Title: Appetite sensations and substrate metabolism at rest, during exercise and recovery: impact of a high-calcium meal.

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Abstract:

The aim of this study was to investigate the effects of the calcium content of a high-carbohydrate, pre-exercise meal on substrate metabolism and appetite sensations before, during and after exercise. Nine active males participated in 2 trials in a double-blind, randomized, crossover design. After consuming a high carbohydrate (1.5 g/kg body mass) breakfast with a calcium content of either 3 (CON) or 9 mg/kg body mass (CAL), participants ran at 60% VO_{2peak} for 60 min. Following exercise, a recovery drink was consumed and responses were investigated for a further 90 min. Blood and expired gas were sampled throughout to determine circulating substrate and hormone concentrations and rates of substrate oxidation. Visual analogue scales were also administered to determine subjective appetite sensations. Neither whole-body lipid oxidation, nor free fatty acid availability differed between trials. The area under the curve for the first hour following breakfast consumption was 16% (95% CI: 0, 35%) greater for fullness and 10% (95% CI: 2, 19%) greater for insulin in the CAL trial but these differences were transient and not apparent later in the trial. This study demonstrates that increasing the calcium content of a high carbohydrate meal transiently increases insulinemia and fullness but substrate metabolism is unaffected.

Keywords: lipid metabolism; calcium; satiety response; hunger; insulin; exercise; randomized-controlled trial; fat oxidation

Introduction

Achieving an optimal body composition is important for many athletes (both elite and recreational), and is achieved via manipulation of both diet and exercise programmes. Calcium intake is associated with reduced fat mass (Schrager 2005), and can accelerate fat loss under energy restriction (Zhu et al. 2013) therefore possibly providing an opportunity for nutritional intervention. This relationship between calcium intake and body fat may be accounted for, in part, by increased faecal fat excretion (Christensen et al. 2009), whole-body lipid oxidation (Gonzalez et al. 2012a) and/or appetite regulation (Major et al. 2009; Bellisle and Tremblay 2011; Gilbert et al. 2011; Ping-Delfos and Soares 2011).

Most literature to date has focussed on chronic calcium supplementation, and less is known about the acute metabolic impact of calcium ingestion. Elevated postprandial lipid oxidation (Soares et al. 2004; Cummings et al. 2006; Ping-Delfos and Soares 2011) and non-esterified fatty acid (NEFA) availability (Soares and Chan She Ping-Delfos 2008) have been observed following ingestion of high calcium and vitamin D meals, in older, overweight/obese populations. This suggests calcium can overcome part of the postprandial suppression of fat oxidation when carbohydrate-containing meals are consumed (Gonzalez et al. 2013). However these investigations were performed at rest, and exercise metabolism in response to acute calcium intake has received little interest.

To date, only one study has investigated this specifically (White et al. 2006). Trained female runners were provided with 5 g/carbohydrate/kg body mass in the 4 h prior to

exercise from 2 meals, the second of which (consumed 1 h prior to exercise) was a test drink either high (500 mg) or low (80 mg) in calcium. No differences were observed in lipid oxidation during exercise. However, evidence from others indicates that NEFA concentrations diverge at 3 h post-consumption of test meals differing in calcium content (Cummings et al. 2006; Ping-Delfos and Soares 2011). As such, the 1 h between test-meal consumption and exercise may not have been long enough to allow for any calcium-related changes. Furthermore, the period of fast prior to test drink consumption was not standardised between-individuals. Therefore we aimed to provide a test meal 3 h prior to exercise and with a lower carbohydrate load, yet still in accordance with sports nutrition guidelines (Burke et al. 2011), to examine the effect of acute calcium intake on subsequent exercise metabolism with diet and activity standardised for 24 h prior to trials.

Considering appetite regulation, some have theorised about calcium specific appetite (Tordoff 2001) which may play a role in energy intake. Evidence is also available that 6 months of milk supplementation augments fasted fullness sensations (Gilbert et al. 2011) and ingestion of a high-calcium and vitamin D breakfast can reduce subsequent 24-h energy intake.

Accordingly, the objective of this study was to investigate the impact of acute calcium intake on postprandial subjective appetite ratings and substrate metabolism during rest, exercise and recovery.

Materials and Methods

Participants

Based on previous findings of a 2.7 g/h (5.4 g for 2 h) difference in lipid oxidation with a high calcium and vitamin D breakfast (Ping-Delfos and Soares 2011) and our own typical error of this postprandial measure equating to 3.37 g over a 2 h period (Gonzalez et al. 2012b) it was calculated that a sample size of 8 would provide statistical power above 80% with an alpha level of 0.05. In order to account for dropouts, the aim was to recruit a sample of 10.

Following completion of informed written consent, ten physically active males were recruited from the student and staff population at Northumbria University. One participant withdrew from the study due to time commitments and therefore nine participants are included in the analysis. Their age, height, body mass, BMI, peak oxygen uptake (V_{O2peak}) and habitual calcium intakes were (mean ± SD) 25 ± 4 years, 180.8 ± 7.8 cm, 79.6 ± 7.1 kg, 24.4 ± 2.5 kg/m^2, 55.1 ± 5.4 ml/kg/min and 1047 ± 487 mg/d, respectively. Eligibility criteria included, young (< 35 y), non-obese (BMI < 30 kg/m^2), self-reported physically active (>30 min of structured exercise, 5 times/week) and no known metabolic or gastrointestinal diseases or food allergies. The protocol was approved by the School of Life Sciences Ethics Committee at Northumbria University.

Preliminary Measurements

Participants undertook two preliminary tests to establish 1) the relationship between oxygen uptake and running speed on a flat treadmill using a 16-min test, and 2) their

110 VO_{2peak} using an incremental treadmill test whereby the gradient was increased by
111 1%/min to exhaustion as previously described in full detail (Williams et al. 1990). On
112 the same day, participants were familiarised with the visual analogue scales (VAS) to
113 later assess subjective appetite sensations in main trials. A food frequency
114 questionnaire, previously validated, and used in athletic populations (Taylor et al. 2009;
115 Bescos Garcia and Rodriguez Guisado 2011), was completed to estimate habitual
116 calcium intake.

117

118 **Experimental Design**

119 Participants completed two trials in a randomized (randomization performed by J.T.G.
120 using an electronic statistical package), double-blind, crossover design separated by ≥ 7
121 d which consisted of a control (CON) and high milk-calcium (CAL) trial. All trials were
122 performed under similar laboratory conditions (mean ± SEM; Temperature: 20.5 ± 1.3
123 and 20.2 ± 1.1 °C; Humidity: 49 ± 3 and 48 ± 3 %; Pressure: 1021 ± 7 and 1021 ± 9
124 mbar for CON and CAL trials, respectively; all $P > 0.05$). Food and fluid diaries were
125 kept for the day preceding the first trial and participants were instructed to replicate this
126 for all subsequent trials. Participants were asked to avoid all foods containing dairy in
127 the final meal prior to trials. Alcohol, caffeine and vigorous activity were prohibited for
128 24 h prior to trials. Compliance to diet and physical activity was assessed by self-
129 completed food diaries and verbal questioning.

130

131 On the morning of the trials, participants arrived in the laboratory at 0730 after a 10-14
132 h fast and a cannula was inserted into an anticubital vein for blood sampling. After

baseline expired gas samples and VAS, participants were served breakfast which consisted of honey nut cornflakes (Tesco, Dundee, UK), skimmed milk (Cravendale, Arla Foods, Denmark) and 100 ml water. The breakfast provided 1.5 g carbohydrate/kg body mass in accordance with current sports nutrition guidelines for pre-exercise meals (Burke et al. 2011). The breakfasts were identical in energy (600 ± 53 kcal), protein (17 ± 2 g), carbohydrate (119 ± 11 g), fat (6 ± 1 g), and vitamin D (7 ± 1 μg) content. On CAL trials, milk-extracted calcium (Capolac®, Arla Foods Ingredients amba, Denmark) was added to the milk to increase calcium content from 3 to 9 mg/kg body mass (CON: 238 ± 21 mg, CAL: 716 ± 74 mg). The calcium powder was completely soluble in milk and the quantities used to increase the calcium content of the meal resulted in negligible increases in protein, carbohydrate and fat (all < 0.5 g) and sodium, magnesium, chloride and potassium (all < 90 mg). Water consumption was *ad libitum* during the postprandial period in the first trial and water intake was replicated for the subsequent trial. Following a 3 h postprandial breakfast period, participants ran on a treadmill at 60% VO_{2peak} for 60 min (Figure 1).

Immediately following exercise, participants ingested 500 ml of chocolate milk as a test drink (Yazoo, Campina Ltd, West Sussex, UK) providing 1500 kJ (18, 63, and 19 % of energy from protein, carbohydrate and fat, respectively) followed by a 90 min recovery period. Chocolate milk was chosen due to its effectiveness as a post-exercise recovery drink (Thomas et al. 2009) and also as a means to investigate potential second-meal effects following the high-calcium breakfast (Soares et al. 2004; Ping-Delfos and Soares 2011).

157 On a separate day, 12 participants (all of the original 10 participants in the study, plus
158 an additional 2 physically active males) performed a triangle taste test to ensure that
159 there was no detectable difference in taste between the breakfasts. In brief, participants
160 tasted three breakfasts, one of which differed from the other two in calcium content.
161 Participants were asked to identify the odd one out of the three. As only 3 out of 12
162 were correct, this was below the 33% considered to be correct by chance.

163

164 **Anthropometric Measurements**

165 Body mass was determined to the nearest 0.1 kg using balance scales (Seca,
166 Birmingham, UK) upon arrival to the laboratory, where participants wore only light
167 clothing. Height was measured to the nearest 0.1 cm using a stadiometer (Seca,
168 Birmingham, UK).

169

170 **Blood Sampling and Analysis**

171 10 ml blood samples were collected at baseline, at 15, 30, 45, 60, 90, 120 and 180 min
172 following breakfast consumption (Figure 1). All samples apart from the time point
173 during exercise were obtained whilst participants were supine to control for changes in
174 plasma volume with posture. Following exercise and test drink ingestion, additional 1
175 ml samples were collected at 5, 10, 15, 20, 25, 30, 45, 60 and 90 min where blood
176 glucose was determined immediately by a glucose analyzer (Biosen C_line, EKF
177 Diagnostics, Magdeberg, Germany). Of the 10 ml samples, a 20 µl capillary tube was
178 filled with whole blood and used to determine blood glucose concentrations, and the
179 remaining whole blood was allowed to stand for 30 min in a non-anticoagulant tube

before being centrifuged at 3000 g and 4°C for 10 min. Aliquots of serum were stored at -80°C for later determination of NEFA (WAKO Diagnostics, Richmond, VA) and insulin (IBL International, Hamburg, Germany) concentrations in duplicate. Intra-assay coefficients of variation were 8.4% and 3.0% for NEFA and insulin, respectively. As exercise of a similar, and indeed greater, intensity and duration has been shown not to result in changes in plasma volume (Broom et al. 2007; Burns et al. 2007; Martins et al. 2007; Broom et al. 2009), it was decided that there was no need to adjust analyte concentrations following exercise and test drink ingestion.

Energy Expenditure and Substrate Oxidation

Substrate metabolism was calculated assuming negligible protein oxidation, with rates of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) using stoichiometric equations and was adjusted during exercise to account for the contribution of glycogen to metabolism (Jeukendrup and Wallis 2005):

$$\text{Rate of lipid oxidation at rest and during exercise (g/min)} = (1.695 \times VO_2) - (1.701 \times VCO_2) \quad (1)$$

$$\text{Rate of carbohydrate oxidation at rest (g/min)} = (4.585 \times VCO_2) - (3.226 \times VO_2) \quad (2)$$

$$\text{Rate of carbohydrate oxidation during exercise (g/min)} = (4.585 \times VCO_2) - (2.962 \times VO_2)$$

(VO_2 and VCO_2 expressed as L/min) (3)

Energy expenditure was calculated based on lipids, glucose and glycogen providing 40.81, 15.64 and 17.36 kJ/g, respectively. At rest, calculations were based on glucose providing all of the carbohydrate for metabolism, whereas during moderate intensity exercise carbohydrate oxidation is met by both glucose and glycogen providing a 20 and 80% contribution, respectively (Jeukendrup and Wallis 2005).

The assumption of negligible protein oxidation was unlikely to affect the estimations of substrate oxidation, as calcium (from dairy or as calcium citrate) does not influence postprandial protein oxidation (Cummings et al. 2006). For further clarification however, the respiratory exchange ratio (RER) is also reported.

Expired gas samples were collected using an online gas analysis system (Metalyzer 3B, Cortex, Germany) calibrated using gases of known concentrations and a 3 L syringe. Participants wore a facemask and after a 5 min stabilisation phase, 10 min samples were obtained and averaged at baseline, every 30 min after breakfast and test drink consumption in line with best practice methods (Compher et al. 2006). Expired gas was continuously sampled throughout exercise and averaged over each 5 min period ignoring the first 5 min to allow for steady-state values. Heart rate was also determined at 15 min intervals throughout exercise via short-range telemetry (Polar Electro UK Ltd., Warwick, UK).

225 **Subjective Ratings**

226 Paper based, 100 mm VAS were completed at baseline, immediately following
227 breakfast and every 30 min thereafter. Questions asked were used to determine hunger,
228 fullness, satisfaction, prospective food consumption (Flint et al. 2000).

229

230 **Statistical Analysis**

231 Due to difficulties with blood collection in one participant, data for blood analytes are
232 presented from 8 participants. Subjective appetite ratings and blood analyte
233 concentrations were converted into time-averaged area under the curve (AUC) using the
234 trapezoidal rule.

235

236 Data were tested for normal distribution using the Anderson-Darling normality test and
237 data not displaying normal distribution were log-transformed prior to statistical analysis.
238 Paired t-tests were used to determine differences at baseline, and differences in total
239 lipid oxidation, carbohydrate oxidation, energy expenditure, and AUCs between trials.
240 Two-way repeated measures ANOVA (trial x time) were used to detect differences for
241 all variables. Holm-Bonferroni step-wise post-hoc test was utilised to determine the
242 location of the variance. Pearson product-moment correlation coefficients were used to
243 determine relationships between insulin concentrations and appetite sensations and
244 between habitual calcium intake and postprandial responses. Statistical significance was
245 set at $P < 0.05$. All results are presented as mean ± SEM

246

Results

Energy Expenditure and Substrate Oxidation

There were no differences in baseline energy expenditure, lipid or carbohydrate oxidation rates, or RER (all $P < 0.05$). No significant interaction effects (trial x time) were detected for either energy expenditure, lipid oxidation or carbohydrate oxidation (all $P > 0.05$). After breakfast consumption, energy expenditure rose to a similar peak in both CON and CAL trials (2.0 ± 0.3 and 2.1 ± 0.3 kcal/min, respectively; $P > 0.05$) and lipid oxidation was suppressed to a similar extent in both trials (postprandial change from baseline: -0.05 ± 0.04 and -0.04 ± 0.03 g/min for CON and CAL, respectively; $P > 0.05$). Lipid oxidation rose throughout exercise with no differences between trials (Figure 2).

There were no differences in total energy expenditure, lipid or carbohydrate oxidation in the postprandial period following breakfast, during exercise, or during recovery (Table 1). There was also no difference in mean heart rate during exercise (CON: 137 ± 6, CAL: 137 ± 6 beats per min; $P > 0.05$).

No significant differences between trials were detected in RER following breakfast, during exercise, or recovery (Table 1; all $P > 0.05$).

Blood Glucose, Serum NEFA and Insulin Concentrations

There were no significant differences between trials in baseline glucose, NEFA or insulin concentrations (all $P > 0.05$).

No significant interaction (trial x time) effects were observed in blood glucose or serum NEFA concentrations at any time point ($P > 0.05$; Figure 3). The 180 min AUC for NEFA was 0.08 ± 0.02 and 0.09 ± 0.03 mmol/L for CON and CAL trials, respectively ($P > 0.05$). Peak insulin concentrations were 339 ± 45 and 351 ± 36 pmol/L in the CON and CAL trials, respectively ($P > 0.05$; Figure 3). The insulin AUC for the first hour was 10 ± 3% greater in CAL vs. CON ($P = 0.02$; Figure 4), but when this was extended to the full 180 min AUC, there was no difference between trials (148 ± 46 and 157 ± 14 pmol/L for CON and CAL trials, respectively; $P > 0.05$).

No differences in blood variables were detected during the recovery period (Table 2, $P > 0.05$)

Subjective Ratings

No significant interaction (trial x time) effects were observed for any subjective appetite ratings (all $P > 0.05$). There was however, a trend for an interaction effect (trial x time), and a significant main effect (trial) detected for fullness sensations ($P = 0.085$ and $P = 0.007$, respectively).

289 The fullness AUC for 0-60, 0-120 and the full 0-180 min postprandial breakfast periods
290 were 16, 14 and 11% greater in CAL vs. CON, respectively (Figure 5 and Table 2; P =
291 0.008, 0.01 and 0.04, respectively). The percentage change in insulin AUC for the 0-60
292 min, showed strong positive relationships with the percentage change in fullness AUC
293 for the same time period (r = 0.95, $P < 0.01$) and for the full 180 postprandial period (r
294 = 0.92, $P < 0.01$). Neither hunger, nor satisfaction, nor prospective consumption
295 differed significantly between trials (Figure 5; all $P > 0.05$). No differences in any
296 appetite sensations were observed during recovery ($P > 0.05$).

297

298 **Relationships with habitual calcium intake**

299 Habitual calcium intake as assessed by the food frequency questionnaire was not
300 associated with any of the high-calcium meal induced-changes in postprandial variables
301 (All $P > 0.05$).

302

303 **Discussion**

304 The main findings of this study demonstrate that increasing the dairy calcium content of
305 a meal does not affect substrate oxidation or energy expenditure before, during or after
306 exercise in physically active males, but transiently increases feelings of fullness and
307 insulin concentrations.

308

309 Acute high-calcium and vitamin D intake has been shown to increase postprandial lipid
310 oxidation in overweight/obese subjects on two occasions (Cummings et al. 2006; Ping-

311 Delfos and Soares 2011). The present study found no significant difference in either
312 postprandial, or exercise lipid oxidation in recreationally active males. Magnitude based
313 inferences suggest a 95% and 92% chance of merely a trivial difference in postprandial
314 and exercise lipid oxidation, respectively. This corroborates the findings in female
315 runners (White et al. 2006). Both these exercise studies involved consumption of high
316 carbohydrate loads prior to exercise (1.5 and 5 g/kg body mass), in accordance with
317 sports nutrition guidelines (Burke et al. 2011). Carbohydrate intake blunts NEFA
318 availability and lipid oxidation at both rest and during exercise (Gonzalez et al. 2013)
319 via insulin-induced suppression of lipolysis. It may be that the action of calcium was
320 eclipsed by the large carbohydrate loads' blunting of NEFA availability. Indeed, NEFA
321 were still suppressed to ~0.05 mmol/L when exercise began (Figure 3C).

322

323 Notwithstanding this, others have reported greater rates of lipid oxidation in the
324 postprandial period following moderate (~70 g) carbohydrate loads (Cummings et al.
325 2006; Ping-Delfos and Soares 2011) with similar levels of calcium intake (difference
326 between CAL vs. CON: ~400 mg in Ping-Delfos & Soares (2011) and ~500 mg in the
327 current investigation) suggesting that calcium may partially overcome the suppression
328 of lipid oxidation after food intake. The 60 min postprandial rise in insulin
329 concentrations seen in the present study (~250%) was less than the ~300% reported by
330 Ping-Delfos & Soares (2011), and the 180 min postprandial change in insulin
331 concentrations were similar to the full 6 h postprandial change in insulin concentrations
332 (both ~230 pmol/L) displayed by Cummings et al. (2006). Thus, with similar
333 postprandial insulinemia and calcium doses, some studies show increased NEFA
334 availability and lipid oxidation with high calcium meals, whereas the present study did
335 not. The likely superior insulin sensitivity of the recreationally active, lean individuals

336 [although 33 % of participants had a BMI of > 25 kg/m^2, their adiposity was likely low
337 given the high VO_2peak (Janssen et al. 2004)] in the current study may have resulted in
338 a more pronounced insulin-induced suppression of lipolysis (Frayn et al. 1997)
339 compared to the overweight populations used previously (Cummings et al. 2006; Ping-
340 Delfos and Soares 2011). We cannot exclude the possibility that differences in expired
341 gas collection (facemask vs. ventilated hood) underlie the discrepancy between the
342 present study, and that of others (Cummings et al. 2006; Ping-Delfos and Soares 2011).
343 Although good practise guidelines suggest there is negligible difference between the use
344 of facemasks and ventilated hoods (Compher et al. 2006).

345

346 In previous studies, postprandial responses to high-calcium meals included lessened
347 suppression of lipid oxidation in the presence of similar insulinemia (Cummings et al.
348 2006; Ping-Delfos and Soares 2011), and the present study demonstrates similar lipid
349 oxidation with higher (albeit modest and transient) insulinemia. Given that this
350 represents a degree of reduced insulin action and metabolic inflexibility, the underlying
351 potential mechanisms deserve discussion. Largely *in vitro* data have suggested that
352 parathyroid hormone and calcitriol are the major regulators of lipolysis and lipid
353 metabolism in response to calcium intake (Zemel 2004; Gonzalez and Stevenson 2012a;
354 Soares et al. 2012). *In vivo* evidence pertaining to these mechanisms in humans
355 however, does not support this. Elevated NEFA availability and lipid oxidation is
356 evident without diverging parathyroid hormone concentrations (Melanson et al. 2005;
357 Ping-Delfos and Soares 2011), and marked changes in calcitriol concentrations do not
358 influence whole body lipid oxidation, circulating NEFA availability or the expression of
359 lipolytic genes in adipose tissue (Boon et al. 2006). Future work is needed to
360 substantiate not only the efficacy of calcium in modulating lipid oxidation but also the

underlying mechanisms. The gastrointestinal peptides glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) may offer some insight. The concentration of these peptides can be enhanced by calcium co-ingestion (Gonzalez and Stevenson 2013), they have been implicated in lipolysis (Timper et al. 2013) and, when manipulated over a period of days, increase lipid oxidation (Boschmann et al. 2009). This makes it tempting to speculate that repeated calcium ingestion with the associated increased exposure to GIP and GLP-1 may lead to a shift in substrate metabolism over a period of days (as is the case with pharmaceutical manipulation (Boschmann et al. 2009)) which is easier to detect than after acute ingestion.

The present study demonstrates that increasing the calcium content of a meal can increase insulin concentrations and feelings of fullness following consumption, whilst other appetite sensations were not significantly affected. We have previously reported that fullness is more susceptible to interventions than hunger and other appetite sensations (Gonzalez and Stevenson 2012b). It may be that fullness is more intimately linked with physiological sensations (such as gastric distension and the metabolic and hormonal milieu), whereas other appetite sensations are, additionally, influenced by psychological factors (Stubbs et al. 2000). Supporting this, the difference in insulinemia between trials was strongly related to the change in fullness sensations. Similar effects have been reported previously (Flint et al. 2007) and suggest that insulin plays a strong role in postprandial fullness sensations. It should however, be acknowledged that the changes observed were relatively small and of a short duration and thus their impact in the real-life situation needs clarification.

385 The acute appetite effects of calcium have previously been inconsistent, with reduced
386 energy intake seen by some using a realistic meal design (Ping-Delfos and Soares
387 2011), but not by others with a very large (~4500 kJ; ~1075 kcal) energy load
388 (Lorenzen et al. 2007). Also, to date, evidence of mechanisms in humans is sparse.
389 Ping-Delfos et al. (2011) found no effect of increased calcium intake on appetite
390 sensations, insulin or leptin concentrations, yet blood was only sampled every hour and,
391 as we have observed a transient effect on insulin and fullness, these authors may have
392 missed the divergence in insulin concentrations. Lorenzen et al. (2007) took more
393 frequent blood samples following meal ingestion. Nevertheless, no effect was observed
394 on AUCs of insulin, or any other appetite-related hormones (namely cholecystokinin,
395 ghrelin, glucagon-like peptide-1 and peptide YY). A potential caveat of Lorenzen et al.
396 (2007) was the energy content of the test meal, which provided 50% of daily energy
397 requirements. This energy load may have caused such large perturbations to
398 postprandial hormone concentrations, that subtle effects were masked.

399

400 Given that there was no significant relationship between the habitual calcium intake and
401 the responses seen, it would suggest that the acute effects of calcium are independent of
402 habitual intake, although to corroborate this a larger study with a wide range of intakes
403 would be necessary.

404

405 It may be regarded as a limitation with the present study that subsequent energy intake
406 (ie. at lunch) was not measured, and therefore interpretations as to the impact of fullness
407 on energy balance are difficult. However, this study provides novel data by
408 demonstrating that milk-calcium can transiently increase satiety concomitant with

409 higher insulin concentrations thereby revealing one of the prospective mechanisms by
410 which calcium intake may affect appetite and provides a platform from which future
411 studies can investigate the effectiveness of calcium intake on metabolism and appetite in
412 athletic and physically active populations. Furthermore, to the best of the authors'
413 knowledge this is the first double-blind study to investigate the acute effects of calcium
414 ingested with a high-calcium meal on substrate metabolism and appetite. It is worthy to
415 note that the calcium supplement was undetectable, and therefore the influence of taste
416 and palatability can be discounted (Bellisle et al. 2012) and differences can be attributed
417 to the calcium content *per se*.

418

419 In conclusion, the present study indicates that, increasing the dairy calcium content of a
420 meal from 0.3 to 0.9 g/kg body mass does not affect substrate metabolism at rest during
421 exercise, or recovery in moderately trained males. However, increasing the calcium
422 content of a meal can augment postprandial fullness, possibly (in part) via greater
423 circulating insulin concentrations. It should be acknowledged that this augmentation is
424 modest and transient. More research is required to understand the long-term
425 implications of these findings, principally regarding energy balance and metabolism.

426

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431

432

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564
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566

Table 1. Energy expenditure and substrate metabolism in the breakfast postprandial period, exercise period and recovery postprandial period.

Trial	Breakfast period (0-180 min)				Exercise period (180-240 min)				Recovery period (240-330 min)			
	EE (kcal)	CO (g)	LO (g)	RER	EE (kcal)	CO (g)	LO (g)	RER	EE (kcal)	CO (g)	LO (g)	RER
CON												
Mean	1436	47.9	16.8	0.85	2951	100.4	30.5	0.87	778	15.4	13.2	0.79
SEM	24	1.5	0.6	0.01	47	2.7	0.8	0.01	12	1.0	0.4	0.02
CAL												
Mean	1446	46.6	17.5	0.84	2964	99.7	31.1	0.86	802	15.6	13.7	0.79
SEM	21	1.9	0.6	0.02	51	3.0	1.0	0.01	14	0.8	0.5	0.02

EE, energy expenditure; CO, carbohydrate oxidation; LO, lipid oxidation; CON, control; CAL, high-calcium. No significant differences were detected between trials by paired t-tests.

585 **Table 2.** Blood glucose concentration and subjective appetite sensations in the breakfast
586 postprandial period and recovery postprandial period.

587

Time Period	**Trial**	**Time-Averaged AUC**				
		Blood glucose ($mmol \cdot L^{-1}$)	Hunger	Fullness	Satisfaction	Prospective consumption
Breakfast Period	CON					
(0-180 min)	Mean	4.44	36	59	58	41
	SEM	0.32	6	7	6	7
	CAL					
	Mean	4.55	33	63*	59	42
	SEM	0.23	5	6	5	6
Recovery Period	CON					
(240-330 min)	Mean	4.60	49	41	41	54
	SEM	0.12	5	5	4	5
	CAL					
	Mean	4.64	51	38	38	57
	SEM	0.14	4	5	4	5

588 AUC, Area under the curve; CON, control; CAL, high-calcium. *Significantly different
589 to CON, $P < 0.05$ as detected by paired t-tests.

590

591

592 **Figure Captions:**

593

594 **Figure 1 –** Schematic representation of main trial protocols. Solid arrows indicate blood
595 samples; dashed arrows represent visual analogue scales.

596

597 **Figure 2 –** Rates of lipid oxidation (**A**), carbohydrate oxidation (**B**) and energy
598 expenditure (**C**) during the control (•) and high-calcium (○) trials. Values are means ±
599 SEM**.**

600

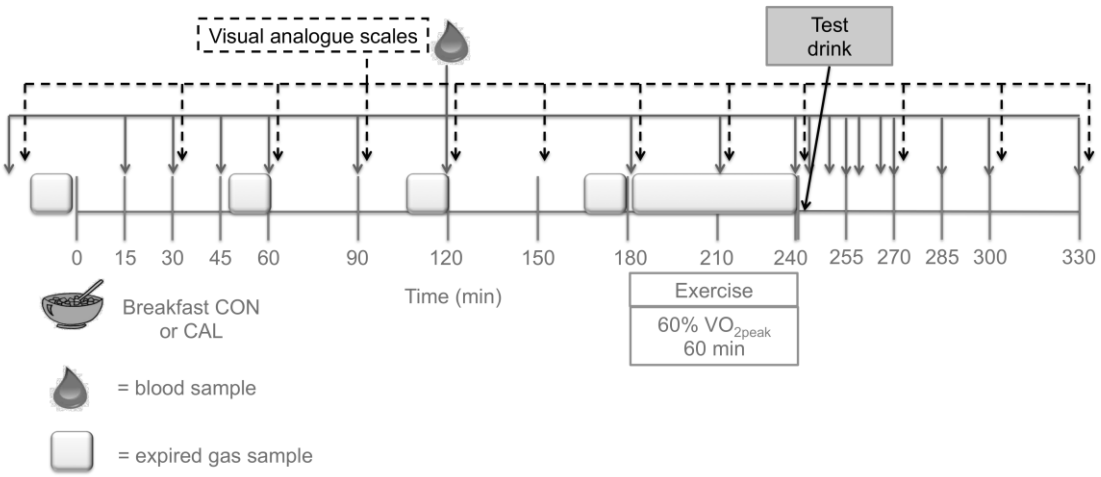
601 **Figure 3 –** Blood glucose (**A**), serum insulin (**B**) and serum non-esterified fatty acid
602 (NEFA; **C**) concentrations for the first 240 min of control (•) and high-calcium (○)
603 trials. Values are means ± SEM.

604

605 **Figure 4 –** Serum insulin time-averaged area under the curve (AUC) for the first 60 min
606 of control (CON) and high-calcium (CAL) trials. Both individual data (○) and group
607 means (•) shown. *Significantly different to CON, $P < 0.05$.

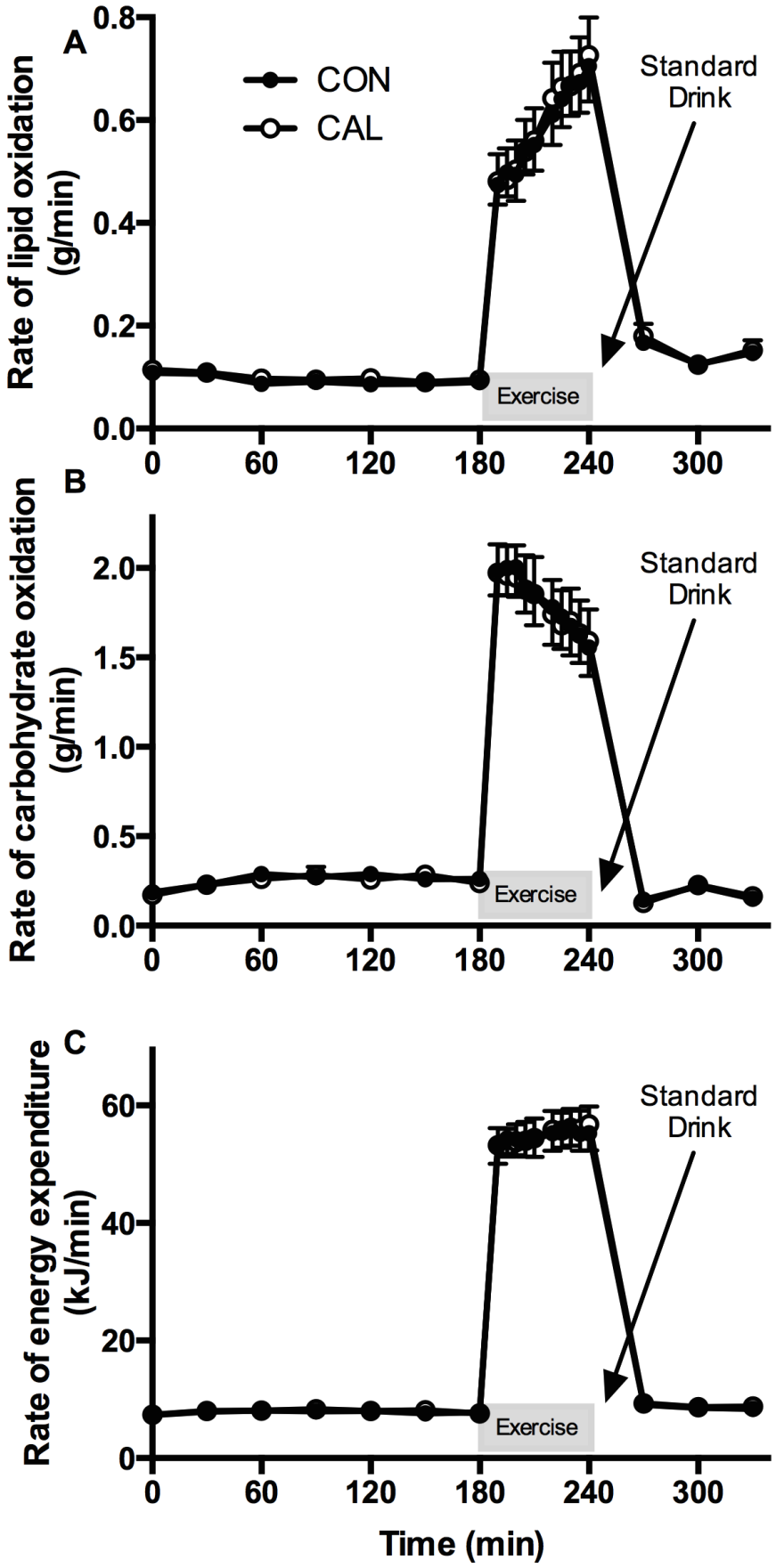
608

609 **Figure 5 –** Hunger (**A**), fullness (**B**), satisfaction (**C**) and prospective consumption (**D**)
610 for the first 240 min of control (•) and high-calcium (○) trials. Values are means ±
611 SEM.

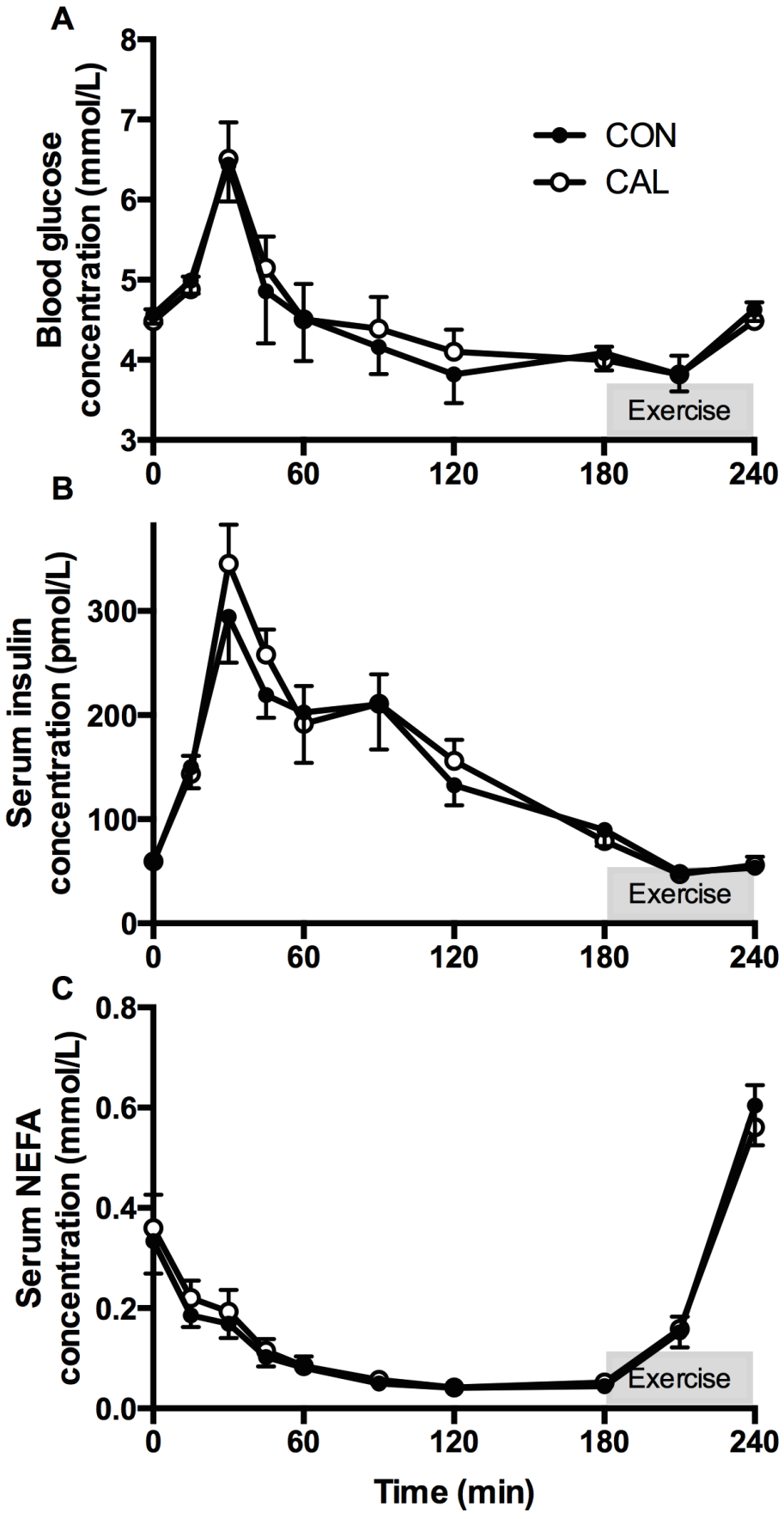

Visual analogue scales
Test drink
0 15 30 45 60 90 120 150 180 210 240 255 270 285 300 330
Time (min)
Exercise
60% VO2peak
60 min
Breakfast CON or CAL
= blood sample
= expired gas sample

612

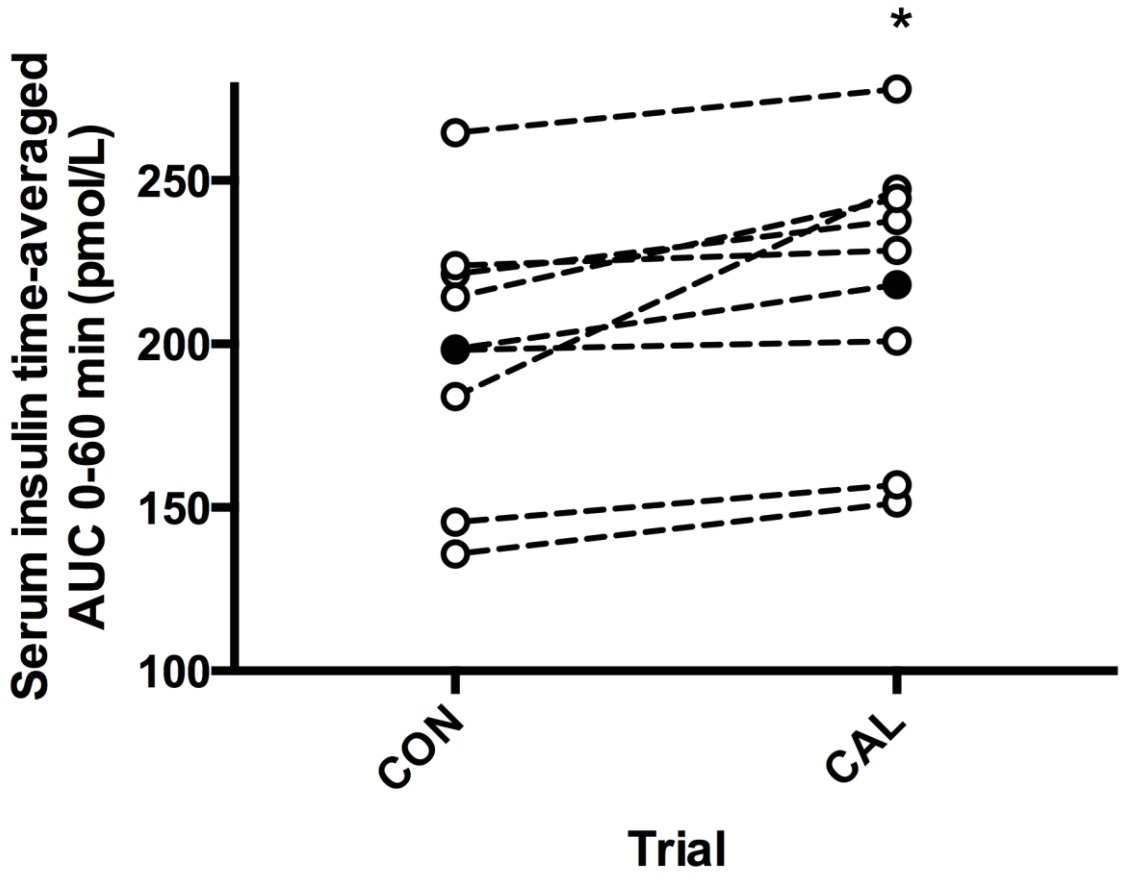
613


A
B
C
CON
CAL
Standard Drink
Exercise
Rate of lipid oxidation (g/min)
Rate of carbohydrate oxidation (g/min)
Rate of energy expenditure (kJ/min)
0.0
0.2
0.4
0.6
0.8
0.5
1.0
1.5
2.0
20
40
60
0
60
120
180
240
300
Time (min)

614


A
Blood glucose concentration (mmol/L)
8
7
6
5
4
3
CON
CAL
Exercise
0
60
120
180
240
B
Serum insulin concentration (pmol/L)
300
200
100
0
Exercise
C
Serum NEFA concentration (mmol/L)
0.8
0.6
0.4
0.2
0.0
Exercise
Time (min)

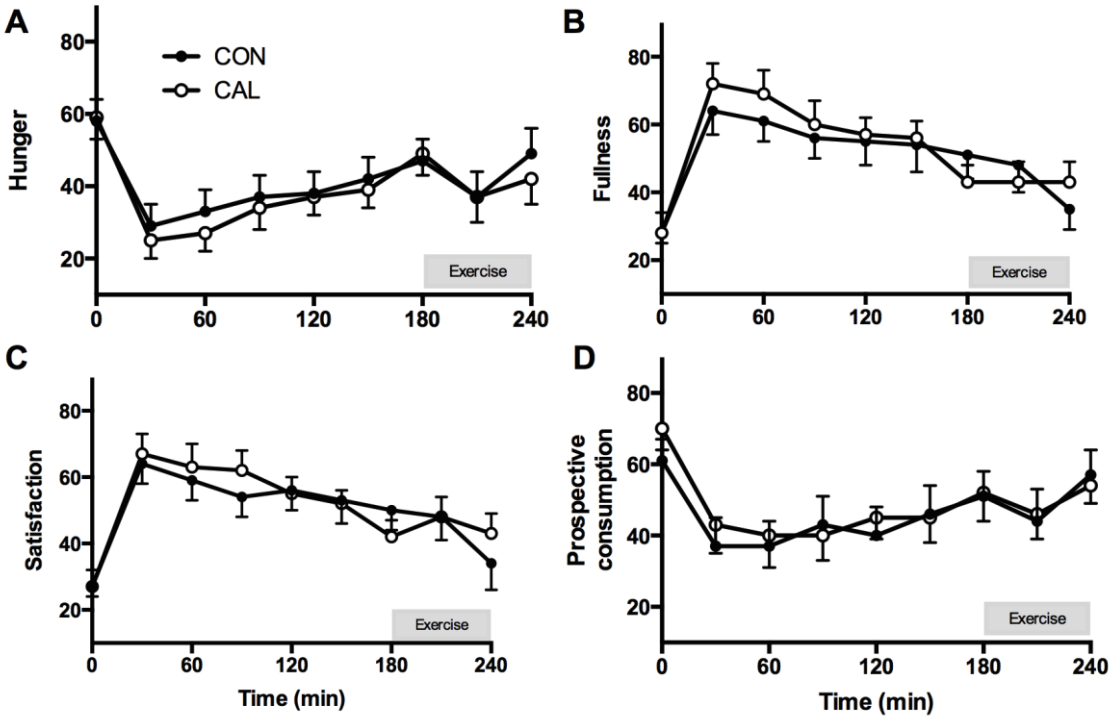
615


*
Serum insulin time-averaged AUC 0-60 min (pmol/L)
250
200
150
100
CON
CAL
Trial

616

617

618


A
Hunger
CON
CAL
Exercise
B
Fullness
Exercise
C
Satisfaction
Exercise
Time (min)
D
Prospective consumption
Exercise
Time (min)

619