CALCIUM INTAKE AND THE OUTCOME OF SHORT – TERM WEIGHT MANAGEMENT

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Summary

Experimental and epidemiological studies suggest that calcium intake is inversely related to weight gain. Calcium of dairy origin has been shown to be more effective in promoting weight loss. However, clinical studies yielded controversial results concerning the role of calcium intake in weight change. The aim of this study was to reveal whether the addition of calcium can affect the outcome of 3-week weight management (WM) with a hypocaloric diet characterized by a decresed calcium intake. Overweight/obese women (n: 67; BMI: 32.2±4.1 kg/m^2; age: 49.1±12.1 years) underwent a 4-week comprehensive WM programme. WM included a 7 MJ/day diet resulting in a stable weight during the 1^{st} week and a 4.5 MJ/day diet with mean daily calcium intake 350 mg during the 2^{nd} – 4^{th} week. Participants were divided in three age- and BMI-matched groups who received placebo or calcium (500 mg/day). Cacium was administered either as carbonate or calcium of dairy origin (Lactoval). There was no significant difference in weight loss in response to WM between the placebo-treated and calcium-treated groups. However, addition of calcium to diet resulted in a lower hunger score in the Eating Inventory as well as a decrease in plasma resistin level. Body composition measured by bioimpedance demonstrated that added calcium lead to a preservation of fat free mass. Nevertheless, a greater loss of fat free mass in the placebo group might be partly due to a greater loss of water.

Key words: obesity – calcium intake – weight change – fat-free mass - hunger – resistin

Introduction

Experimental studies have demostrated that calcium intake lowers body weight in rats and mice (Bursey *et al.* 1989, Shi *et al.* 2001). Several observational studies have shown an inverse relationship between dietary calcium intake or intake of dairy products and body weight (Barger-Lux *et al.* 2001, Davies *et al.* 2000, Jacqmain *et al.* 2003, Lin *et al.* 2000, Zemel *et al.* 2000, Zemel *et al.* 2004). Davies *et al.* (2000) analyzed four observational, two cross-sectional and two longitudinal studies and confirmed significant negative association between calcium intake and body weight. According to this analysis, differences in calcium intake could explain 3% of the variance in body weight. CARDIA study revealed a negative relationship between calcium intake or dairy consumption and obesity and insulin resistance syndrome (Pereira *et al.* 2002). Recent results of Liu *et al.* (2005) also indicate that the intake of calcium and dairy products may be associated with lower prevalence of metabolic syndrome in middle-aged and older women. In observational studies on the role of calcium intake confounding factors, such as dairy protein, should be taken into account. In our recent study (Kabrnova *et al.* 2004) in 208 obese individuals (BMI: 40.0 $\pm$ 7.7 kg/m^2) body weight changes over a 3- to 6-month weight management (WM) were negatively related to changes in the intake of both dietary calcium (-0.210, $p = 0.003$) and protein (-0.289, $p = 0.000$).

A few of the intervention studies conducted on the role of calcium intake on body weight and body composition yielded conflicting results (Boon *et al.* 2005, Rajpathak *et al.* 2006, Thompson *et al.* 2005, Chan *et al.* 1995). Studies have also demonstrated that calcium from dairy products is more effective in reducing body weight than calcium from supplements (Zemel *et al.* 2004, Zemel *et al.* 2000).

Different mechanisms were proposed to mediate the effects of dietary calcium on body weight changes. Formation of faecal fatty acid complexes to reduce fat absorption may represent an important mechanism through which calcium affects body weight regulation

(Jacobsen *et al.* 2005). Elevation of faecal fat excretion in response to increased calcium intake should be considered especially in individuals with an excessive fat intake. Intracellular Ca^{2+} is a key regulator of lipid metabolism. Its elevated intracellular concentrations stimulate the expression and activity of lipogenic enzymes and reduce lipolysis with a subsequent increased accumulation of fat in adipocytes (Zemel *et al.* 2000). Dietary calcium-induced suppression of 1.25-dihydroxyvitamin D diminishes the entry of Ca^{2+} into adipocytes and as a consequence also diminishes fat storage in adipocytes (Xue *et al.* 2001, Zemel 2003). Calcium might affect energy balance by stimulating an expression of uncoupling proteins in adipocytes (UCP2) and skeletal muscles (UCP3) (Yu *et al.* 2003, Zemel *et al.* 2000, Zemel 2003). Calcium also affects fat oxidation. Melanson *et al.* (2003) demonstrated that calcium intake correlated positively with 24-hr fat oxidation, during both sleep and moderate physical activity.

Food intake, energy balance and body weight as well as insulin sensitivity is regulated by complex neurohormonal signals (Druce and Bloom 2006). Adherence to the WM programme is greatly influenced by psychobehavioural factors, among which the eating behaviour plays a crucial role. However, an association between calcium intake and hormonal and psychobehavioural factors has not yet been evaluated. The aim of the present study was to evaluate whether a calcium supplement (500 mg/day) would influence a change in body weight and body composition in response to a 3-week WM programme with strictly defined and supervised calorie intake. The impact of calcium intake on metabolic, hormonal and psychobehavioral parameters was also evaluated.

Subjects and methods

Sixty seven overweight/obese women (BMI: 32.2$\pm$4.1 kg/m^2; age: 49.1$\pm$12.1 years) participated in a 4-week WM programme in the Spa Obesity Unit. Subjects with diabetes,

uncompensated thyroid dysfunction and those treated with drugs affecting water balance (diuretics, hormonal contraceptive and replacement therapy etc.) were excluded from the study.

The comprehensive WM included a precisely defined low energy diet, daily physical activity supervised by a physiatrist and cognitive behavioural modification of lifestyle. Energy and nutrient content of meals prepared in the central kitchen during the entire period of study was calculated by the PC programme „Nutrition“. This software covers almost 3000 food items and evaluates the intake of energy, macronutrients and micronutrients. All subjects were advised to eat all of the meals served in four daily portions in the spa dining room. Mean daily energy intake before initiation of the spa treatment was calculated to be about 7.0 MJ. Therefore the patients were provided a 7 MJ/day diet during the 1st week of WM. Only those patients who exhibited a stable weight during the first week entered the trial and received a hypocaloric diet providing 4.5 MJ/day (protein 25.3%, fat 28.7%, carbohydrate 46.0%) with a low calcium supply (350 mg/day) over the subsequent 3-week period. This diet yielded a 2.5 MJ deficit in comparison with the pretreatment week.

Participants were divided in three age- and BMI-matched groups who received either a placebo (P group, n = 21) or calcium (500 mg/day), either as carbonate (C group, n = 25) or calcium of dairy origin Lactoval (L group, n = 21). Lactoval was prepared from milk and contained calcium as phosphate (70%), lactate (10%) and citrate (20%). Tablets were analyzed in the Dairy Research Institute and their calcium content corresponded to declared values. A dietitian distributed placebo or calcium tablets to the patients in three daily doses. The dietitian also checked that the patients took the tablets immediately after receiving them.

The studied women were predominatly perimenopausal. The amount of women in menopause was comparable among the groups.

Anthropometric, biochemical, hormonal and psychobehavioural investigations were conducted before and after a 3-week WM. Body weight and body composition were analyzed by a bipedal-bimanual Body Composition Analyzer Tanita BC-418MA (Tanita Inc., Tokyo, Japan). Anthropometric measurements included body weight, height, waist and hip circumference, subscapular, triceps, biceps and suprailiac skinfolds. BMI was calculated according to the formula: body weight (kg)/height (m^2).

Eating behaviour was evaluated by the Eating Inventory (Stunkard and Messick 1985) which assesses three behavioural traits: 1. dietary restraint - deliberate control of intake, 2. disinhibition - measure of loss of control over food intake (for example in response to stress, anxiety, depression and alcohol intake), 3. perceived hunger - awareness of and susceptibility to hunger. Beck Depression Inventory (Beck *et al.* 1961) was used to evaluate the level of depression.

Blood samples for biochemical and hormonal investigations were taken in the morning after a 12-hour overnight fast. Biochemical indexes (blood glucose, glycosylated haemoglobin, uric acid, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, C reactive protein) were assessed by the standard laboratory procedures. Hormonal levels (TSH, fT3, fT4, insulin, C peptide, prolactin, growth hormone, total ghrelin, IGF-1, cortisol, sex hormone binding globulin /SHBG/, leptin, ghrelin, peptide YY, neuropeptide Y, adiponectin, resistin) were analyzed by radioimmunoassays.

Protocol of the study was reviewed and accepted by the Ethics Committee of the Institute of Endocrinology. All patients were informed about the study design and signed an informed consent form concerning their participation in the study.

Statistical analysis: Results are expressed as means $\pm$ SD. Differences in parameters before and after WM as well as differences between the groups were assessed using the Kruskal-Wallis robust analysis of variance (ANOVA) followed by Kruskal-Wallis multiple

comparisons. Differencres between groups were assessed by Mann-Whitney test. Changes were evaluated by Wilcoxon's paired test.

Results

The baseline data was similar in all three groups for body mass index (C group: 32.39±4.35 kg/m^2, L group: 32.42±4.22 kg/m^2, P group: 32.36±4.86 kg/m^2), fat stores (C group: 41.3±4.9%; L group: 41.7±6.4%; P group: 42.0±5.7%), as well as for the plasma leptin levels (C group: 21.7±8.4 ng/ml; L group: 21.5±9.3 ng/ml; P group: 20.3±9.1 ng/ml) which under stable weight conditions reflect body fat stores. An average weight loss of 3.8 ± 1.6 kg was achieved in response to WM. Table 1 summarizes significant changes in anthropometric, psychobehavioral and hormonal parameters in the whole cohort. Decreases in body weight, BMI, body circumferences and skinfolds were demonstrated. In response to WM, the Beck depression score, hunger scores and disinhibition scores decreased whereas the restraint score increased. Significant decreases in serum leptin and NPY levels were shown. A significant decline in fasting blood glucose and insulin concentrations was demonstrated together with a significant rise in SHBG level, whereas no significant changes in serum adiponectin (- 0.06 ± 2.69 mg/l) and resistin (- 0.18 ± 0.78 µg/l) levels were observed.

No significant differences were observed in any of the values concerning anthropometric, body composition, psychobehavioural, biochemical and hormonal parameters when the three groups differing in the calcium intake were compared both before and after the weight reduction. Only selected data from 52 measured parameters are shown in Table 2.

As shown in Table 3, significant decreases in body weight, BMI and fat mass were observed in both the calcium-treated groups and in the group receiving a placebo. No significant differences in the decreases in anthropometric indexes and fat mass were

demonstrated between the three groups of patients. On the other hand, a significant decline in FFM was shown in a group treated with a placebo (-1.46 $\pm$ 3.36 kg, p = 0.006) while both groups provided with additional calcium did not exhibit any significant changes in FFM. Hunger score decreased significantly in both groups treated with calcium (calcium carbonate group: -1.54 $\pm$ 2.59, p = 0.010; Lactoval group: - 1.76 $\pm$ 2.98, p = 0.017) whereas a decline of hunger score in placebo treated group was not significant (-0.38 $\pm$ 2.56).

Figure 1 demonstrates a significant difference in the change of resistin level in response to WM between the placebo-treated group and the joint cohort including both calcium-treated groups. In contrast to the WM-induced increase in resistin level in the placebo-treated group, a decrease in mean resistin level was demonstrated in the joint calcium-treated group.

Discussion

The main finding of the present study is that an administration of a calcium supplement in a daily dose of 500 mg does not result in an increased weight loss during short-term weight management. When discussing the results, we should consider both advantages and limitations of our study. In all previous studies calcium has been supplemented as calcium carbonate, calcium citrate or calcium citrate-malate although calcium phosphate represents a major source of calcium in dairy products. No previuous studies employed administration of calcium of dairy origin or calcium phosphate. In our study patients were given besides calcium carbonate tablets prepared from the milk which contained calcium as phosphate, lactate and citrate. However, we failed to see any significant difference in weight loss between the groups supplemented with 500 mg calcium provided as calcium carbonate or as calcium of dairy origin. The second advantage of our study was that we had an opportunity to maintain all of the subjects on the same diet providing a daily energy

deficit of 2.5 MJ with an average daily calculated calcium intake of 350 mg. In previously published interventional studies the quantity of calcium obtained from each daily meal had not been evaluated and only the role of supplemented calcium or high dairy product consumption had not been considered.

Zemel (2004) reported on weight loss reached over 24 weeks in obese subjects assigned to three different calorie-restricted diets prescribing a daily energy deficit of 500 kcal: low dairy, high dairy and calcium-supplemented low dairy. Accelerated weight and fat loss in response to energy restriction was observed in the high dairy consumers and calcium-supplemented groups in comparison with low dairy low-calcium consumers (Zemel 2004). It could be objected that our study was conducted over a short period of time and that the amount of supplemented calcium was not high enough to affect the weight loss. The duration of our intervention, only 3 weeks, was rather short. We were unable to extend the duration of the supervised in-patient stay over usual 4-wk period of the spa treatment. The lack of evidence for the role of calcium in promoting weight loss in our study might be due to a rather high daily energy deficit which could surpass the effects of calcium deficiency over a short-time period of WM. In many interventional studies patients received $\geq$ 1000 mg calcium/day. Our calcium-supplemented patients received on average a total daily dose of 850 mg calcium which was shown to be sufficient for potentiating weight reduction as well as for its beneficial effects on body composition. According to Thompson *et al.* (2005) diets higher than 800 mg of calcium in dairy products or higher in fiber and lower in glycaemic index do not enhance weight reduction beyond what is seen with calorie restriction alone. The Amsterdam Growth and Health Longitudinal Study which followed a cohort of men and women from age 13 years in 1977 to age 36 years in 2000 suggested a threshold of approximately 800 mg/day above which calcium intake has no additional beneficial effect on body composition (Boon *et al.* 2005). Barr (2003) in a Medline search between the years 1966

– 2001 identified 17 randomized trials of calcium supplementation in subjects without caloric restriction. In most studies, no differences in body weight or body composition were detected between the calcium and placebo treated or untreated groups. Recker *et al.* (1996) in a 4-yr study detected a significant difference in body weight change. Postmenopausal women receiving 1.2 g calcium/day lost 0.35 kg/yr more than did the control group.

Body composition in this study was evaluated by the bioimpedance method which is greatly influenced by the hydration of the examined subjects. Therefore patients which were treated with drugs which could affect water balance were not included in the study. Some studies raised questions about the use of BIA for evaluation of body composition in obese subjects as well as its changes in response to the weight management (Kyle UG *et al*. 2004). However, recent study of Jebb *et al.* (2006) declares that BIA is a useful method to measure body composition changes during clinical weight management programmes. BIA was also classified as a useful tool for body composition assessment in extremely obese subjects before and after massivre weight loss induced by gastric bypass surgery (Das *et al.* 2003). In our study, a significant decrease in FFM was demonstrated in the placebo-treated group, whereas loss of FFM was demonstrated as not being significant in both calcium-treated groups. It cannot be excluded that the higher loss of body weight in the placebo group (-4.34 kg in the placebo group versus -3.58 kg in both calcium treated groups) was partly due to the differences in water balance. However, there has been no data available about the association of low calcium intake with the loss of water.

On the other hand, the protective effect of calcium intake on FFM was reported by Heaney *et al.* (2002) in a 3-yr calcium intervention trial in young women given either 1500 mg calcium per day or a placebo. The group as a whole had gained weight at a 3-yr follow-up; there was no significant difference in weight gain between the calcium supplemented and

control groups. However, the weight gain in the calcium supplemented women consisted primarily in an increase in fat free mass, while the placebo treated women accumulated twice as much body fat. High dairy/calcium diets in 3 weight loss studies conducted by Zemel *et al.* (Obes Res 2004, Int J Obes 2005, Obes Res 2005) induced not only higher fat loss but also markedly reduced the loss of FFM compared with the low dairy/calcium diet. Preservation of FFM by dairy products could be attributable to the high content of branched chain amino acids in proteins of dairy origin. Branched chain amino acids, especially leucine, play a key role the regulation of muscle protein synthesis (Layman and Walker 2006). It is difficult to find an explanation how dietary calcium per se might influence preservation of FFM during the weight loss studies. Calcium-induced suppression of 1,25-dihydroxyvitamin D levels has been proposed as a mechanism leading to reduction of visceral adiposity as 1,25-dihydroxyvitamin D has been shown to stimulate 11β-hydroxysteroid dehydrogenase-1 (Morris and Zemel 2005). However, it seems unlikely that such a calcium-induced inhibition of local cortisol production in adipose tissue could play any role in modulation of overall body protein catabolism.

In addition**,** a significant decline in the hunger score of the Eating Inventory was demonstrated in calcium-treated groups, but not in the placebo-treated group. This change in perception of hunger could contribute to a better long-term outcome of weight management in calcium-treated patients. However, change in hunger score does not necessarily implicate change in energy intake which was provided the same for all participants in our study. The observed decline in the hunger score cannot be attributed to changes in fasting concentrations of hormones involved in food intake regulation. No significant differences in profile of these hormones were seen between the calcium and placebo treated individuals. However, we did not examine postprandial hormonal responses which could affect both satiety and hunger feelings. Ping-Delfos *et al.* (2004) revealed that a high dairy calcium and vitamin D diet did

not affect subjective sensations of hunger and satiety in the immediate postprandial period, but spontaneous food intake over the subsequent 24h period was significantly reduced.

Previous studies demonstrated that a diet characterized by a higher calcium intake or higher dairy intake, especially low-fat dairy intake, may lower the risk of type 2 diabetes (Choi *et al.* 2005, Pittas *et al.* 2006). A combined daily intake of >1,200 mg calcium and >800 IU vitamin D was associated with a 33% lower risk of type 2 diabetes with RR of 0.67 (0.49-0.90) compared with an intake of <600 mg and 400 IU calcium and vitamin D, respectively (Pittas *et al.* 2006). Among the mechanisms involved in lowering the risk of type 2 diabetes, hormonal mechanisms affecting insulin sensitivity should be considered (Lu *et al.* 2006, Heilbronn *et al.* 2004, Silha *et al.* 2003). A significant difference in the change of resistin levels in response to a negative energy balance between calcium treated and placebo treated individuals was shown in our study. Calcium mediated differences in resistin response to WM might play a role in reducing the risks of type 2 diabetes and metabolic syndrome.

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Table 1 – Anthropometric, psychobehavioural and hormonal indexes and their changes in response to weight management in the whole cohort.

	All subjects						
	Before		After		Difference		Significance
Variable	mean	SD	mean	SD	mean	SD	p
Weight (kg)	84.60	12.81	80.78	12.48	-3.82	1.63	0.000001
BMI (kg/m^2)	32.39	4.48	30.92	4.33	-1.47	0.63	0.000001
Waist (cm)	98.83	11.92	93.64	11.43	-5.19	2.28	0.000001
Hip (cm)	115.49	9.20	112.12	8.97	-3.37	1.61	0.000001
WHR	0.86	0.07	0.83	0.07	-0.02	0.02	0.000001
Fat (kg)	35.81	9.52	32.01	8.83	-3.80	2.83	0.000001
Fat (%)	41.65	5.69	38.98	6.12	-2.67	2.89	0.000001
FFM (kg)	48.79	4.68	48.48	5.32	-0.31	3.18	0.035526
Beck	10.38	6.37	7.56	6.43	-2.82	4.16	0.000003
Restraint	10.03	4.54	12.94	4.57	2.91	4.30	0.000004
Hunger	4.08	3.28	2.83	2.73	-1.24	2.71	0.000641
Disinhibition	6.61	2.97	4.86	2.70	-1.74	2.55	0.000008
Glucose (mmol/l)	5.13	1.56	4.83	1.24	-0.29	1.70	0.05
Insulin (mIU/l)	8.36	4.45	7.83	4.81	-0.53	5.99	0.05
SHBG (nmol/l)	61.49	44.21	79.08	53.73	14.89	26.72	0.000001
Leptin (µg/l)	21.21	8.98	15.36	7.13	-5.85	6.45	0.000001
NPY (pmol/l)	101.8	52.31	84.14	41.08	-17.7	31.49	0.000003

Table 2 – Comparison of selected anthropometric, psychobehavioural and hormonal characteristics in placebo-treated group (group P) and in groups treated with calcium carbonate (group C) or with calcium of dairy origin (Lactoval, group L) before and after weight reduction. No significant differences in measured parameters were observed between the groups both before and after weight reduction.

Variable	Before							After						
	group C		Group L		Group P		ANOVA	Group C		Group L		Group P		ANOVA
	mean	SD	mean	SD	mean	SD		mean	SD	mean	SD	mean	SD	
Weight (kg)	84.95	11.75	83.43	11.65	85.37	14.83	NS	81.61	11.56	79.57	11.49	81.03	14.22	NS
BMI (kg/m^2)	32.39	4.35	32.42	4.22	32.36	4.86	NS	31.11	4.19	30.90	4.10	30.72	4.68	NS
Fat (kg)	35.57	9.12	35.38	9.39	36.51	10.03	NS	31.93	7.68	31.38	9.42	32.72	9.38	NS
Fat (%)	41.30	4.98	41.70	6.39	42.01	5.69	NS	38.69	4.54	38.71	7.58	39.58	6.03	NS
FFM (kg)	49.37	3.61	48.08	4.29	48.85	5.90	NS	49.69	5.38	48.19	4.95	47.40	5.33	NS
Beck	10.75	5.76	8.29	3.43	12.05	8.41	NS	7.42	5.35	5.72	3.94	9.57	8.61	NS
Restraint	10.38	3.83	9.81	4.03	9.86	5.62	NS	13.54	4.36	12.71	3.76	12.48	5.39	NS
Hunger	4.54	3.04	4.24	2.99	3.38	3.68	NS	3.00	2.84	2.47	2.06	3.00	3.13	NS
Disinhibition	6.79	3.04	6.95	2.90	6.05	2.90	NS	5.42	2.96	4.81	2.95	4.29	1.88	NS
Leptin (µg/l)	21.74	8.47	21.47	9.31	20.34	9.14	NS	17.51	7.67	15.13	7.51	13.13	5.01	NS
NPY (pmol/l)	108.30	54.23	99.07	42.45	97.17	57.96	NS	92.09	38.87	84.26	41.20	74.92	41.51	NS
Adiponectin (mg/l)	10.66	3.09	13.07	7.35	9.99	4.22	NS	11.09	3.70	12.35	6.27	10.39	4.02	NS
Resistin (µg/l)	2.68	0.89	2.33	0.65	2.15	0.65	NS	2.08	0.54	2.28	0.80	2.37	0.99	NS

Table 3 – Changes in selected anthropometric and psychobehavioural characteristics in placebo-treated group (group P) and in groups treated with calcium carbonate (group C) or with calcium of dairy origin (Lactoval, group L). Kruskal-Wallis ANOVA as well as Kruskal-Wallis mutliple comparisons found no significant differences in changes between the groups.

	Group C			Group L			Group P		
Variable	Mean	SD	P	Mean	SD	P	mean	SD	p
Weight (kg)	-3.34	1.79	0.00002	-3.87	1.62	0.00006	-4.34	1.37	0.00006
BMI (kg/m^2)	-1.29	0.69	0.00002	-1.51	0.65	0.00006	-1.64	0.49	0.00006
Fat (kg)	-3.64	3.83	0.00002	-4.00	2.73	0.00006	-3.80	1.45	0.00006
FFM (kg)	0.32	3.49	0.64738	0.11	2.45	0.53124	-1.46	3.36	0.00630
Restraint	3.17	3.47	0.00068	2.90	4.15	0.00767	2.62	5.44	0.05544
Hunger	-1.54	2.59	0.01013	-1.76	2.98	0.01680	-0.38	2.56	0.44642
Disinhibition	-1.38	2.73	0.04447	-2.14	2.61	0.00421	-1.76	2.39	0.00482

Figure 1 – Change of resistin level in response to WM in the placebo-treated group (group P) and joint calcium-treated group (group CL).