

## The acute effects of different sources of dietary calcium on postprandial energy metabolism

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Dairy Ca intake has been shown to be superior to elemental Ca in increasing the loss of body fat during energy restriction. We questioned whether the mechanisms involved an increase in postprandial energy expenditure, fat oxidation and/or a greater lipolysis. The acute effects of different sources of Ca were examined in eight subjects, aged 47–66 years and BMI 27.6–36.1 kg/m<sup>2</sup>, in a three-way cross-over study. Subjects were randomly provided breakfast meals either low in dairy Ca and vitamin D (LD; control), high in non-dairy Ca (calcium citrate) but low in vitamin D (HC) or high in dairy Ca and vitamin D (HD). Diet-induced thermogenesis, fat oxidation rates (FOR), carbohydrate oxidation rates (COR), insulin, glucose, NEFA and glycerol were measured hourly over a 6 h postprandial period. Postprandial data were calculated as a change ( $\Delta$ ) from the fasting value. Results showed that  $\Delta$ NEFA was significantly different between meals (LD  $-1.50$  (SEM 0.26), HC  $-1.22$  (SEM 0.32), HD  $-0.94$  (SEM 0.27) mmol/l  $\times$  6 h;  $P=0.035$ ), with a lesser suppression following both high-Ca meals.  $\Delta$ FOR was significantly higher following the two high-Ca meals (LD  $-6.5$  (SEM 2.2), HC 2.93 (SEM 2.34), HD 3.3 (SEM 2.5) g  $\times$  6 h;  $P=0.005$ ), while reciprocally  $\Delta$ COR was significantly lower.  $\Delta$ Glycerol was less suppressed following the high-Ca meals but statistical significance was not achieved. No differences in diet-induced thermogenesis, insulin or glucose were observed. Regardless of source, Ca intake acutely stimulated postprandial fat oxidation; and there was a lesser suppression of NEFA following these meals.

### Calcium: Vitamin D: Thermogenesis: Fat oxidation: Obesity

The epidemic of obesity, and its co-morbidities of dyslipidaemia, insulin resistance and CVD, is now a global problem. The prevalence of overweight and obesity in Australia increases with age and is greatest in the 55–64-year age group (Thorburn, 2005). Overall, it is more common for Australians to have a weight problem, with 48% men and 30% women being overweight and a further 19% men and 22% women, obese (National Health and Medical Research Council, 1997). Interestingly, 50% of the adult Australian population are consuming Ca at well below the recommended dietary intake (Australian Bureau of Statistics, 1998). Vitamin D, a key regulator in Ca status, is also of concern. Recent Australian data state that 70% of the elderly and institutionalised Australian population have a frank or sub-optimal vitamin D status, while 23% younger Australians seem to have a poor vitamin D status (Nowson & Margerison, 2002). The co-existence of poor Ca status and overweight or obesity presents a tantalising paradigm for public health intervention.

The regulation of Ca homeostasis is important to a large number of physiological processes, from blood clotting to muscle contraction to enzyme secretion. Over the years the role of Ca in osteoporosis and hypertension has been well established. However, until recently the role of Ca in obesity and type 2 diabetes was unheard of. Zemel *et al.* (2000)

propose that intracellular Ca holds the key to regulation of insulin sensitivity, lipid storage and synthesis. Interestingly Zemel's tenet arose from clinical trials on hypertension, where it was observed that increasing the intake of Ca from about 400 to 1000 mg/d resulted in significant weight loss. Zemel speculated that the increased calcitrophic hormones (vitamin D<sub>3</sub>) secondary to a low Ca intake stimulated adipocyte Ca influx, which in turn stimulated fatty acid synthase activity and hence lipid storage (Zemel *et al.* 2000). If this is true, increasing Ca intake should lower calcitrophic hormones, reduce intracellular Ca and protect against fat gain.

A number of epidemiological studies have supported Zemel's original findings with a strong inverse correlation between Ca intake and adiposity (Parikh & Yanovski, 2003) and that individuals with the lowest Ca intake tend to have the highest body weight. Data from the National Health and Nutrition Examination Survey III also showed a strong inverse association for relative risk of obesity and Ca intakes (Zemel *et al.* 2000). Similar relationships have also been demonstrated in the Australian population, based on an examination of the data from the National Nutrition Survey 1995 (Soares *et al.* 2004a). The results indicated that Australian men and women with higher Ca intakes had lower BMI as well as waist circumferences, on controlling for various confounders

**Abbreviations:** COR, carbohydrate oxidation rate; FOR, fat oxidation rate; HC, high non-dairy Ca (calcium citrate) and low vitamin D; HD, high dairy Ca and vitamin D; LD, low dairy Ca and vitamin D (control).

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(Soares *et al.* 2004a). Retrospective analyses lend support to the thesis that Ca may influence the regulation of body weight. Davies *et al.* (2000) re-evaluated the results from five clinical studies (one intervention and four observational studies) looking at dietary Ca and bone mineral content. The results of Davies *et al.* (2000) showed a significant negative association between Ca intake and weight for all age groups; however, it is important to point out that with these early studies, weight or fat loss was not the major end point. More recently three clinical studies have documented that a high-dairy-Ca diet accelerated fat loss, particularly from the abdominal region. These results were obtained under energy-deficit conditions (Zemel, 2004, 2005a,b), as well as during weight maintenance (Zemel *et al.* 2005b). The studies of Thompson *et al.* (2005) and Harvey-Berino *et al.* (2005), however, did not replicate these outcomes. Both studies concluded that a high-Ca diet did not augment weight loss above that obtained from energy restriction alone. Clearly, the issue is far from settled.

The present study examined the acute effects of increasing Ca intake on postprandial thermogenesis and substrate oxidation in overweight and obese subjects. It was based on the hypothesis that an increase in Ca intake would stimulate fat oxidation and thermogenesis, and that Ca from dairy sources would be superior to elemental (non-dairy) Ca in these effects.

## Methods

### Study design

The study was a single-blind, within-subject randomised comparison of the acute responses to three isoenergetic mixed meals. The interval between trials ranged from 2 to 3 weeks, and subjects were instructed to maintain their habitual intake and activity patterns throughout the study.

### Subjects

Eight subjects, six men and two postmenopausal women, aged between 47 and 66 years and BMI within the range 27.6–36.1 kg/m<sup>2</sup>, who were residents of Perth, WA, were recruited by advertisement in the local media (for subject details, see Table 1). Subjects were screened by a telephone questionnaire and selected individuals underwent an appointment with

the chief investigator to explain the test-day protocol and familiarise the subjects with indirect calorimetry measurements. Inclusion criteria included: (i) absence of clinical signs or symptoms of chronic disease; (ii) history of weight stability ( $\pm 2$  kg for the preceding 12 months); (iii) not on medication affecting metabolic rate or body composition; (iv) resting diastolic blood pressure  $< 90$  mmHg; (v) for the women, at least 2 years postmenopausal; (iv) for the women, not on hormone replacement therapy. All subjects gave written informed consent to participate in the study. The Human Research Ethics Committee of Curtin University of Technology (HR 245-2001) approved the protocol. All measurements were made in the clinical rooms of the Bentley Campus of Curtin University (Perth, WA, Australia).

### Test meals

The composition of the test breakfasts is provided in Table 2. The meals contained the following foods to meet the percentage macronutrients and Ca goals (Table 2): white bread (Goodman Fielder, Malaga, WA, Australia); butter (Bowland Dairies Pty Ltd, Rowville, Vic, Australia); Adelphi ham and tomato (Coles Supermarket, Karawara, West Australia); muesli bar (Uncle Toby's, Wahgunyah, Vic, Australia). One meal was supplemented by a low-Ca, low-vitamin D UHT milk (LD; control). One meal was supplemented with a high-Ca, high-vitamin D milk (HD). All milks were provided by Murray Gouldburn Co-Operative Co. Ltd (Brunswick, Vic, Australia). One meal was supplemented with orange juice (Berri Ltd, Carlton, Vic, Australia), and consumed with two tablets of Citracal (Mission Pharmacal Australia Pty Ltd, Sydney, NSW, Australia) (HC). The solid components of all meals were of a similar weight (about 400 g) and fluids consumed were of identical volumes (200 ml). The macronutrient content of the breakfast meals was determined using Foodworks analysis package 3.0 (Xyris Software, Highgate Hill, Qld, Australia). All subjects completed a palatability questionnaire that enquired about amount, taste, and overall acceptability of each meal. Each answer was scored on a 15 cm visual analogue scale anchored by the most negative to the most positive response for each question.

### Anthropometry and body composition measurements

Standing height was measured using a stadiometer fixed to the wall and recorded to the nearest 0.1 cm. Body weight was measured after an overnight fast on each occasion, immediately after voiding, with subjects wearing underwear and a light surgical gown, on a digital balance and recorded to the nearest 100 g. Waist and hip circumferences were measured as described by Norton & Olds (2000). Body composition was determined by dual-energy X-ray absorptiometry (Lunar DPX-L, Madison, WI, USA) at a certified commercial bone densitometry centre.

### Measurement protocol

Subjects were requested to abstain from any strenuous exercise for 36 h before the measurement. All subjects were provided with 2 litres de-ionised water for *ad libitum* consumption 12 h before the study. They were provided with

**Table 1.** Physical and metabolic characteristics of the ten subjects\*

(Mean values with their standard errors)

	Mean	SEM
Age (years)	53.7	2.28
Weight (kg)	92.4	6.97
BMI (kg/m <sup>2</sup> )	32.5	0.97
Fat mass (kg)	31.6	2.82
Fat-free mass (kg)	63.3	7.23
Waist circumference (cm)	108.1	3.76
Serum vitamin D <sub>3</sub> (nmol/l)	75.5	7.62
Fasting triacylglycerol (mm)	1.7	0.025
Fasting LDL (mm)	3.4	0.06
Fasting HDL (mm)	0.7	0.0058

\* Six males and two females.

**Table 2.** Nutrient composition of the test meals  
(Mean values with their standard errors)

Diet...	LD		HC		HD	
	Mean	SEM	Mean	SEM	Mean	SEM
Energy content (kJ)	2391.0	0.028	2386.9	0.29	2372.8	0.46
Protein (g)	20.7	0.004	20.7	0.002	21.1	0.004
Protein (% total energy)	14.7	0.002	14.7	0.001	15.1	0.001
Total fat (g)	22.0	0.003	21.6	0.009	22.1	0.001
Total fat (% total energy)	34	0.004	33.4	0.013	34.5	0.005
Carbohydrate (g)	69.9	0.00	70.7	0.016	68.3	0.03
Carbohydrate (% total energy)	46.8	0.005	47.4	0.006	46.0	0.01
Fibre (g)	4.9	0.001	4.9	0.002	4.7	0.005
Ca (mg)	175.8	0.04	575	0.001	531.7	0.05
Vitamin D (nmol/l)*	40		45		364	
Volume (ml) <sup>†</sup>	365		350		362	
Weight (g)	401.1	0.05	408.1	0.032	408.7	0.04

LD, low dairy Ca and vitamin D (control); HC, high non-dairy Ca (calcium citrate) and low vitamin D; HD, high dairy Ca and vitamin D.

\*Vitamin D content was calculated using US data for one sample of each of the three test meals (Institute of Medicine Food and Nutrition Board, 1999).

<sup>†</sup>Volume was calculated by the analysis of one meal as a sample of each of the three test meals.

the same low-fat meal containing < 50 mg Ca, which was consumed on the night before each trial day. Subjects arrived at the laboratory after a 12 h overnight fast, and emptied their bladder (the time was recorded); the subjects were then weighed. They lay supine for a mandatory 30 min rest period, while the Deltatrac II metabolic monitor (Datex Ohmeda, Helsinki, Finland) was calibrated. At the end of this rest period, the canopy was placed over the head of the subject and they were asked to remain awake and motionless, as far as possible, for a 30 min RMR measurement. This protocol yields an RMR not very different from a BMR measurement obtained immediately on awaking, after an overnight stay in the laboratory (Soares *et al.* 1989).

After the RMR measurement, the plastic canopy was removed and a fasting blood sample (15 ml) was collected. Following the blood collection subjects made a fasting collection of urine. They were given a breakfast meal (Table 2), which they consumed within 10 min. The palatability questionnaire was completed at this time. After 20 min into each postprandial hour, subjects returned to the supine position and rested for 10 min. The canopy was then placed over their head and measurements made for the last 30 min of the hour. The first 5 min was not included in subsequent data analysis. In between measurements subjects were allowed to sit up in bed and listen to music, or read. Some elected to sit at a table close to the bed, where they did craft work or read a book. An intermittent measurement protocol provides accurate postprandial data relative to that obtained by continuous minute-to-minute measurement over the postprandial period (Piers *et al.* 1992). De-ionised water was allowed *ad libitum* over the first postprandial visit, and the amount consumed was noted. These amounts were kept constant for subsequent visits. All subjects made two separate urine collections, one between 0 and 3 h and another 3–6 h after the meal. The weight and duration of all collections were noted, urines were acidified and a sample was frozen at  $-80^{\circ}\text{C}$ . Total urinary N excretion was estimated by the Kjeldahl technique. All subjects were offered a buffet meal before they returned home.

#### Resting metabolic rate

RMR was measured by indirect calorimetry using a Deltatrac II metabolic monitor (Datex Ohmeda), an open-circuit ventilated canopy measurement system. The measurement was conducted under standardised conditions, as in our previous studies (Piers *et al.* 2002) with subjects lying (a) at complete physical rest; (b) in a thermally neutral environment; (c) 12–14 h after their last meal and a minimum of 8 h sleep; (d) awake and emotionally undisturbed; (e) without disease and fever. The within-subject CV in RMR was 3.3% in the present study. The Deltatrac II was calibrated on the morning of each experimental day. Performance of the instrument was also checked at regular intervals during the study period by monitoring the  $\text{CO}_2$  produced: $\text{O}_2$  consumed ratio, during a 30 min ethanol burn. The mean ratio for twenty ethanol burns was 0.663 (SD 0.008), with a CV of 1.2%.

#### Diet-induced thermogenesis and substrate oxidation rates

Diet-induced thermogenesis was measured as described previously (Piers *et al.* 1992), and expressed in absolute values (kJ/6 h) and as a percentage of the energy in the breakfast meal. Whole-body substrate oxidation rates were calculated at rest (fasting), and for every hour up to 6 h postprandial, using measures of  $\text{O}_2$  consumption,  $\text{CO}_2$  production and urinary N excretion in that postprandial phase. The equations of Ferranini (1988) were used to calculate energy expenditure and substrate oxidation rates.

#### Blood assays

Venous blood samples were drawn at baseline and at the end of each 30 min RMR measurement. Blood for determination of insulin, total cholesterol, LDL-cholesterol (by calculation), HDL-cholesterol and triacylglycerol, NEFA and glycerol was left to stand for 30 min at room temperature and then centrifuged at 3000 g for 10 min. Serum was extracted and the samples stored

at  $-80^{\circ}\text{C}$  until later analysis. A finger prick blood sample was also taken and blood glucose was measured using Accu-Chek Active glucose strips (Roche Diagnostic, Castle Hill, NSW, Australia). Serum insulin was measured by ELISA based on two monoclonal antibodies (Dako Diagnostic, Ely, Cambs, UK) according to the manufacturer's instructions. Serum triacylglycerol and total cholesterol were measured by enzymic colorimetric kits (TRACE Scientific Ltd, Melbourne, Vic, Australia). Serum HDL-cholesterol was determined after prior precipitation of apo B-containing lipoproteins with phosphotungstic acid and  $\text{MgCl}_2$  by enzymic colorimetry (TRACE Scientific Ltd). Serum LDL-cholesterol was determined by using a modified version of the Friedewald equation:  $\text{LDL-cholesterol} = ((\text{total cholesterol}) - (\text{HDL-cholesterol}) - (0.46 \times (\text{triacylglycerol})))$  (Bairaktari *et al.* 2000). NEFA and glycerol were determined by an enzymic colorimetric method (WAKO NEFA-C Kit; Novachem Pty Ltd, Collingwood, Vic, Australia and Randox Ltd, Crumlin, Co. Antrim, UK). Individual  $\text{CV}_{\text{intra}}\%$  were  $<2.1\%$  (glucose),  $\text{CV}_{\text{intra}}\%$  and  $\text{CV}_{\text{inter}}\%$   $<5\%$  (insulin),  $<2\%$  (total cholesterol),  $<2\%$  (HDL),  $<2\%$  (triacylglycerol) and  $<5\%$  for both NEFA and glycerol.

#### Statistical analysis

All data are presented as means with their standard errors, unless otherwise stated. Change between fasting and fed states was calculated by subtracting the fasting value  $\times$  duration of measurement from the total postprandial value over 6 h. Since the intervals of measurement in the postprandial period were equal, this summary statistic was analogous to determining the incremental area under the curve (Mathews *et al.* 1990). Total postprandial fat and carbohydrate oxidation were also adjusted for their respective fasting values using the method of Ravussin & Bogardus (1989). A repeated-measures ANOVA was used to determine statistical significance, which was set at the 5% level. *Post hoc* testing used the least significant difference procedure. Pearson's correlation coefficients for all anthropometric, body composition and metabolic variables were separately calculated for each meal. Data were analysed using the SPSS for Windows statistical software package (version 11; SPSS Inc., Chicago, IL, USA).

## Results

The physical characteristics of the subjects are shown in Table 1. Sensory evaluation of the test meals showed no significant difference in the scores for 'amount' (LD 9.3 (SEM 3.2) cm; HC 7.3 (SEM 3.0) cm; HD 6.7 (SEM 2.6) cm), 'taste' (LD 12.8 (SEM 2.5) cm; HC 12.7 (SEM 2.4) cm; HD 11.8 (SEM 3.3) cm) and 'overall acceptability' of each meal (LD 12.9 (SEM 2.5) cm; HC 14.1 (SEM 0.7) cm; HD 13.5 (SEM 1.2) cm).

#### Effect of breakfast meals

**Energy expenditure and substrate oxidation.** There was no significant difference in body weight or RMR between the three trials. However, basal fat oxidation rate (FOR) was significantly higher following the low-Ca meal, and carbohydrate oxidation rate (COR) significantly lower, despite randomisation of the test meals (Table 3). Diet-induced thermogenesis, in absolute terms (kJ/6 h) or as percentage of energy in meal (%), was not different between test meals (Table 3). There was a significant rise in RQ following all meals, but the change in RQ was significantly ( $P=0.029$ ) lower by 12.7 and 7.8% following the HC and HD meals, respectively (Table 4). Fat oxidation was significantly suppressed after each meal, but the suppression was significantly less by 38 and 44% following the HC and HD meals, respectively (Table 4). Reciprocally, the increase in carbohydrate oxidation ( $\Delta\text{COR}$ ) was significantly ( $P=0.027$ ) lower by 60% (HC) and 55.4% (HD) compared with the LD meal (Table 4). Sum of postprandial FOR adjusted for fasting values was also significantly different between meals ( $P=0.039$ ), with the rank order meal LD  $<$  meal HC  $<$  meal HD (Table 4). The sum of postprandial COR adjusted for fasting values showed a trend to be lowest following the HD meal ( $P=0.06$ ; Table 4).

**Substrates and hormones.** There were no significant differences between the three meals for basal or postprandial glucose or insulin concentrations (Table 4; Fig. 1 (A and B)). Following all meals, postprandial NEFA levels were suppressed, with the HD meal significantly less suppressed over the 6 h postprandial period relative to the LD meal (LD  $-1.50$  (SEM 0.26) mmol/l; HC  $-1.22$  (SEM 0.32) mmol/l; HD  $-0.94$  (SEM 0.27) mmol/l;  $P=0.035$ ) (Fig. 2 (A)). Serum glycerol was less suppressed

**Table 3.** Fasting measurements before the three test meals (Mean values with their standard errors for eight subjects)

Meal...	LD		HC		HD		Significance by ANOVA for repeated measures ( <i>P</i> )
	Mean	SEM	Mean	SEM	Mean	SEM	
RMR (kJ/h)	298.5	23.1	292.9	24.1	296.1	22.4	0.31
RQ	0.80 <sup>a</sup>	0.02	0.89 <sup>b</sup>	0.02	0.85 <sup>b</sup>	0.02	0.009
Protein oxidation (g/h)	3.0	0.4	3.33	0.2	3.8	0.7	0.26
Fat oxidation (g/h)	4.1 <sup>a</sup>	0.5	1.9 <sup>b</sup>	0.7	2.9 <sup>ab</sup>	0.6	0.003
Carbohydrate oxidation (g/h)	5.3 <sup>a</sup>	1.2	10.2 <sup>b</sup>	1.6	7.4 <sup>ab</sup>	1.1	0.026
Serum glucose (mmol/l)	5.25	0.2	5.09	0.98	5.13	0.14	0.22
Serum insulin ( $\mu\text{IU/ml}$ )	11.83	4.20	13.11	4.84	10.63	4.29	0.31
Serum NEFA (mmol/l)	0.65	0.076	0.627	0.076	0.615	0.092	0.54
Serum glycerol ( $\mu\text{mol/l}$ )	77.86	5.45	73.73	5.84	76.39	7.99	0.27

LD, low dairy Ca and vitamin D (control); HC, high non-dairy Ca (calcium citrate) and low vitamin D; HD, high dairy Ca and vitamin D.

<sup>a,b</sup> Mean values within a row with unlike superscript letters were significantly different ( $P<0.05$ ; *post hoc* least significant difference procedure).



**Table 4.** The influence of the source of calcium on postprandial thermogenesis and substrate oxidation (Mean values with their standard errors for eight subjects)

	LD		HC		HD		Significance by ANOVA for repeated measures ( <i>P</i> )
	Mean	SEM	Mean	SEM	Mean	SEM	
Thermogenesis (kJ/6 h)*	155.3	26.3	170.8	32.4	165.7	19.5	0.59
DIT (%)	6.5	1.1	7.2	1.4	7.0	0.8	0.58
Change in RQ*	0.32 <sup>a</sup>	0.09	-0.039 <sup>b</sup>	0.08	-0.024 <sup>b</sup>	0.11	0.029
Change in protein oxidation (g/6 h)*	-11.5	2.4	-13.7	0.7	-16.7	4.4	0.31
Change in carbohydrate oxidation (g/6 h)*	34.1 <sup>a</sup>	7.6	13.6 <sup>b</sup>	7.5	15.2 <sup>b</sup>	7.1	0.032
Adjusted sum of carbohydrate oxidation (g/6 h)†	68.0	5.1	72.5	4.8	59.8	7.5	0.061
Change in fat oxidation (g/6 h)*	-6.5 <sup>a</sup>	2.2	2.9 <sup>b</sup>	2.3	3.3 <sup>b</sup>	2.5	0.005
Adjusted sum of fat oxidation (g/6 h)†	14.1 <sup>a</sup>	1.8	18.2 <sup>ab</sup>	1.7	20.9 <sup>b</sup>	2.4	0.039
Change in glucose (mmol/l)	2.04	1.25	1.65	0.50	1.73	1.02	0.76
Change in insulin (μIU/ml)*	181.93	50.18	188.18	31.90	193.09	40.16	0.73
Change in NEFA (mmol/l)*	-1.50 <sup>a</sup>	0.26	-1.22 <sup>ab</sup>	0.32	-0.94 <sup>b</sup>	0.27	0.035
Change in glycerol (μmol/l)*	-48.29	27.0	0.99	25.62	2.31	26.78	0.31

LD, low dairy Ca and vitamin D (control); HC, high non-dairy Ca (calcium citrate) and low vitamin D; HD, high dairy Ca and vitamin D.

DIT, diet-induced thermogenesis.

<sup>a,b</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ; *post hoc* least significant difference procedure).

\* Calculated as sum of postprandial values over 6 h - (fasting value per h × 6).

† Calculated as sum of postprandial values over 6 h adjusted for fasting baseline.

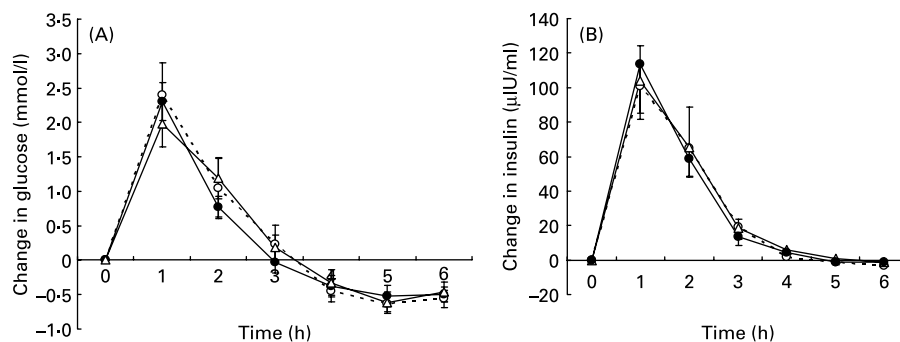
after the HD meal by almost 70 % but results did not attain statistical significance (LD -48.30 (SEM 27.0) μmol/l; HC 1.0 (SEM 25.62) μmol/l; HD 2.3 (SEM 26.78) μmol/l;  $P = 0.31$ ) (Fig. 2 (B)). Pearson's correlations did not detect any significant relationships between variables.

## Discussion

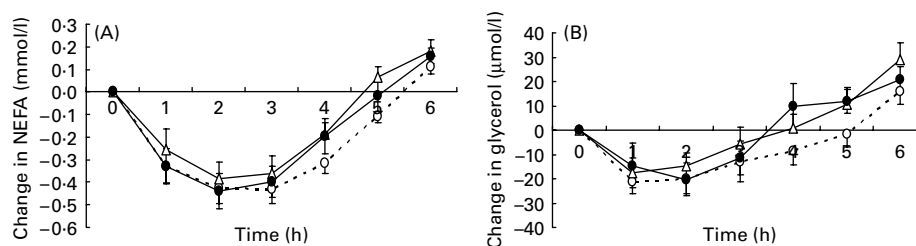
The regulation of fat balance is critical to energy balance and the equilibrium of fat stores in the body (Flatt, 1995). Fat balance is determined by the body's ability to match fat oxidation to fat intake. Any dietary factor that can stimulate fat oxidation over a prolonged period would hence reduce fat stores, provided there was no compensatory increase in fat intake. We pursued the idea that Ca and other ingredients in dairy products may help alter body composition if they could either stimulate whole-body fat oxidation, increase postprandial energy expenditure or accelerate mobilisation of fat stores (lipolysis).

For this purpose the control test meal was low in dairy (and Ca) and vitamin D (LD). The second experimental meal (HC) was a high-Ca meal using calcium citrate (pharmacological). There is evidence to indicate that Ca is better absorbed as calcium citrate in both fasting and mixed-meal situations (Sakhaee *et al.* 1999). In addition, this meal was served with orange juice to maximise the absorption of the calcium citrate tablets (Mehansho *et al.* 1989; Heaney *et al.* 2005). The third meal (HD) was high in dairy and hence Ca with additional vitamin D (see p. 2). Vitamin D was added to enhance the absorption of Ca (Holick, 2004) and to mimic the US practice of fortifying all dairy products with vitamin D (Newmark *et al.* 2004).

There were no differences in the taste preference and overall acceptability of each meal. Palatability of a test meal is known to affect insulin responses and ultimately substrate utilisation (Sawaya *et al.* 2001). The amount and volume of each meal were also similar (Table 2) and led to similar gastric emptying, based on the paracetamol absorption test



**Fig. 1.** (A) Changes from fasting values for glucose responses in eight subjects, following breakfast meals low in dairy and Ca (○), no dairy but high in Ca (●) and high in dairy and Ca (Δ). Values are means, with their standard errors represented by vertical bars. Repeated-measures ANOVA shows a significant effect of time ( $P = 0.014$ ), no significant effect of meal ( $P = 0.886$ ) and no meal × time interaction ( $P = 0.293$ ). (B) Changes from fasting values for insulin responses in eight subjects, following breakfast meals low in dairy and Ca (○), no dairy but high in Ca (●) and high in dairy and Ca (Δ). Values are means, with their standard errors represented by vertical bars. Repeated-measures ANOVA shows a significant effect of time ( $P = 0.003$ ), no significant effect of meal ( $P = 0.729$ ) and no meal × time interaction ( $P = 0.604$ ).



**Fig. 2.** (A) Changes from fasting values for NEFA responses in eight subjects, following breakfast meals low in dairy and Ca ( $\circ$ ), no dairy but high in Ca ( $\bullet$ ) and high in dairy and Ca ( $\Delta$ ). Values are means, with their standard errors represented by vertical bars. Repeated-measures ANOVA shows a significant effect of time ( $P=0.005$ ), a significant effect of meal ( $P=0.035$ ) and no meal  $\times$  time interaction ( $P=0.593$ ). (B) Changes from fasting values for glycerol responses in eight subjects, following breakfast meals low in dairy and Ca ( $\circ$ ), no dairy but high in Ca ( $\bullet$ ) and high in dairy and Ca ( $\Delta$ ). Values are means, with their standard errors represented by vertical bars. Repeated-measures ANOVA shows a significant effect of time ( $P=0.012$ ), no significant effect of meal ( $P=0.28$ ) and no meal  $\times$  time interaction ( $P=0.538$ ).

(Cummings *et al.* 2004). Hence, neither palatability nor gastric emptying of the test meals is expected to confound the outcomes.

#### Fat oxidation following calcium

We observed that subjects acutely fed a high-dairy or high-Ca meal exhibited a lesser suppression of FOR over a 6 h postprandial period, when compared with a low dairy Ca meal. These findings have been confirmed by our group using a sequential meal design (Soares *et al.* 2004b). The present results also compare favourably with evidence from the literature. In a cross-sectional study, Melanson *et al.* (2003) showed a positive correlation ( $r$  0.38;  $P=0.03$ ) between acute Ca intake and fat oxidation using whole-body room calorimetry. Moreover, in a prospective study of energy restriction, these authors showed a greater 24 h fat oxidation following high Ca intakes (Melanson *et al.* 2005). One long-term study has also demonstrated a greater FOR in subjects who followed a high-Ca diet for 1 year (Gunther *et al.* 2005). In contrast, Jacobsen *et al.* (2005) manipulated Ca and protein intakes over 2-week periods and did not find any change in 24 h energy expenditure or fat oxidation between diets.

In the present study, circulating levels of NEFA were less suppressed following both high-Ca meals (Fig. 2 (A)), which paralleled the changes in fat oxidation. The similar glycaemic and insulinaemic responses following all three meals (Figs. 1 (A and B)) would suggest that the relatively elevated NEFA levels did not result from a reduced insulin action. Frayn (1998) and Coppack *et al.* (1994) have argued that following a mixed meal, chylomicron triacylglycerol is preferentially acted upon by adipose tissue lipoprotein lipase. While re-esterification into triacylglycerol does occur, much of this NEFA fails to be 'trapped' within adipose tissue and finds itself back in circulation.

A role for the sympathetic nervous system in postprandial events cannot be discounted. Sympathetic nervous system activity contributes to meal-induced thermogenesis, and is a potent stimulator of adipose tissue lipolysis (Coppack *et al.* 1994). Although not statistically significant, the postprandial changes in serum glycerol were similar between the two high-Ca meals, and about 70% higher relative to the low-Ca meal (Fig. 2 (B)). Evidence of a significantly higher glycerol level following high Ca intakes has also been obtained under hypoenergetic conditions (Zemel *et al.* 2005a,b), as well as during weight maintenance (Zemel *et al.* 2005b). While we acknowledge that measurements of circulating glycerol are not the best

quantitative index of adipose tissue lipolysis, the consistency of effects across studies merits further investigation. We anticipated a higher diet-induced thermogenesis between meals, but could not demonstrate such an effect. Hence higher intakes of Ca acutely modulate the type of fuel being utilised in the postprandial state, without a change in energy production. The Ca content of the meals tested in the present study (530–575 mg) would in all probability exceed what is habitually consumed at individuals meals. Whether this would result in an attenuation of the effect on fat oxidation remains a possibility, but requires dose–response studies for confirmation. Second, an increased frequency of Ca consumption would increase the net amount absorbed for the day. Hence both factors are important when extrapolating results from acute meal-based studies to dietary prescription for body-weight regulation.

In any study involving the manipulation of a nutrient, it is important to be reassured that the results are not biased by poor absorbance of that nutrient. We have examined three markers of Ca bioavailability; intact parathyroid hormone suppression, serum ionised Ca and urinary Ca excretion. The rank order of bioavailability was similar across all methods with meal LD < meal HD < meal HC (Cummings & Soares, 2005). The observations that FOR following the HD meal were similar to after the HC meal, despite poorer bioavailability of Ca, may indicate the presence of other bioactive components in dairy products that influence fat oxidation (Zemel, 2002, 2003; Parikh & Yanovski, 2003).

To the best of our knowledge, the present prospective study is the first to show a greater postprandial FOR following high-Ca meals, both dairy and non-dairy. The lesser postprandial suppression of NEFA would drive the increase in the fat oxidation, and the trend for a greater lipolysis would serve to maintain the relatively higher NEFA levels following such meals. Such data provide a mechanistic framework for understanding how the intake of Ca-rich foods may lead to a greater fat loss in obese human subjects undergoing energy restriction. Further confirmation of the acute, and chronic benefits of Ca are required to place these observations on a firmer footing.

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