for this discrepancy could be the difference in habitual calcium intake of participants. Indeed, based on results showing that women consuming less than 600 mg calcium/d had an increased percentage body fat<sup>(4)</sup>, the existence of a threshold of usual calcium intake below which a supplementation in this mineral would effectively promote fat mass loss is possible. The first objective of the present study was to investigate this hypothesis and to assess the impact of a calcium plus vitamin D (calcium+D) supplementation on the outcome of a weight-reducing programme in female low- and very low-calcium consumer (VL-CC) overweight/obese subjects.

Up to now, the potential effect of calcium on energy balance and adiposity has been explained by a suppression of calcitriol (1,25-dihydroxyvitamin D) which decreases intra-adipocyte calcium influx and concentration<sup>(5)</sup> leading to a reduced lipogenic gene expression and stimulation of lipolysis and adipocytes uncoupling protein 2 expression<sup>(6,7)</sup>.

Moreover, a diet high in calcium was also shown to increase 24 h lipid oxidation<sup>(8)</sup> and to reduce lipid absorption due to the intestinal formation of calcium-fatty acids insoluble ‘soaps’ that are excreted in the faeces<sup>(9)</sup>. From another perspective, the idea of a ‘calcium-specific appetite control’ was proposed by Tordoff<sup>(10)</sup> who documented the possibility that low calcium intake or stores might trigger episodes of increased desire to eat and stimulate motivation to seek out or choose calcium-containing items, and potentially other nutrients and/or food ‘recognised’ as calcium-rich<sup>(10)</sup>. Although a calcium-specific appetite exists in rats<sup>(11)</sup> it is not known if in man such a specific appetite control could contribute to the calcium-induced effect on adiposity. Thus, a secondary objective of the present study was to determine whether a possible contribution of calcium+D supplementation to fat mass loss could be related to changes in spontaneous energy/nutrient intake.

## Methods

Habitual calcium intake of participants was carefully assessed in a two-step process. First, a pre-screening evaluation

**Abbreviations:** calcium+D, calcium plus vitamin D; VL-CC, very low-calcium consumer.

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questionnaire on usual consumption of food high in calcium was administered during a telephone conversation to people who answered recruitment advertisements. Second, a 3 d dietary record administered at baseline<sup>(12)</sup> was explained by a nutritionist and a computerized version of the Canadian Nutrition File (version 0.99 02.09.97) was used to assess macro- as well as micronutrient content of food<sup>(13)</sup>. A sample of 234 women residing in the Québec City metropolitan area corresponded to the study inclusion criterion for habitual calcium intake which was originally set at  $\leq 600$  mg/d (assessed with the pre-screening evaluation questionnaire), but increased to  $< 800$  mg/d to increase recruitment rate. Other inclusion criteria have been reported elsewhere<sup>(14)</sup>.

The study protocol and weight-loss intervention ( $-2900$  kJ/d, non-macronutrient specific) have been described in detail elsewhere<sup>(14)</sup>. Briefly, eighty-four women were initially enrolled and randomized in a double-blind manner to receive the calcium+D supplement (Caltrate<sup>®</sup> 600+D; Wyeth Consumer Healthcare Inc., Madison, NJ, USA) or the placebo coupled to a 15-week energy restriction. The calcium+D supplement tablets were composed of 600 mg elemental calcium and 5  $\mu$ g vitamin D and were taken before breakfast and lunch (one tablet each time) for a daily total of 1200 mg calcium and 10  $\mu$ g vitamin D from the supplement. Nineteen women dropped out after enrolment (eleven from the active group and eight from the placebo group), and two participants were excluded from the final analysis within total blindness of their assignment to one or the other treatment group, due to significant well-documented deviations from protocol guidelines. Their exclusion had no marked effect on the results. Moreover, six and four women from calcium+D and placebo groups, respectively, were excluded from the buffet meal analyses due to missing data. The study protocol was approved by the Laval University Ethics Committee (2001-213-R-2).

Baseline (week 0) anthropometric variables and RMR were measured, as previously described<sup>(14,15)</sup>. Approximately 3 h after consumption of a standardized breakfast, energy and macronutrient intake during an *ad libitum* buffet-type meal test (buffet) were measured as previously described<sup>(16)</sup>. In our laboratory, this buffet has been shown to have a high reproducibility and to be a reliable method for assessment of macronutrient preferences<sup>(17)</sup>. These measurements were repeated after 15 weeks of intervention.

Statistical analyses were performed using software from the SAS Institute (version 9.1.2; Cary, NC, USA). The cut-off value of 600 mg was used to determine VL-CC ( $\leq 600$  mg/d) and low-calcium consumer ( $> 600$  and  $< 800$  mg/d) status based on results obtained previously in our laboratory<sup>(4)</sup>. A two-way ANOVA with repeated measure on one factor (time) was used to assess the effects of treatment (calcium+D and placebo) and time, and their interaction on anthropometric variables. A paired *t* test between groups on changes in relevant variables was used to assess 95 % CI. Pearson correlation analyses were performed in VL-CC groups to assess the relationship between change in lipid intake (g) during the buffet and fat mass change.  $P < 0.05$  was considered significant.

## Results

Characteristics of the calcium+D and placebo groups at baseline and after the weight-loss programme have been presented

elsewhere<sup>(14)</sup> and essentially revealed significant but comparable changes in anthropometric variables between groups (results not shown). However, when only the VL-CC were considered in the analyses, significant time  $\times$  treatment interaction effects were observed for changes in body weight, BMI, fat mass and percentage fat (Table 1). These variables were adjusted for baseline body weight which did not affect the significance of the results except for percentage fat ( $P = 0.06$ ).

Although VL-CC calcium+D and placebo groups differed significantly for baseline habitual calcium intake ( $P < 0.0001$ ), analysis of 3 d diaries revealed no other difference in habitual energy and macronutrient intake. Analysis of the spontaneous energy and macronutrient intake during the buffet in week 15 showed that compared to baseline, the calcium+D group consumed significantly less lipids whereas the placebo group consumed more lipids, although not significantly (Table 1). This resulted in a between-group difference for change in total energy intake totalizing approximately 1000 kJ (Table 1). No significant change or interaction effect was observed for RMR.

Significant positive correlation between changes in lipid intake during the buffet and in fat mass was observed in the VL-CC groups (Fig. 1).

## Discussion

The present results showed, in agreement with others<sup>(3,18)</sup>, that a calcium+D supplementation induced no statistically significant increase in fat mass loss in response to an energy restriction. On the other hand, and also in accordance with studies in low-calcium consumers<sup>(1,2)</sup>, women in the present study characterized by a calcium intake  $< 600$  mg/d who received the calcium+D experienced a weight and fat loss more than four times that observed in their placebo counterparts who did not lose weight significantly despite their efforts to comply with the dietitian's nutritional guidelines. Others have suggested that energy restriction might be a prerequisite for calcium supplementation to exert its effect on energy balance<sup>(19)</sup>. The present results agree with this hypothesis but further suggest that in this condition a calcium+D supplementation might be necessary for people with very low-calcium intake to achieve a successful weight loss, as previously suggested by the threshold hypothesis<sup>(4,18)</sup>. Indeed, expected fat mass change calculated from a model that takes into consideration prescribed energy deficit<sup>(20)</sup> was achieved in calcium+D only (4.4 and 4.7 kg for expected and actual, respectively).

To our knowledge, the present study is the first to report in human subjects a possible association between calcium and vitamin D supplementation, body fat and variables influenced by appetite control. Indeed, in response to the calcium+D supplementation, the VL-CC group decreased their spontaneous lipid intake during the buffet, which is a test mimicking free-living conditions of *ad libitum* access to food, whereas their placebo counterparts increased their lipid intake (not significantly). This suggests a macronutrient-specific effect of the calcium+D supplementation on spontaneous energy intake, and ties up with the concept of a 'calcium-specific appetite control' recently proposed by Tordoff<sup>(10)</sup>. Although this concept has not been well characterized in man, appetite for specific minerals and for calcium in particular<sup>(11)</sup> exists in

**Table 1.** Characteristics of very low-calcium consumer (VL-CC; habitual calcium intake  $\leq 600$  mg/d) calcium plus vitamin D (calcium+D) and placebo groups in baseline and after the weight-loss programme‡  
(Mean values and standard deviations)

	VL-CC calcium+D (n 7)						VL-CC placebo (n 6)						<i>P</i> <			
	Week 0		Week 15		Change		Week 0		Week 15		Change					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Time	Treat	Inter§	95% CI
Body weight (kg)	78.0	6.0	72.2***	4.9	-5.8	2.6	83.0	12.3	81.6††	13.9	-1.4	-2.4	0.0003	0.01	0.009	-5.7, -1.7
BMI (kg/m <sup>2</sup> )	29.8	1.2	27.6***	1.3	-2.2	0.9	32.1†	3.8	31.6†††	4.5	-0.5	0.9	0.0002	0.12	0.008	-2.2, -0.7
Waist circumference (cm)	97.1	3.8	91.5	4.4	-5.6	3.3	104.7	9.3	101.2	11.1	-3.5	2.9	0.0002	0.03	0.25	-6.6, -2.8
Fat mass (kg)	29.0	4.1	24.3**	3.7	-4.7	2.3	31.8	7.4	30.6††	8.7	-1.2	2.4	0.36	0.0009	0.02	-4.8, -1.3
Fat-free mass (kg)	49.0	4.9	47.9	4.3	-1.1	1.5	51.2	5.8	51.0	5.7	-0.2	0.7	0.09	0.85	0.20	-1.4, 0.1
Percentage fat	37.2	4.2	33.7**	4.3	-3.5	2.2	38.0	4.3	36.9†	4.8	-1.0	2.0	0.003	0.70	0.06	-3.8, -0.9
Energy intake (kJ)	3655	1544	2893	758	-182	330	3994	1851	4245	2282	26	349	0.49	0.97	0.36	-320, 164
Lipid intake (g)	45	14	27*	7	-18.2	12.5	41	21	50	30	7.5	14	0.98	0.24	0.02	-19, 8
RMR																
kJ/24 h per kg fat-free mass	131.8	10.5	128.4	9.6	-0.8	5.4	133.9	7.1	131.0	8.8	-2.9	9.6	0.59	0.46	0.72	-7.1, 3.3
kcal/24 h per kg fat-free mass	31.5	2.5	30.7	2.3	-0.2	1.3	32.0	1.7	31.3	2.1	-0.7	2.3	0.59	0.46	0.72	-1.7, 0.8

Inter, time x treatment interaction; Treat, treatment effect.

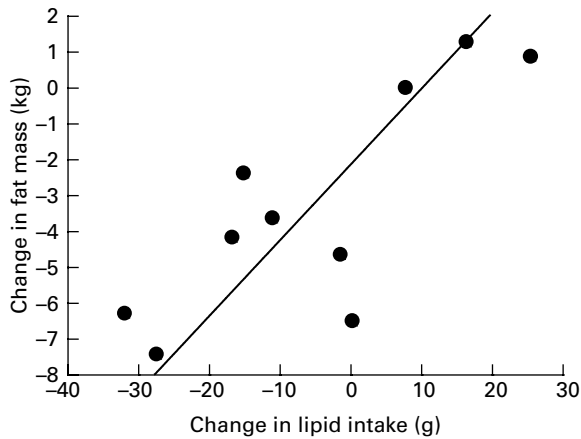
Mean values were significantly different from those of week 0: \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.0001.

Mean values were significantly different from those of the VL-CC calcium+D group for the same time period: †*P*<0.05, ††*P*<0.01, †††*P*<0.0001.

‡ For details of subjects and procedures, see Methods.

§ Adjusted for body weight in baseline.

|| n 5 for calcium+D and placebo groups.



**Fig. 1.** Correlation between changes in lipid intake during the *ad libitum* buffet and in fat mass in very low-calcium consumer (habitual calcium intake  $\leq 600$  mg/d) calcium plus vitamin D and placebo groups ( $r$  0.80;  $P=0.005$ ).

deficient animals<sup>(10)</sup>. Since in man the main source of calcium is food, a specific appetite driven-behaviour is likely to be accompanied by an increase in energy intake despite being incidental to the need for calcium as observed in animals<sup>(10)</sup>. Accordingly, an adequate calcium intake could reverse this tendency and favour the success of a weight-reducing programme. Interestingly, the lower than expected total body weight loss in placebo women suggests that these women decreased their food intake by approximately 625 kJ/d<sup>(20)</sup>, indicating an inability to comply with the  $-2900$  kJ/d prescribed energy deficit.

As for the specific effect on lipids, one can only speculate that the co-occurrence of fats and calcium in an abundance of food, especially dairy food, might orientate calcium-deficient individuals towards a selection of high-fat food as a result of the learnt association between these two nutrients. Indeed, it is believed that if an innate behavioural mechanism to respond to calcium deficiency exists in man, it would be associated with the complex flavour profile that characterizes most appreciable sources of calcium<sup>(10)</sup>.

In accordance with others<sup>(9)</sup>, change in RMR could not explain the difference in body weight loss between groups. Alternatively, assuming that a calcium-induced increase in faecal fat excretion would have accounted for an energy loss of approximately 350 kJ/d, as observed by others<sup>(9)</sup>, it could have only partially explained the approximately 141 MJ, or approximately 1.35 MJ/d difference in body mass loss between the VL-CC groups (based on an energy equivalent of fat mass and fat free mass corresponding to 39.5 and 7.6 MJ/kg, respectively<sup>(21)</sup>). In this regard, change in total lipid consumption during the buffet represents a difference in energy intake between groups which averaged approximately 1000 kJ. Assuming that participants' diet during the 15 weeks was of a similar macronutrient composition as what they ate during the buffet, which averaged approximately half of their reported daily energy intake, the effect of calcium supplementation on macronutrient preference appears to be the variable measured in the present study that most substantially explained the difference in body weight loss between VL-CC calcium+D and placebo groups. Moreover, according to the respiratory quotient and food quotient concept<sup>(22)</sup> the

effect of calcium+D supplementation on spontaneous fat intake means that this group would have been more prone to be in negative fat balance over time. In support of this, change in lipid intake during the buffet was positively correlated with change in fat mass that was the best indicator of variation in fat balance in this study. However, given the small group of participants in the present study and the methods used for gathering information on habitual calcium consumption, the effect of very low-calcium intake on macronutrient preference deserves replication.

In conclusion, in the context of a weight-reducing programme, a calcium+D supplementation was necessary in female overweight/obese VL-CC to reach significant fat mass loss that seemed to be partly explained by a decrease in lipid intake. We propose that this change in lipid intake could be influenced by a calcium-specific appetite control.

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