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PAPER

Relation between calcium intake and fat oxidation in adult humans

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OBJECTIVE: To determine if total calcium (Ca^{2+}) intake and intake of Ca^{2+} from dairy sources are related to whole-body fat oxidation.
DESIGN: Cross-sectional study.
SUBJECTS: A total of 35 (21 m, 14 f) non-obese, healthy adults (mean $\pm$ s.d., age: 31 $\pm$ 6 y; weight: 71.2 $\pm$ 12.3 kg; BMI: 23.7 $\pm$ 2.9 kg m^{-2}; body fat: 21.4 $\pm$ 5.4%).
MEASUREMENTS: Daily (24 h) energy expenditure (EE) and macronutrient oxidation using whole-room indirect calorimetry; habitual Ca^{2+} intake estimated from analysis of 4-day food records; acute Ca^{2+} intake estimated from measured food intake during a 24-h stay in a room calorimeter.
RESULTS: Acute Ca^{2+} intake (mg $\cdot$ kcal^{-1}) was positively correlated with fat oxidation over 24 h ($r = 0.38$, $P = 0.03$), during sleep ($r = 0.36$, $P = 0.04$), and during light physical activity ($r = 0.32$, $P = 0.07$). Acute Ca^{2+} intake was inversely correlated with 24-h respiratory quotient (RQ) ($r = -0.36$, $P = 0.04$) and RQ during sleep ($r = -0.31$, $P = 0.07$). After adjustment for fat mass, fat-free mass, energy balance, acute fat intake, and habitual fat intake, acute Ca^{2+} intake explained $\sim$10% of the variance in 24-h fat oxidation. Habitual Ca^{2+} intake was not significantly correlated to fat oxidation or RQ. Total Ca^{2+} intake and Ca^{2+} intake from dairy sources were similarly correlated with fat oxidation. In backwards stepwise models, total Ca^{2+} intake was a stronger predictor of 24 h fat oxidation than dairy Ca^{2+} intake.
CONCLUSION: Higher acute Ca^{2+} intake is associated with higher rates of whole-body fat oxidation. These effects were apparent over 24 h, during sleep and, to a lesser extent, during light physical activity. Calcium intake from dairy sources was not a more important predictor of fat oxidation than total Ca^{2+} intake.
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Keywords: body weight regulation; lipolysis; room calorimeter; energy expenditure

Introduction

There is little understanding of the optimal dietary composition necessary to promote weight loss and prevent weight gain. While much attention has been focused on macronutrient intake and body weight regulation, particularly dietary fat,[1] an emerging body of literature suggests that dietary calcium (Ca^{2+}) may play a role in the regulation of body weight and body fat. In an analysis of the first National Health and Nutrition Examination Survey (NHANES I), McCarron *et al*[2] reported that body weight was inversely related to self-reported Ca^{2+} intake. In subsequent cross-sectional studies, self-reported Ca^{2+} intake was shown to be inversely related with body fat mass,[3] body weight,[4] and the relative risk of obesity in NHANES III.[5] Low self-reported Ca^{2+} intake was later shown to predict gains in body fat in children[6] and young women.[7] In humans, greater weight loss was observed with Ca^{2+} supplementation in placebo-control trials of the effect of dietary Ca^{2+} on osteoporotic risk,[4] and an unexpected 4.9 kg weight loss was observed in a clinical trial investigating the anti-hypertensive effects of increasing dietary Ca^{2+}.[5] It has been estimated that a 1000 mg Ca^{2+} intake difference is associated with an 8 kg difference in mean body weight, and that Ca^{2+} intake explains approximately 3% of the variance in body weight.[4] Thus, there is an increasing interest in understanding the mechanism by which dietary Ca^{2+} potentially regulates body weight and fat mass.

It has been hypothesized that high Ca^{2+} diets protect against fat gain by creating a balance of lipolysis over

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lipogenesis in adipocytes.[5] Ca^{2+} homeostasis is maintained by the concerted actions of parathyroid hormone (PTH), calcitonin, and the vitamin D metabolite 1α,25-dihydroxy-vitamin D_3 (1α,25-$(OH)_2D_3$). When serum Ca^{2+} levels fall below normal (8.5–10.5 mg dl^{-1}), counter-regulatory increases in PTH promote increased bone resorption, decreased Ca^{2+} excretion in the kidneys, and increased formation of 1α,25-$(OH)_2D_3$. Both 1α,25-$(OH)_2D_3$ and PTH stimulate increases in intracellular concentrations of Ca^{2+} ($[Ca^{2+}]_i$) in human and murine adipocytes.[5] *In vitro* data demonstrate that lipolysis and lipogenesis in adipocytes are regulated by Ca^{2+}-dependent mechanisms. Agouti protein exerts a potent inhibition of forskolin-induced lipolysis, which is blocked by treatment with the calcium channel blocker nitrendipine.[8] Treatment of 3T3-L1 adipocytes with recombinant agouti protein increases fatty acid synthase (FAS, a key enzyme in *de novo* fatty acid synthesis) mRNA levels 1.5-fold, and this effect is attenuated by treatment with nitrendipine.[9] Thus, it is hypothesized that low dietary Ca^{2+} leads to increased $[Ca^{2+}]_i$, mediated by changes in circulating 1α,25-$(OH)_2D_3$ and PTH, thereby reducing lipolysis and enhancing lipogenesis in adipocytes.[5]

A mechanistic link between calcium intake and adiposity is supported by observations in both mice and humans. In mice on an obesity-promoting diet, those animals on high-Ca^{2+} diets compared to animals on a low-Ca^{2+} diet had lower $[Ca^{2+}]_i$ in adipocytes,[10] and gained less weight[5,10] and fat pad mass,[5,10] despite no differences in food intake. In obese humans, basal adipocyte concentrations of $[Ca^{2+}]_i$[11] and circulating 1α,25-$(OH)_2D_3$[12–14] are elevated. A mechanistic link between Ca^{2+} intake and lipolysis is supported by the observation that mice on the high-Ca^{2+} diets have higher levels of forskolin-stimulated glycerol release.[5,10] Although FAS expression and activity are lower in adipocytes from mice on high-Ca^{2+} diets,[5,10] a contribution from *de novo* lipogenesis in the development of human obesity remains doubtful.[15]

Implicit in the hypothesis that high Ca^{2+} intake promotes maintenance of lower body fat mass in humans by enhancing lipolysis is the assumption that high-Ca^{2+} diets promote greater rates of whole-body fat oxidation. In Pima Indians, a low rate of fat oxidation has been shown to be predictive of future weight gain.[16] To our knowledge, the association between Ca^{2+} intake and whole-body fat oxidation in humans has not been previously examined. Using data from subjects previously studied in our whole-room calorimeter, we examined the association between 24-h fat oxidation and (a) self-reported (habitual) Ca^{2+} intake, and (b) measured (acute) Ca^{2+} intake to test the hypothesis that a higher intake of dietary Ca^{2+} is associated with higher levels of fat oxidation. As data in humans[17] and animals[6,10] suggest that dairy sources of calcium exert a stronger effect than non-dairy sources, we also considered the effects of total and dairy calcium separately. To eliminate the effect of variations in fat oxidation during the day because of the timing of meals and activity, the association between Ca^{2+} intake and sleeping fat oxidation was also examined. Finally, we examined fat oxidation during light physical activity to determine if fat oxidation during exercise is related to Ca^{2+} intake.

Methods

Subjects

Data from healthy, normal-weight subjects, who previously completed a 24-h stay in the whole-room calorimeter, were used in the current analyses. Subjects were moderately active (3–5 h per week of exercise, as determined from self-report), and between 20 and 45 y of age. Smokers or individuals reporting a history of diabetes, cardiovascular disease, or metabolic disorders known to affect intermediary metabolism were excluded. A health history and physical examination was performed to confirm that there were no medical reasons for exclusion. Subjects provided informed written consent. The study protocols were approved by the Colorado Multiple Institutional Review Board and the Scientific Advisory Board of the General Clinical Research Center (GCRC) at the University of Colorado Health Sciences Center.

All subjects had completed one 24-h stay in the whole-room calorimeter under similar conditions. A standardized walking and stepping protocol was performed each day between 14.20 and 16.30 to account for activity level outside the calorimeter. This protocol consisted of 10-min periods alternating between either walking or stepping and sitting quietly. Subjects were free to move about the calorimeter during other times of the day, but primarily this time was spent in sedentary behavior (reading, writing, or watching television). Subjects were instructed to remain awake and not to nap or perform any exercise other than that prescribed by the protocol. During each stay in the calorimeter, subjects consumed a diet designed to achieve energy balance, estimated from fat-free mass. The composition of the diet was 30% energy fat, 15% energy protein, and 55% energy carbohydrate. Subjects were permitted to select their food preferences (eg some subjects avoided dairy products), so there was a wide range of Ca^{2+} intake.

Measurements

Body composition. Body composition was determined by hydrodensitometry, with residual volume measured simultaneously using the open-circuit nitrogen-dilution technique.[18] Nitrogen was measured using a Med-Science 505-D Nitralizer (St Louis, MO, USA). Percent body fat was estimated from body density (average of 7–10 repeat measurements) using the revised equation of Brozek *et al*.[19]

Daily (24 h) energy expenditure and substrate oxidation. Total daily energy expenditure (EE) and substrate oxidation were determined from oxygen consumption and carbon

dioxide production measured in a whole-room calorimeter. Gas concentrations were determined from the flow rate and the differences in CO_2 and O_2 concentrations between entering and exiting air using Hartman and Braun (Frankfurt, Germany) oxygen (Magnos 4 G) and carbon dioxide (Uras 3 G) analyzers. Values were corrected for temperature, barometric pressure, and humidity. Urine was collected for the duration of the calorimeter stay and analyzed for total nitrogen concentration, which was then used to determine 24-h protein oxidation.[20] EE and substrate oxidation were calculated from oxygen consumption and the respiratory quotient (RQ) based on the equations of Jequier *et al*.[21] Values for all indices were averaged over 1 min intervals and recorded to a data file. The operation of the calorimeter was controlled and data collected minute by minute using a customized program operating on a personal computer. An advantage of the room calorimeter is that it permits the determination of EE and substrate oxidation during different segments of the day (eg during sleep and the walking/stepping protocol). The accuracy and precision of the calorimeter is evaluated regularly by burning propane at a variable rate. Calibration tests consistently demonstrate a 97–98% recovery of the predicted values for oxygen consumption and carbon dioxide production. Quick response rates (in the order of 1–2 min) are observed, allowing for an accurate determination of EE and substrate oxidation over short intervals (eg $\geq$30 min).

Energy and macronutrient intake. Habitual Ca^{2+} intake was determined from 4-day food diaries completed over four consecutive days that included a weekend day. Each subject was individually trained by a dietitian to weigh and record all food and beverage intakes. The diaries were completed 2–4 weeks before subjects were studied in the room calorimeter. Subjects were instructed to consume their usual diets during the measurement period. In the presence of the subject, the dietitian reviewed the completed food intake records for clarity and completeness. Caloric and macronutrient contents of the 4-day food were determined using Food Intake Analysis Software (FIAS, Version 3.98, University of Texas

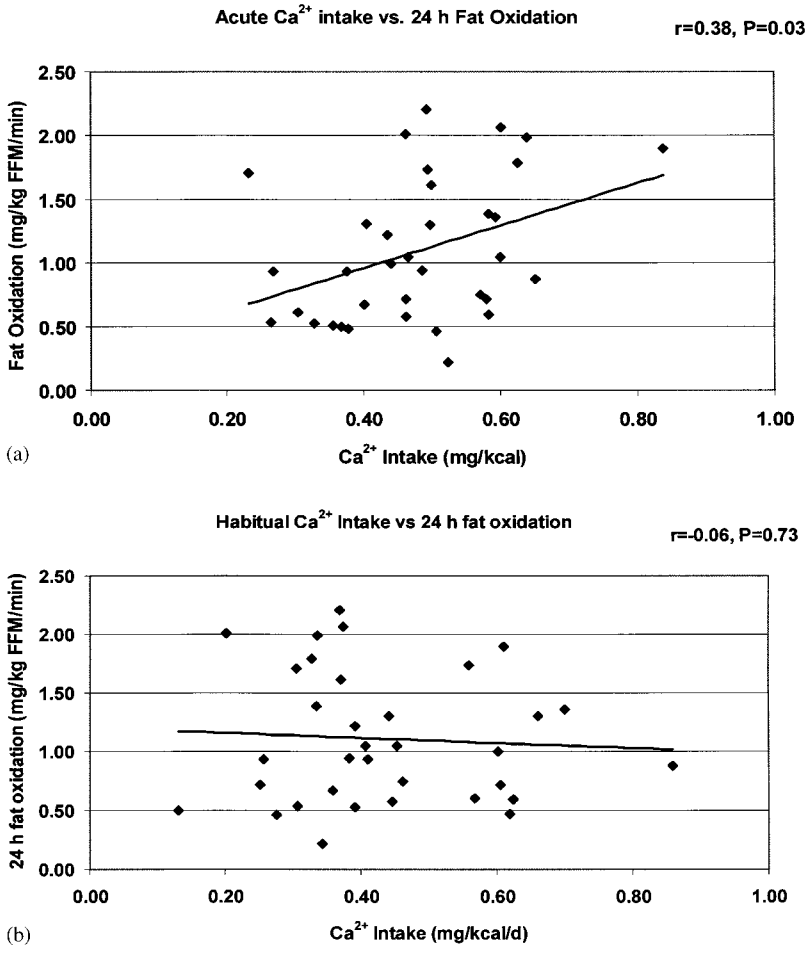

Figure 1 Relation between 24-h fat oxidation and acute (a) and habitual (b) Ca^{2+} intake.

Health Sciences Center, Houston, TX, USA). Acute Ca^{2+} intake was determined from measured food intake during the calorimeter stay. Diets consumed in the calorimeter were developed using Diet Planner (Version 2.12, San Francisco, CA, USA), which provides macro- and selected micronutrient data, based on the gram weight of food consumed. Calcium intake from dairy sources was calculated from the estimated intake of milk, cheese, ice cream, and yogurt. To minimize estimation errors, only whole-food sources of dairy Ca^{2+} were used in the estimation of dairy Ca^{2+}; dairy Ca^{2+} from mixed or prepared foods (eg lasagna) was not used.

Statistical analysis. Statistical analyses were done using SAS (SAS Institute Inc., Cary, NC, USA, 2000). To minimize the effects of body weight, calcium intake is expressed relative to total calories consumed (mg kcal^{-1}), and fat oxidation relative to fat-free mass (gm kg FFM^{-1} min^{-1}). Unadjusted correlation coefficients between Ca^{2+} intake and measures of fat oxidation were determined using Pearson correlations. To further examine the relationship between Ca^{2+} intake and fat oxidation, multiple regression models were developed adjusted for factors known to affect 24 h fat oxidation (fat mass, fat-free mass, energy balance, and acute and habitual fat intake), and partial correlations adjusting for these factors were used to estimate variance in outcomes that is explained by Ca^{2+}. Finally, to consider the effects of dairy *vs* total Ca^{2+} intake on fat oxidation, backwards stepwise multiple regression was performed using total (acute and habitual) and dairy (acute and habitual) Ca^{2+} as predictors.

Results

A total of 35 subjects (21 m, 14 f) were studied (mean $\pm$ s. d., age: 31 $\pm$ 6 y; weight: 71.2 $\pm$ 12.3 kg; BMI: 23.7 $\pm$ 2.9 kg m^{-2}; body fat: 21.4 $\pm$ 5.4%). Mean self-reported Ca^{2+}

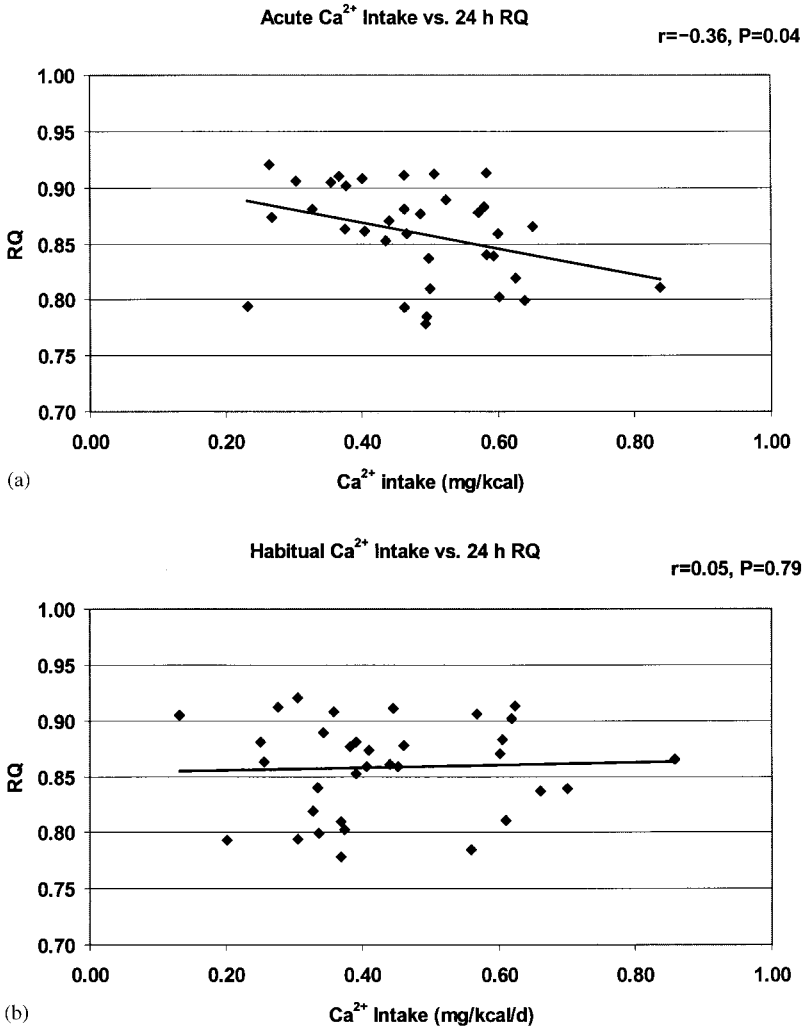

Figure 2 Relation between 24-h RQ and acute (a) and habitual (b) Ca^{2+} intake.

intake (mean ± s.e.m.) was 1222 ± 116 mg day^{-1} (range, 485–4109 mg day^{-1}). Self-reported dairy calcium intake was 664 ± 94 mg day^{-1}, corresponding to 50.3 ± 3.6% of total calcium intake. During the 24 h stay in the whole-room calorimeter, acute Ca^{2+} intake was 1046 ± 55 mg day^{-1} (range, 477–1768 mg day^{-1}). Acute dairy Ca^{2+} intake was 640 ± 44 mg day^{-1}, which was 59.5 ± 1.7% of total Ca^{2+} intake. Self-reported Ca^{2+} intake was greater than Ca^{2+} intake measured in the calorimeter ($P=0.09$), but were significantly correlated whether expressed in absolute (mg day^{-1}, $r=0.46$, $P<0.01$) or relative (mg kcal^{-1}, $r=0.35$, $P=0.04$) terms. Neither habitual ($r=0.04$) nor acute ($r=0.26$) Ca^{2+} intake were significantly correlated with 24-h EE.

The association between Ca^{2+} intake and 24-h fat oxidation and RQ is illustrated in Figures 1 and 2. Acute Ca^{2+} intake (mg kcal^{-1}) was positively correlated with 24-h fat oxidation (Figure 1a, $r=0.38$, $P=0.03$) and inversely correlated with 24-h RQ (Figure 2a, $r=-0.36$, $P=0.04$). Acute dairy Ca^{2+} intake was also significantly correlated with 24-h fat oxidation ($r=0.35$, $P=0.04$). In the adjusted multiple regression model, total acute Ca^{2+} (partial $r=0.33$, $P=0.08$) and acute dairy Ca^{2+} (partial $r=0.31$, $P=0.11$) were positively correlated with 24-h fat oxidation. Neither habitual total Ca^{2+} intake (mg·kcal^{-1}, Figures 1b and 2b) nor habitual dairy Ca^{2+} intake (data not shown) were significantly related to 24-h fat oxidation or RQ. After adjustment, total habitual (partial $r=-0.23$, $P=0.22$) and habitual dairy Ca^{2+} (partial $r=-0.20$, $P=0.30$) were not significant predictors of 24-h fat oxidation.

Similar to the 24-h data, acute Ca^{2+} intake was positively correlated with sleeping fat oxidation (Figure 3a, $r=0.36$, $P=0.04$) and inversely correlated with sleeping RQ (Figure 4a, $r=-0.31$, $P=0.07$). Acute dairy Ca^{2+} intake was also significantly correlated with sleeping fat oxidation ($r=0.45$, $P<0.01$). After adjustment, total acute Ca^{2+} (partial $r=0.29$, $P=0.13$) and acute dairy Ca^{2+} (partial $r=0.38$, $P=0.04$)

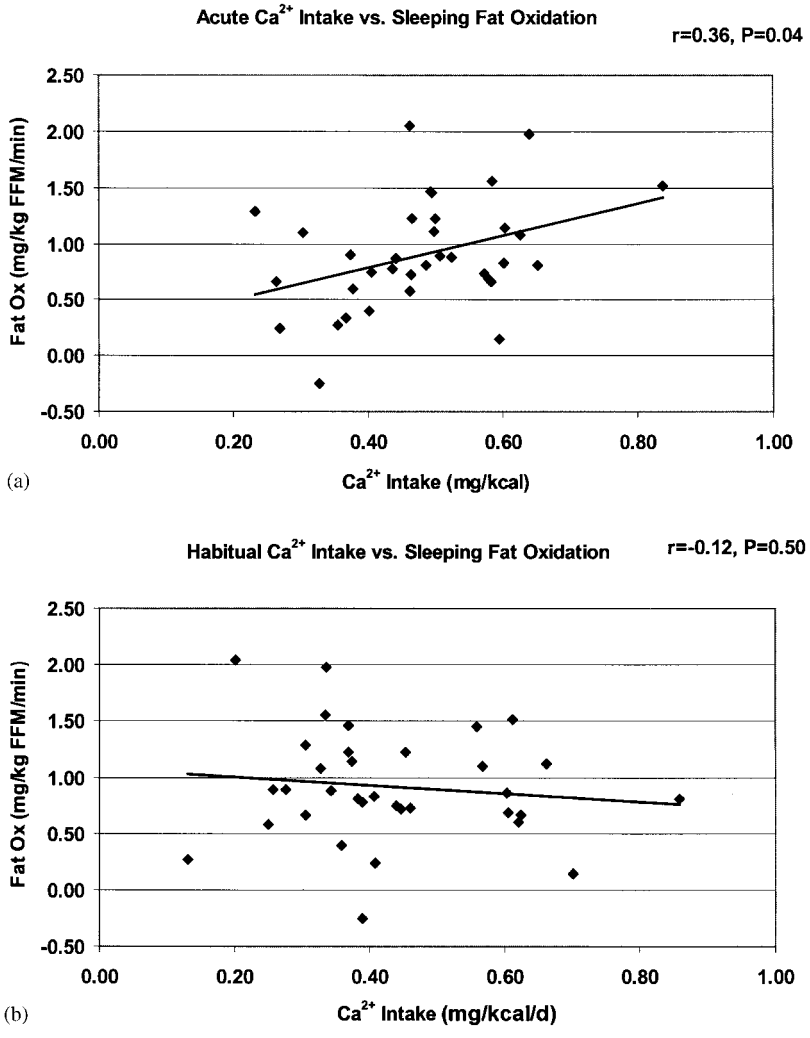

Figure 3 Relation between sleeping fat oxidation and acute (a) and habitual (b) Ca^{2+} intake.

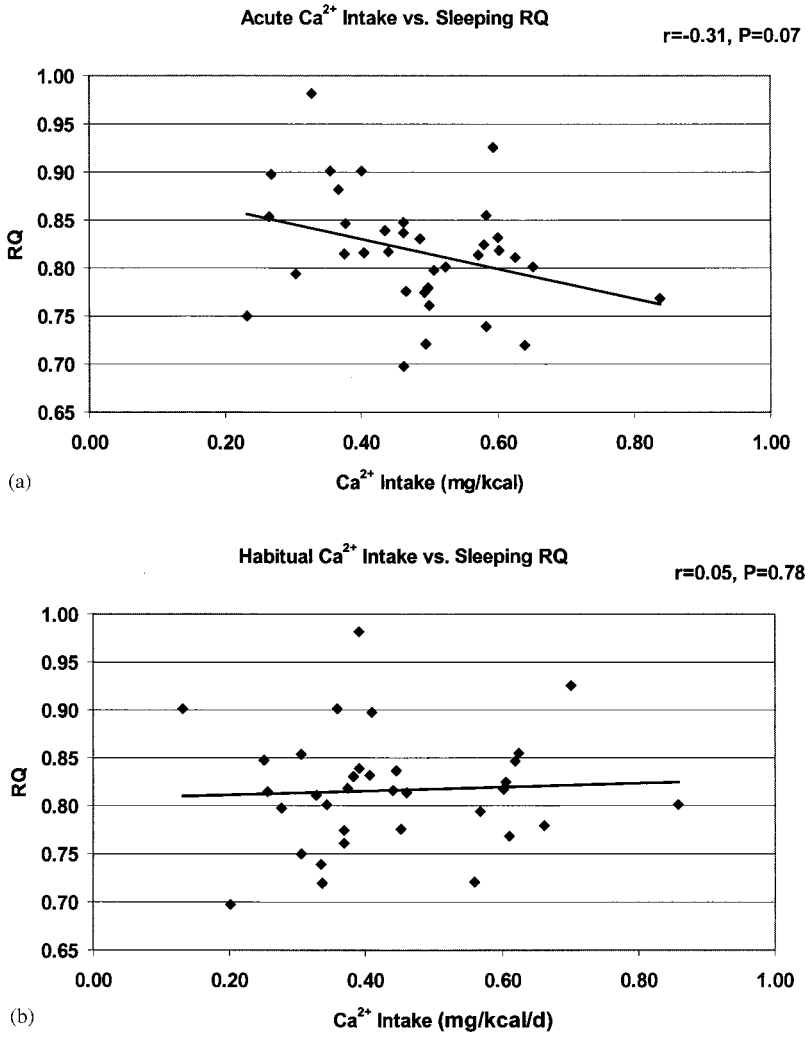

Figure 4 Relation between sleeping RQ and acute (a) and habitual (b) Ca^{2+} intake.

intakes were weakly but positively correlated with sleeping fat oxidation. Habitual Ca^{2+} intake was not related to sleeping fat oxidation (Figure 3b) or RQ (Figure 4b). After adjustment, total habitual Ca^{2+} (partial $r=-0.23$, $P=0.07$) and habitual dairy Ca^{2+} (partial $r=-0.31$, $P=0.11$) were not significant predictors of sleeping fat oxidation.

Acute Ca^{2+} intake was positively correlated with walk/step fat oxidation ($r=0.32$, $P=0.07$) and inversely correlated with walk/step RQ ($r=-0.25$, $P=0.16$, Figure 5a), although these correlations were not significant. After adjustment, acute total Ca^{2+} (partial $r=0.25$, $P=0.20$) and acute dairy Ca^{2+} (partial $r=0.18$, $P=0.35$) explained less than 5% of the variance in walk/step fat oxidation. Habitual Ca^{2+} intake (Figure 5b) was not related to walk/step RQ. Similarly, neither acute nor habitual dairy Ca^{2+} intake were significantly correlated with walk/step RQ or fat oxidation, even in the adjusted regression models (data not shown).

In the backwards stepwise multiple regression models, acute ($P=0.02$) and habitual ($P=0.04$) total Ca^{2+} intake remained significant predictors of 24-h fat oxidation, whereas acute and habitual dairy Ca^{2+} were not. However, sleeping fat oxidation was best predicted by total habitual ($P=0.01$) and acute dairy ($P<0.01$) Ca^{2+} intake.

Discussion

In healthy, young, non-obese humans, acute Ca^{2+} intake is significantly and positively related to fat oxidation measured using whole-room, indirect calorimetry. Thus, the findings of the current study are consistent with the hypothesis that high dietary Ca^{2+} diets protect against fat mass gain by promoting lipolysis,[5] which may in turn promote increased fat oxidation. To our knowledge, this is the first study in humans to report an association between Ca^{2+} intake and fat oxidation.

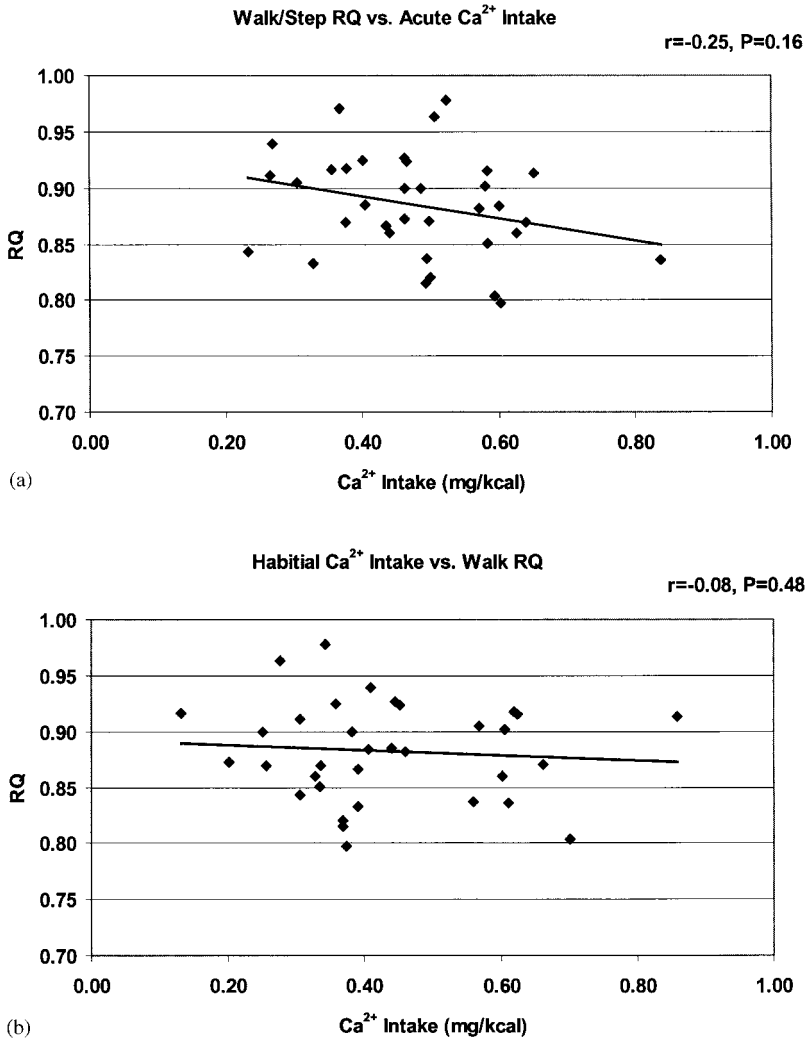

Figure 5 Relation between RQ during the standardized walking and stepping routine and acute (a) and habitual (b) Ca^{2+} intake.

Interestingly, habitual self-reported Ca^{2+} intake was not related to fat oxidation. This could be because of problems with self-report, or with imprecision for estimating Ca^{2+} intake from food recall methods. Self-report errors were not a factor in our estimate of acute Ca^{2+} intake, although estimating Ca^{2+} from measured intake would still introduce errors. Alternatively, the significant association of fat oxidation with acute but not habitual Ca^{2+} intake may suggest that the effects of dietary Ca^{2+} on adipocyte metabolism occur rather quickly. $1\alpha,25\text{-}(OH)_2D_3$ exerts physiological effects through post-nuclear transcription,[22] and also generates rapid non-genomic signal transductions, including stimulation of $[Ca^{2+}]_i$, via a putative vitamin D receptor expressed in a wide variety of cells.[24–27] Glycerol release from human adipocytes is suppressed only after 2 h treatment with $1\alpha,25\text{-}(OH)_2D_3$. Pretreatment with a specific antagonist (1β-dihydroxyvitamin D) of the putative membrane vitamin D receptor blocks this effect, whereas treatment with an agonist ($1\alpha,25$-dihydroxylumisterol$_3$) specific for this receptor makes the effect more pronounced.[28] Thus, it is possible that the association between acute Ca^{2+} intake and 24-h fat oxidation in the current study can be attributed to non-genomic effects of $1\alpha,25\text{-}(OH)_2D_3$.

Because the current study was a cross-sectional analysis, we were not able to determine if Ca^{2+} intake exerts a direct effect on fat oxidation. Indeed, it is possible that dietary Ca^{2+} only serves as a marker for other nutrients that directly modulate lipolysis and fat oxidation. Direct *in vivo* effects of alterations in dietary Ca^{2+} on circulating levels of PTH and $1\alpha,25\text{-}(OH)_2D_3$, changes in intracellular Ca^{2+} concentrations, and the subsequent effects on lipolysis and fat oxidation remain to be demonstrated.

It has been suggested that the beneficial role for dietary Ca^{2+} in weight management is markedly greater from dairy *vs* non-dairy sources of Ca^{2+}.[5,17] However, in the current analysis, the correlations between fat oxidation and Ca^{2+}

intake were nearly identical whether total or dairy Ca^{2+} was used. Moreover, total, but not dairy, Ca^{2+} remained as significant predictors of 24-h fat oxidation in backwards stepwise regression models. However, acute dairy Ca^{2+} intake was a significant predictor of sleeping fat oxidation in the backwards models. Thus, although our data do not support an additive effect of dairy Ca^{2+} on fat oxidation, we cannot exclude the possibility that the effects of dairy Ca^{2+} on fat oxidation are only apparent at rest.

In summary, in this cross-sectional analysis we found that subjects with higher intakes of dietary calcium during a 24-h period also had higher rates of fat oxidation during that period. After adjustment for factors known to affect fat oxidation measured with room calorimetry, intake of this single micronutrient explained $\sim$10% of the variance in fat oxidation between individuals. Although these results do not show directly that calcium promotes fat oxidation, the findings are consistent with the hypothesis that high intakes of calcium are associated with lower levels of fat mass.

Acknowledgements

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