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Weight loss and calcium intake influence calcium absorption in overweight postmenopausal women[12–3]

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Abstract

Background—Weight loss (WL) reduces bone mass and increases fracture risk. Mechanisms regulating calcium metabolism during WL are unclear.

Objective—The objective was to assess the effect of 6 wk of WL at 2 different amounts of calcium intake [normal (NlCa): 1 g/d; high (HiCa): 1.8 g/d] on true fractional calcium absorption (TFCA), bone turnover, and bone-regulating hormones in overweight postmenopausal women.

Design—Seventy-three women (body mass index, 26.9 ± 1.9 kg/m^2) were recruited either to consume a moderately energy-restricted diet (WL group) or to maintain their body weight [weight-maintenance (WM) group] and were randomly assigned to either the HiCa or the NlCa group in a double-blind manner. Subjects underwent weekly diet counseling, and measurements were taken at baseline and after 6 wk.

Results—Fifty-seven women completed the study and had a baseline TFCA of 24.9 ± 7.4%. Energy restriction significantly decreased the total calcium absorbed ($P < 0.05$) in the WL group ($n = 32$) compared with the WM group ($n = 25$; analysis of covariance). Regression analysis showed that a greater rate of weight loss suppressed TFCA and the total calcium absorbed ($P < 0.05$) in the HiCa group. The women in the NlCa WL group absorbed inadequate amounts of calcium (195 ± 49 mg/d), whereas the women in the HiCa WL group absorbed adequate amounts (348 ± 118 mg/d). Parathyroid hormone explained 22% of the variance in calcium absorbed in the NlCa group only.

Conclusions—We suggest that WL is associated with elevated calcium requirements that, if not met, could activate the calcium-parathyroid hormone axis to absorb more calcium. Normal intakes of calcium during energy restriction result in inadequate total calcium absorption and could ultimately compromise calcium balance and bone mass.

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All authors contributed to the interpretation of the results and the data analysis. SAS was responsible for the study design. MC, CSR, and SAS participated in the data collection and laboratory and statistical analysis and were primary writers of the manuscript. No author had any financial or personal interest in a company or organization sponsoring the research.

Keywords

Calcium absorption; bone turnover; diet; postmenopausal status; weight loss

INTRODUCTION

Numerous studies over the past decade indicated that weight loss of ≥5% is associated with a decrease in bone mass (1–5). In addition, fracture risk is increased among women who lose weight (6, 7). It was suggested that there is a continuous mobilization of bone during energy restriction (5), which can explain the reduction in bone mass with weight loss. Previous studies in our laboratories determined that, during weight reduction in obese postmenopausal women, bone turnover is elevated along with changes in serum hormone profiles such as elevations in parathyroid hormone (PTH) and reductions in the concentrations of sex steroids (4, 5). We observed that calcium supplementation (1 g/d) suppressed the weight loss–associated increase in bone turnover (5). At a constant calcium intake, one possible mechanism driving the rise in PTH is a reduction in intestinal calcium absorption. Calcium supplementation could act to suppress bone resorption by overcoming the decrease in calcium absorption. Consistent with this hypothesis, our studies in rats (8) showed a decrease in intestinal calcium absorption with energy restriction.

The aims of this study were to determine whether intestinal calcium absorption is altered by short-term moderate energy restriction at 2 different amounts of calcium intake and to better understand the regulation of calcium metabolism, PTH, and bone turnover during weight loss in overweight postmenopausal women.

SUBJECTS AND METHODS

Subjects

Seventy-three weight-stable (≥3 mo), overweight [body mass index (BMI; in kg/m^2): 25–29.9], postmenopausal (≥3 y) women were recruited into either a weight-loss (WL) or weight-maintenance (WM) program. Advertising in local newspapers was done every 6 mo over a 3-y period. Telephone screenings and eligibility questionnaires assessed medical and nutrition history, and women with disease states (including osteoporosis, as assessed by dual-energy X-ray absorptiometry) or with use of medications known to influence calcium or bone metabolism were excluded. There were ≈10–15 women per group and 7 groups during the years 2000–2003. Written informed consent was obtained from each volunteer. The study was approved by Rutgers University Institutional Review Board.

Study design

Participants underwent a 1-mo stabilization period, during which they were instructed to consume a total of 1.0 g calcium/d and asked to maintain body weight. A standard multivitamin and mineral supplement (Sentury-Vite; Pharmavite Corp, Mission Hills, CA) for older adults (>50 y) was provided throughout the study to standardize nutritional status in all subjects. The supplement contained 200 mg calcium, 10 μg vitamin K, 5 μg vitamin D, 100 mg magnesium, and 48 mg phosphorus as well as other standard nutrients at their

recommended amounts. With the use of food-frequency questionnaires, we evaluated habitual calcium intake and made recommendations to adjust intake to ≈0.8–1.0 g/d in all subjects. At the end of the stabilization period, baseline measurements were performed. Subjects in the WL group then started on a standard nutrition education and behavior modification weight-reduction program under the supervision of a registered dietitian that included weekly instruction (n = 10–15/group). Diet counseling and sample collection were conducted every 6 mo over a 3-y period (in April or October) in an effort to minimize seasonal effects on 25-hydroxyvitamin D [25(OH)D] (9). WL was achieved through a reduced energy intake while maintaining habitual exercise levels. Women in the WL group were required to lose ≥2.5% body weight. Before (baseline) and after 6 wk of weight reduction or maintenance, calcium absorption and body weight were measured, and 3-d dietary intake records and fasting blood and second-morning-void urine samples were collected.

In addition to the multivitamin and mineral supplement (0.2 g calcium), women were randomly assigned in a double-blinded manner (before 1-mo stabilization) to receive an additional daily supplement of calcium citrate containing either 0.2 g or 1.0 g calcium (Mission Pharmacal, San Antonio, TX). Subjects were instructed to consume 4 placebo tablets and 1 calcium tablet (200 mg calcium/tablet) or 5 calcium tablets each day (2 in the morning and 3 in the evening). Therefore, the total supplemented calcium was 0.4 g/d and 1.2 g/d in the normal calcium (NlCa) and high calcium (HiCa) groups, respectively. Assuming that calcium from food sources during energy restriction was ≈800 mg/d, as instructed, the goal for total calcium intake (dietary and supplemental) was 1.2 g/d, which is recommended for this age group (9), and 1.8 g/d in the NlCa and HiCa groups, respectively.

Laboratory methods

True fractional calcium absorption—Dual stable-isotope methods were used to determine true fractional calcium absorption (TFCA). On the day of the calcium absorption test, women were admitted at 0700 after an overnight fast. After blood collection (10 mL), subjects were asked to void and then were served a standard breakfast (170 mg calcium) to be consumed in its entirety. This meal contained ^{43}Ca that had been mixed in milk and allowed to equilibrate ≥12 h before the test. Immediately after breakfast, ^{42}Ca was injected intravenously over ≈3 min. Syringes containing the isotope solution (to be mixed in the milk or infused intravenously) were weighed before and after administration on a precision balance scale. Complete urine collection was monitored in each subject throughout the following 24 h, and the ratio of each isotope to ^{44}Ca was determined in oxalate-precipitated aliquots of the pooled 24-h urine by using high-resolution, inductively coupled plasma mass spec-trometry.

Calculations—TFCA (a) was calculated from the pooled 24-h urine samples with the use of the following equations (10):

$$\alpha = \text{Fraction oral tracer in urine over time} / \text{fraction intravenous tracer in urine} \quad (1)$$

$$\alpha = ([\text{Calcium concentration} \times vol \text{ sample} \times NA \text{ oral} \times \Delta\% \text{ excess oral}]/\text{oral dose})([\text{calcium concentration} \times vol \text{ sa}$$

and

$$\alpha = (NA \text{ oral} \times \text{intravenous dose} \times \Delta\% \text{ excess oral})(NA \text{ intravenous} \times \text{oral dose} \times \Delta\% \text{ excess intravenous}) \quad (3)$$

where NA = the natural abundance of the isotope, the Δ% excess = [(observed ratio – NA ratio)/NA ratio] × 100, and the ratios are relative to Ca^{44}.

The following equation was used to estimate daily absorbed calcium:

$$\text{Estimated daily absorbed calcium} = TFCA \times \text{average calcium intake/d} \quad (4)$$

where calcium intake/d is averaged from supplements and food records (2 weekdays and 1 weekend day, nonconsecutive).

Biochemical analyses—Serum concentrations of PTH, estradiol, estrone (Diagnostic System Laboratories, Webster, TX), 25(OH)D, and 1,25 dihydroxyvitamin D [1,25$(OH)_2$D] (DiaSorin, Stillwater, MN) were measured with the use of radioimmunoassay. Urinary calcium excretion and creatinine (no. 587 and 555; Sigma, St Louis) were measured in 24-h urine samples on the day of the calcium absorption test. CVs are <15% as reported by the manufacturers. Markers of bone resorption (urinary pyridinium crosslinks, pyridinoline and deoxypyridinoline) were measured with the use of HPLC after hydrolyzed samples were submitted to a prefractionation procedure (11). Peaks were detected with the use of fluorescence (12) and quantitated by using external standards. Values are corrected for creatinine excretion to adjust for differences in the concentration of spot urine samples (CV of 8% and 13% for pyridinoline and deoxypyridinoline, respectively). Serum *N*-telopeptide of type I collagen (sNTx) was measured by using an enzyme-linked immunosorbent assay (Osteomark, OSTEX International Inc, Seattle; CV: 4.6%). Bone formation was evaluated by measuring serum osteocalcin with the use of a radio-immunoassay with a CV < 8% (Biomedical Technologies, Inc, Stoughton, MA).

Statistics

The group (WM compared with WL) and calcium intake (NlCa compared with HiCa) effects were assessed by using two-factor analysis of covariance (ANCOVA) with the week 6 measurements examined as the dependent variable. Because of differences in baseline body weight ($P < 0.08$), estrone ($P < 0.01$), 25(OH)D ($P < 0.05$), 1,25$(OH)_2$D ($P < 0.05$), and sNTx ($P < 0.06$) between groups, these variables were included as covariates in the model. Because of the potential effect of age, season, and year of recruitment on calcium absorption, these variables were also included as covariates. When appropriate, multiple comparisons were performed by using Tukey's post hoc test. In addition, the percentage change from baseline to week 6 was assessed (two-way ANCOVA). Pearson correlation coefficients, comparisons of regression lines, and stepwise multiple regressions were used to evaluate the associations between changes in the different variables measured. To evaluate the effect of season of recruitment on either baseline values or the changes, we conducted a separate analysis by using these variables as the independent factors (one-factor analysis of

variance). $P < 0.05$ was considered significant. Data are presented as mean ± SD unless otherwise indicated. All analyses were conducted with use of the SAS statistical package (version 8.2; SAS Institute Inc, Cary, NC).

RESULTS

A flow diagram of the women who were eligible, recruited, randomly assigned, and excluded from analysis is shown in **Figure 1**. Baseline characteristics of the 57 subjects included in the study are shown in **Table 1**. The mean age was 61 ± 5 y (range: 52–75 y). Initial BMI averaged 26.9 ± 1.9. Twenty-one of the subjects were studied during the spring and summer months and 36 during the fall and winter months. Some baseline values were influenced by the season of recruitment. For example, women recruited after the summer months (in October) presented with greater concentrations of serum 25(OH)D ($P < 0.05$) and lower concentrations of serum PTH ($P < 0.02$) and tended to show greater concentrations of urinary calcium excretion ($P < 0.08$), but, as expected (13), the season did not affect calcium absorption. These differences notwithstanding, the changes in all variables from baseline were not affected by the season of recruitment (data not shown). It was ascertained that age (52–75 y) did not influence baseline characteristics, nor did the year of recruitment. In addition, we examined baseline variables for women who lost weight faster (–0.67 to –1.31 kg/wk) rather than slower (–0.30 to –0.66 kg/wk) and found no differences in baseline characteristics between the groups.

Weight loss and nutrient intake

Women allocated to the WL group lost an average of 3.4 ± 1.3 kg (4.7% ± 1.8% of initial body weight) with an average rate of weight change at –0.7 ± 0.2 kg/wk. Women in the WM group maintained their weight within 0.3 ± 1 kg ($P < 0.0001$ compared with women in WL group).

Intake of all nutrients was not significantly different at baseline between the groups (**Table 2**). As expected, total calcium intake increased more in the HiCa-supplemented group (Table 2). Total intake of energy, protein, fat, and carbohydrates decreased in the WL group, as expected with the weight-reduction program. There were no other differences between groups in the change in intake and no significant interactions between calcium amount and weight group.

Calcium absorption and excretion

TFCA and other calcium variables at week 6 (final values) are shown in **Table 3**. TFCA response tended to be influenced by energy restriction, showing lower values and less estimated absorbed calcium in women in the WL group (272 ± 118 mg/d) than in women in the WM group (306 ± 153 mg/d; $P \leq 0.06$). When the percentage change (not shown in the table) in the absorbed calcium from baseline to week 6 was examined in women consuming 1 g calcium/d, there was a decrease for the WL group (–13.4% ± 30.4%; $P < 0.01$) but not for the WM group (–3.2% ± 30.3%). In women consuming high amounts of calcium, there was a significant percentage increase ($P < 0.001$) in absorbed calcium from baseline for both the WL (52.2% ± 48.7%) and WM (74.8% ± 44.2%) groups. Not surprisingly, the absorbed

calcium was significantly higher ($P < 0.0001$) in the HiCa group (379 ± 138 mg/d) than in the NlCa group (210 ± 64 mg/d; Table 3). This finding is despite a tendency ($P < 0.06$) to reduce the percentage change in TFCA when consuming a HiCa diet (–18.7% ± 15.5%) compared with NlCa diet (–10.4% ± 18.8%). Calcium supplementation did not significantly affect 24-h urinary calcium excretion in any of the groups.

Biochemical assays and bone turnover

Calcium-regulating hormones and bone turnover markers after weight change (final values) are shown in Table 3. Serum concentrations of $1,25(OH)_2D$ were higher ($P < 0.05$) in women who lost weight (129.6 ± 41.3 pmol/L) than women in the WM group (106.3 ± 33.6 pmol/L) and did not change significantly with weight loss. Serum estrone was lower ($P < 0.02$) in the WL group (43.7 ± 18.9) than in the WM group (66.3 ± 28.7; Table 3). In addition, the percentage change in serum estrone decreased more ($P = 0.01$) in the WL group (–8.9% ± 16.8%) than in the WM group (–0.9% ± 18.9%). No other hormones were affected by the amount of calcium intake or energy restriction. For serum osteocalcin, the higher calcium intake prevented a rise (HiCa: –2.5% ± 11.7%) compared with normal calcium intake (NlCa: 7.9% ± 13.1%) ($P < 0.001$; not shown in the table), and final values tended to be lower in the HiCa group (Table 3). There were no significant changes in bone resorption markers (urinary cross-links or sNTx). In addition, 24-h urinary creatinine at baseline was 8.5 ± 2.5 mmol/d and 8.1 ± 2.5 mmol/d in the WL and WM groups, respectively, and did not change significantly as a result of weight loss.

Correlation and multiple regression analyses

The correlations between the average change of weight and both TFCA and the total estimated absorbed calcium after 6 wk are shown in **Figure 2**. In the HiCa group, the rate of weight loss was directly associated with a decline in TFCA ($P < 0.02$) and the estimated daily absorbed calcium ($P < 0.05$). In addition, the slopes for HiCa (Figure 2) are significantly different from the NlCa slopes for both TFCA ($P < 0.01$) and daily absorbed calcium ($P < 0.02$). In the same group (HiCa), a negative weight change tended to be associated with higher serum osteocalcin concentrations ($r = –0.385$, $P < 0.05$). No other serum or urinary variables correlated with the changes in body weight in the HiCa group. In women consuming normal amounts of calcium, a negative weight change was associated with higher serum $1,25(OH)_2D$ ($r = –0.554$, $P < 0.01$) as well as lower serum estrone ($r = 0.623$, $P < 0.001$) and estradiol ($r = 0.448$, $P < 0.05$). Consistent with these results, the increase in $1,25(OH)_2D$ correlated with decreases in serum estrone ($r = –0.358$, $P < 0.05$) in this same group (NlCa).

Multiple regression analysis on the changes in TFCA showed that for the NlCa group changes in serum PTH and estradiol together explained 36% of the variance in TFCA (**Table 4**). None of the variables measured explained the variance in TFCA in the HiCa group.

DISCUSSION

The present study evaluated the effects of 6 wk of weight reduction at 2 different amounts of calcium intake on calcium absorption, bone turnover, and calcium-regulating hormones in overweight postmenopausal women. We show that the effect of weight loss at ≈0.7 kg/wk on calcium absorption depends on the amount of calcium intake. At a 1.0 g calcium/d intake, which is above the reported <0.8 g/d intake in this population (14), calcium absorption was maintained, likely at the expense of an increase in the calcium-PTH axis (22% of the change from baseline can be predicted by regression analysis). In contrast, with a calcium intake that exceeds current recommendations, weight loss was associated with a decrease in fractional calcium absorption, yet there was no up-regulation of calcitropic hormones, and total estimated calcium absorbed was sufficient.

We observed that a greater weight loss per week was associated with a diminished ability to increase the amount of absolute calcium absorbed than in situations of weight maintenance. Taking into account the current daily calcium recommendations for this population (1200 mg/d) and considering normal absorptive efficiency for this age group as 20% (13), we estimate that postmenopausal women need to absorb ≈240 mg calcium/d. This amount is also consistent with achieving a zero calcium balance (the goal in adults), when considering the daily net losses through the urine (≈100–200 mg) (15–17) and feces (≈130–150 mg) (15, 18, 19). The mean value of estimated calcium absorbed after 6 wk of weight loss was ≈19% below the estimated requirement when consuming 1 g calcium/d but not for the women who maintained their weight. This inadequate dietary calcium intake could induce calcium release from bone, resulting in net bone loss. These data suggest that there is a higher calcium requirement during weight loss than under weight-stable conditions.

It is well known that an increase in calcium intake decreases the efficiency of calcium absorption (20). Heaney et al (13) observed an inverse association between calcium intake and calcium absorption when they evaluated a large number of studies involving calcium intakes that ranged from ≈0.2–≈2.3 g/d. The amount by which calcium intake increased in the present study (from ≈0.9 to ≈1.8 g/d) may not be large enough to substantially affect calcium absorption. In the analysis by Heaney et al (13), there was no effect of calcium intake on calcium absorption within the range of calcium intakes used in the present study. Our data indicate that the rate of weight loss is associated with a decrease in calcium absorption at high calcium intake (1.8 g/d). Consistent with these findings, we previously observed a decrease in calcium absorption in a rat model of energy restriction with high calcium intake (8). In addition, under conditions of inadequate calcium intake, there is typically an increase in the calcium-PTH axis and calcium absorption (21). The present results suggest that the maintenance (as opposed to a decrease) of calcium absorption in dieting women with “normal” calcium intakes was explained in part (22%) by an elevated calcium-PTH axis, indicating an increased need for calcium. It is possible that in women consuming 1 g calcium/d, calcium absorption was reduced at a time point before 6 wk, thereby activating the calcium-PTH axis and restoring calcium absorption values back to baseline values. Urinary calcium excretion did not change to compensate for the changes in calcium absorption. These data suggest that with weight loss, 1.0 g calcium/d intake does not meet the increased demands. In agreement with our results, others (22) have shown that

calcium supplementation during weight reduction (1.0 g/d) did not protect from loss of bone mass, suggesting that the recommended amounts of calcium intake are insufficient during weight loss.

The mechanism underlying a possible increase in calcium requirements during weight reduction is unclear. We found an association between the rate of weight loss and a decrease in estrone and estradiol in the group with normal calcium intake. A decrease in sex hormones with weight loss could be a mediator of decreases in calcium absorption observed in this group. The effect of weight loss on sex hormones in postmenopausal women is likely due to a decrease in fat mass able to locally synthesize the hormone (23). Six weeks of weight loss is a short time to observe a substantial loss of fat. A greater effect on sex hormone concentrations is expected later during weight loss, as previously observed (4, 24), and could account for the small association with weight loss. The decrease in estrogen could further impair the efficiency of calcium absorption.

It is also possible that energy restriction affects calcium requirements because of some catabolism (25, 26) or a decrease in insulin-like growth factor-1 (26, 27), leading to an imbalance in bone turnover, with decreased bone formation (27). In addition, there is evidence of increased concentrations of serum cortisol in fasting healthy young women (28) and in women with anorexia nervosa (29, 30). The abovementioned changes could also play a role in inducing a reduction in calcium absorption and in the increase in bone turnover or net bone loss observed with moderate weight loss (4, 31, 32). It is important that our previous studies showed that bone turnover increased during weight only loss when calcium intake was at 0.7 g/d and that this increase could be suppressed with 1.0 g calcium/d supplementation (5). Even though bone resorption markers were not suppressed in the current study with the higher (1.8 g/d) compared with normal (1.0 g/d) calcium intake, it is possible that calcium supplementation has a more pronounced effect if subjects have a lower baseline intake (5, 22). Alternatively, it is possible that 6 wk of energy restriction is too brief a period in which to observe a significant response in bone markers (5). Nevertheless, our data during energy restriction show that an inadequate amount of calcium absorbed at 1 g/d (Table 3) could ultimately result in an increase in both bone turnover and loss.

The relative increase in serum $1,25(OH)_2D$ with weight loss is intriguing. Our measurements were done at an early stage of weight loss, and there could be an acute release of vitamin D stored in adipose tissue available for conversion to the active metabolite (33). Although vitamin D status was within normal range in our overweight subjects, it was shown that the obese have lower serum vitamin D concentrations and secondary hyperparathyroidism, possibly because of the deposit of vitamin D in adipose tissue (33–35). In addition, we have shown higher 25(OH)D concentrations after energy restriction in obese but not lean rats (8). Hence, increased serum vitamin D during weight loss could be expected.

In summary, weight loss is associated with an increase in the demands for calcium intake beyond the usual intake and possibly above current recommendations. We observed that, in over-weight women losing weight, the intake of 1.0 g calcium/d elicits a relative increase in the calcium-PTH axis, which likely occurs secondary to a reduction in calcium absorption in

the initial weeks of energy restriction. At a calcium intake of 1.8 g/d, the total absorbed calcium is sufficient, despite a decrease in the efficiency of intestinal calcium absorption during weight loss. To our knowledge, this is the first study that examines the effects of energy restriction on calcium absorption, bone turnover, and calcium-regulating hormones. Because of the high prevalence of women on weight-loss diets, these findings have important clinical implications and emphasize that an adequate calcium intake should be a priority in efforts to achieve healthy weight loss and to prevent the detrimental effects on bone.

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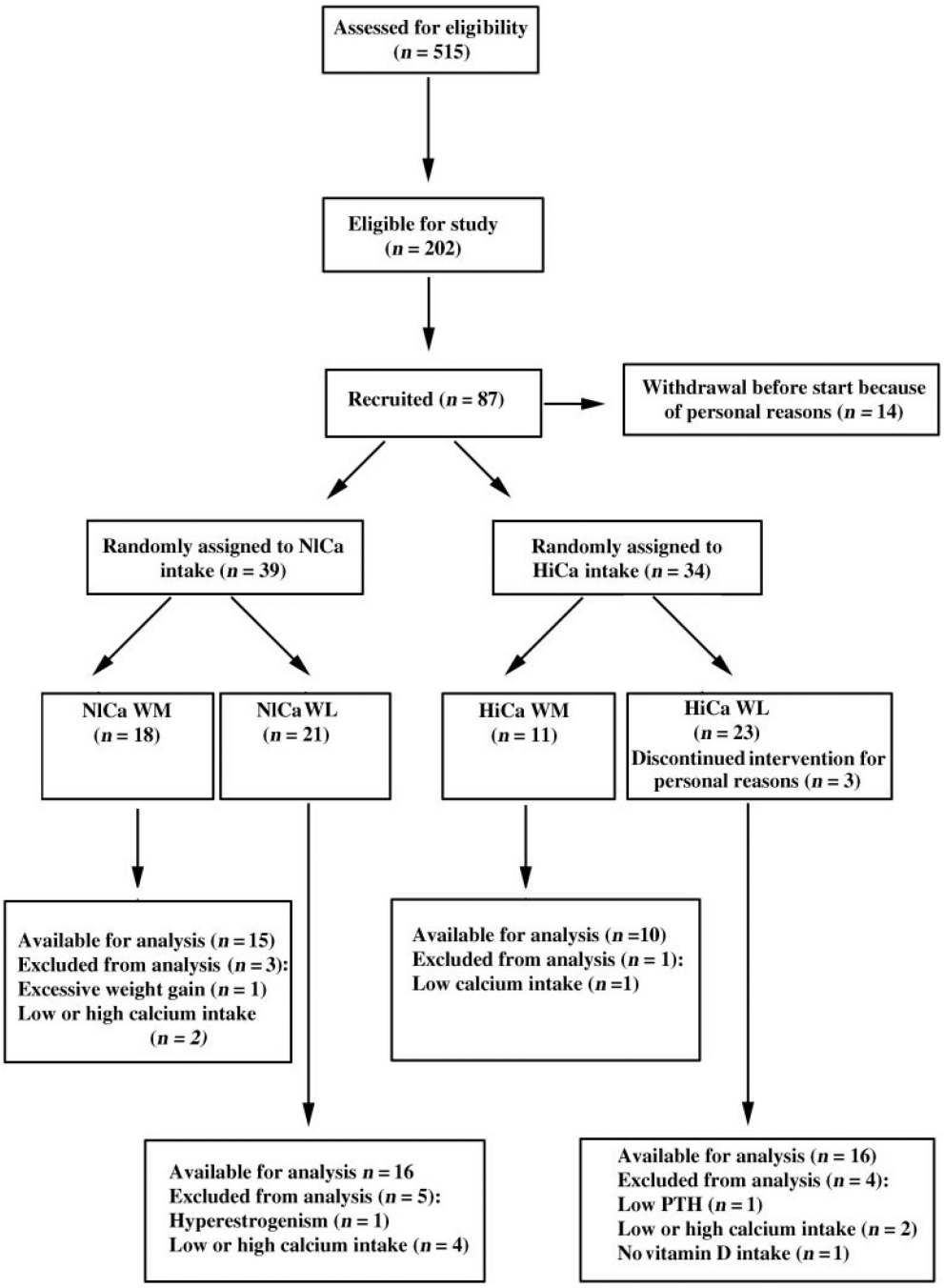

FIGURE 1.

Flow diagram of subjects in study. Excessive weight gain: >2 kg; hyperestrogenism: estradiol >80 pg/mL; low PTH (parathyroid hormone): <10 pg/mL; low calcium intake: <500 mg/d; high calcium intake: >1500 mg/d. NlCa, normal calcium intake; HiCa, high calcium intake; WM, weight maintenance; WL, weight loss.

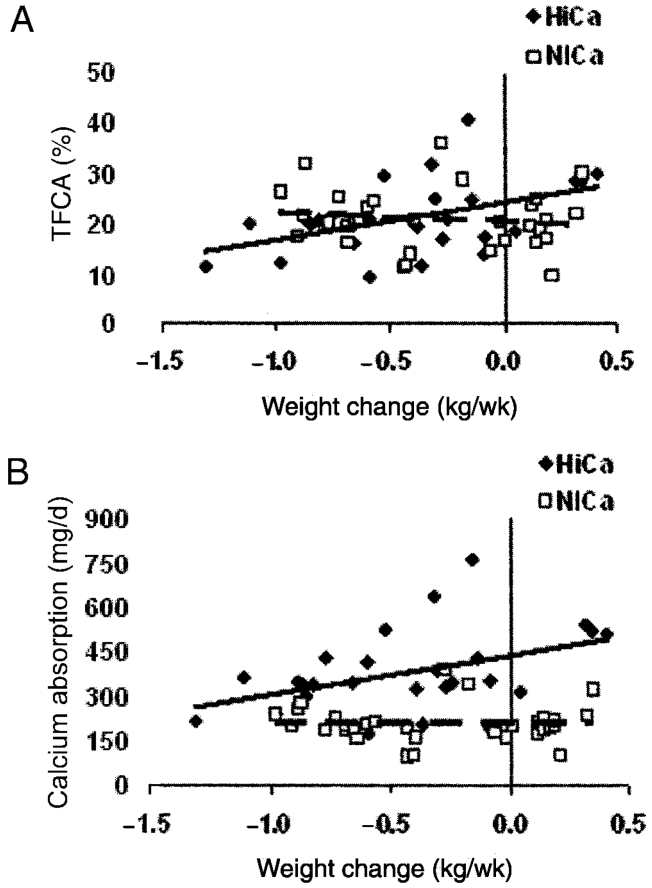

FIGURE 2.

(A) Association between the rate of weight change and true fractional calcium absorption (TFCA) [high calcium group (HiCa): $r = 0.46$, $P = 0.018$; normal calcium group (NlCa): $r = –0.05$, P = NS] and (B) estimated amount (mg) of calcium absorbed (HiCa: $r = 0.43$, $P = 0.028$; NlCa: $r = –0.01$, P = NS) after 6 wk of dietary intervention in 57 postmenopausal overweight women. Diamonds and solid line represent HiCa ($n = 26$), open squares and dashed line represent NlCa ($n = 31$).

TABLE 1

Baseline characteristics of study participants[1]

	NlCa group		HiCa group		*P*[2]
	WM (*n* = 15)	**WL (*n* = 16)**	**WM (*n* = 10)**	**WL (*n* = 16)**	
Age (y)	60.3 ± 5.2	62.3 ± 5.3	63.0 ± 4.1	59.1 ± 6.0	0.2086
Body weight (kg)	68.0 ± 7.7	74.5 ± 7.3	70.9 ± 5.1	71.5 ± 5.8	0.0725
BMI (kg/m^2)	26.6 ± 2.0	27.3 ± 1.75	27.0 ± 1.7	26.9 ± 2.2	0.8447
Calcium intake (mg/d)	961 ± 273	1067 ± 321	891 ± 218	983 ± 230	0.4247
TFCA (%)	25.1 ± 8.8	22.8 ± 5.2	28.6 ± 8.6	24.5 ± 6.8	0.2771
Calcium absorption					
(mmol/d)	6.0 ± 2.4	6.0 ± 1.7	6.7 ± 3.2	5.9 ± 1.7	0.8293
(mg/d)	238 ± 94	238 ± 69	267 ± 130	237 ± 70	
Urine calcium					
(mmol/d)	3.3 ± 1.2	2.6 ± 1.7	4.1 ± 2.3	2.7 ± 1.9	0.1386
(mg/d)	133 ± 47	103 ± 68	163 ± 91	107 ± 74	
Estrone (pmol/L)	76.7 ± 27.3^a	47.6 ± 16.3^b	50.8 ± 14.1^b	51.4 ± 29.4^b	0.0044
Estradiol (pmol/L)	55.8 ± 15.6	47.9 ± 15.7	56.6 ± 12.8	48.7 ± 17.2	0.3214
25(OH) vitamin D (nmol/L)	96.6 ± 19.9^a	85.8 ± 21.9^{a,b}	81.4 ± 14.6^{a,b}	74.1 ± 22.0^b	0.0284
1,25(OH)$_2$ vitamin D (pmol/L)	92.9 ± 42.9^a	135.8 ± 41.4^b	117.0 ± 34.0^{a,b}	102.3 ± 32.4^{a,b}	0.0158
PTH (pmol/L)	3.2 ± 1.5	3.0 ± 1.8	2.8 ± 1.3	3.7 ± 2.5	0.6415
Osteocalcin (nmol/L)	2.9 ± 0.9	3.0 ± 0.9	2.4 ± 0.9	3.2 ± 0.7	0.1365
PYD/creatinine (nmol/mmol)	30.6 ± 11.6	28.3 ± 14.4	33.0 ± 24.7	26.4 ± 7.5	0.7000
DPD/creatinine (nmol/mmol)	10.2 ± 4.8	8.3 ± 4.0	11.2 ± 7.9	8.5 ± 3.6	0.4102
sNTx (nmol BCE)	14.4 ± 5.4	10.8 ± 3.1	15.4 ± 6.7	11.8 ± 4.7	0.0590

[1] Data ($\bar{x}$ ± SD) for n = 31 normal calcium (NlCa) group and n = 26 high calcium (HiCa) group. WM, weight maintenance; WL, weight loss; TFCA, true fractional calcium absorption; PTH, parathyroid hormone; PYD, pyridinoline; DPD, deoxypyridinoline; sNTx, serum *N*-telopeptide of type I collagen; BCE, bone collagen equivalent.

[2] One-factor ANOVA (comparing the 4 groups to each other) with Tukey post hoc test if $P < 0.05$. Values in the same row with different superscript letters are significantly different, $P < 0.05$.

TABLE 2

Nutrient intake at baseline and after 6 wk of weight maintenance (WM) or weight loss (WL) in postmenopausal women randomly assigned to normal calcium (NlCa) and high calcium (HiCa) groups[1]

	NlCa group (*n* = 31)			HiCa group (*n* = 26)		
		6 wk			6 wk	
	Baseline	WM	WL	Baseline	WM	WL
Energy (kcal)[2]	1632 ± 378	1409 ± 424	1080 ± 182	1706 ± 401	1408 ± 449	1252 ± 381
Protein (g)[2]	75.4 ± 18.5	70.6 ± 15.5	54.9 ± 9.0	72.2 ± 18.9	62.9 ± 24.2	59.4 ± 14.7
Fat (g)[3]	58.2 ± 34.2	47.5 ± 22.9	29.4 ± 11.1	62.5 ± 26.6	47.7 ± 24.6	39.4 ± 27.1
Carbohydrates (g)[3]	208.1 ± 63.0	184.6 ± 55.0	156.2 ± 30.7	216.1 ± 51.8	183.8 ± 42.0	172.5 ± 38.8
Calcium (mg)[3,4,5]	1015 ± 299	1002 ± 203	973 ± 237	948 ± 226	1776 ± 183	1803 ± 190
Phosphorus (mg)	1148 ± 356	982 ± 203	954 ± 517	1080 ± 243	847 ± 317	867 ± 263
Vitamin D (μg)	3.0 ± 2.3	2.1 ± 1.3	2.3 ± 1.3	2.8 ± 2.8	2.4 ± 2.0	2.2 ± 1.6
Magnesium (mg)	249.0 ± 91.9	217.0 ± 58.9	190.3 ± 59.2	255.3 ± 61.9	196.7 ± 60.1	212.7 ± 65.9
Sodium (mg)[6]	2468 ± 666	2291 ± 924	1791 ± 625	2477 ± 827	2073 ± 614	2009 ± 1074
Vitamin K (μg)	129.3 ± 111.6	121.8 ± 103.9	115.3 ± 75.5	84.3 ± 63.4	90.0 ± 104.9	90.1 ± 57.2

[1] All values are $\bar{x}$ ± SD. Patient intake was estimated from 3-d food records. Baseline values are not significantly different between groups, and there are no significant interactions (calcium concentration by weight group).

[2] Effect of weight loss (WL compared with WM) (two-factor ANOVA): $P < 0.01$.

[3] Effect of weight loss (WL compared with WM) (two-factor ANOVA): $P < 0.07$.

[4] Effect of calcium supplementation (HiCa compared with NlCa), $P < 0.0001$ (two-factor ANOVA).

[5] Includes supplemented calcium at 0.4 g/d (NlCa) and 1.2 g/d (HiCa).

[6] Does not include salt from shaker.

TABLE 3

Body weight, calcium intake and absorption, calcium-regulating hormones, and bone turnover after ≈6 wk of weight maintenance (WM) or weight loss (WL) in postmenopausal women with 2 different amounts ofcalcium intake [normal (NlCa), 1.0 g/d; high (HiCa), 1.8 g/d][1]

	NlCa group		HiCa group		*P*[2]		
	WM (*n* = 15)	WL (*n* = 16)	WM (*n* = 10)	WL (*n* = 16)	Calcium amount	Group	Calcium × group interaction
Body weight (kg)	68.4 ± 7.5	71.2 ± 7.1	70.9 ± 5.5	67.9 ± 5.7	0.3227	<0.0001	0.9494
Total calcium intake (mg/d)	1047 ± 144	973 ± 237	1776 ± 183	1803 ± 190	<0.0001	0.9820	0.6264
TFCA (%)	21.5 ± 6.6	20.6 ± 5.5	24.0 ± 8.1	19.4 ± 6.3	0.6099	0.0617	0.7694
Calcium absorption							
(mmol/d)	5.6 ± 1.9	4.9 ± 1.2	10.7 ± 4.0	8.7 ± 3.0	<0.0001	0.0532	0.3345
(mg/d)	223.7 ± 75.5	195.4 ± 48.8	429.3 ± 159.8	348.3 ± 118.0			
Urine calcium							
(mmol/d)	3.2 ± 1.8	2.8 ± 1.5	3.5 ± 1.9	3.7 ± 2.3	0.2594	0.7881	0.5771
(mg/d)	128.6 ± 72.1	111.6 ± 59.7	140.4 ± 77.0	146.3 ± 90.7			
Estrone (pmol/L)	77.8 ± 28.5	40.7 ± 10.4	48.9 ± 19.6	46.3 ± 24.4	0.3359	0.0137	0.1097
Estradiol (pmol/L)	54.8 ± 15.1	41.5 ± 8.8	51.8 ± 7.7	43.8 ± 15.1	0.1720	0.1718	0.1763
25(OH) vitamin D (nmol/L)	98.4 ± 26.0	94.6 ± 27.7	80.1 ± 16.2	76.9 ± 18.2	0.1639	0.2239	0.7501
$1,25(OH)_2$ vitamin D (pmol/L)	99.4 ± 35.0	145.2 ± 41.3	116.9 ± 29.8	113.8 ± 36.2	0.9663	0.0464	0.3262
PTH (pmol/L)	3.7 ± 1.3	2.8 ± 1.7	3.0 ± 1.8	3.0 ± 2.0	0.6152	0.3708	0.3433
Osteocalcin (nmol/L)	3.1 ± 1.1	3.3 ± 1.1	2.4 ± 1.1	3.0 ± 0.7	0.0655	0.3645	0.0117
PYD/creatinine (nmol/mmol)	27.2 ± 9.5	28.7 ± 14.8	27.6 ± 17.3	22.3 ± 6.9	0.5467	0.4769	0.7043
DPD/creatinine (nmol/mmol)	10.1 ± 3.3	9.2 ± 4.9	8.8 ± 5.6	6.7± 2.3	0.1071	0.0952	0.7839
sNTx (nmol BCE)	15.6 ± 8.6	14.5 ± 4.8	15.3 ± 4.8	13.4± 5.4	0.9238	0.3554	0.7011

[1] All values are $\bar{x}$ ± SD. NlCa group, *n* = 31; HiCa group, *n* = 26. TFCA, true fractional calcium absorption; PTH, parathyroid hormone; PYD, pyridinoline; DPD, deoxypyridinoline; sNTx, serum *N*-telopeptide of type I collagen; BCE, bone collagen equivalent.

[2] Two-factor analysis of covariance (covariates: body weight, estrone, 25-hydroxyvitamin D, 1,25 dihydroxyvitamin D, sNTx, age, year, and season of recruitment).

TABLE 4

Multiple linear stepwise regression analysis for the change in calcium absorption with weight loss in overweight postmenopausal women consuming 1.0 g calcium[1]

Variable	β coefficient	*P*	Partial R^2
Change in calcium absorption			
PTH	0.525	0.0019	0.22
Estradiol	0.366	0.0239	0.13
Model R^2	—	0.0021	0.36

[1] PTH, parathyroid hormone. $n = 31$. For the group with higher calcium intake (1.8 g/d, $n = 26$), none of the measured variables reached significance for inclusion in the model.