Breakfast and Exercise Contingently Affect Postprandial Metabolism and Energy Balance in Physically Active Males.

Authors and Affiliations

Javier T. Gonzalez[1*], Rachel C. Veasey[1], Penny L.S. Rumbold[2] and Emma J. Stevenson[1]

[1]Brain, Performance and Nutrition Research Centre, School of Life Sciences, Northumbria University, Northumberland Building, Newcastle upon Tyne, NE1 8ST, UK

[2] Department of Sport and Exercise Sciences, School of Life Sciences, Northumbria University, Northumberland Building, Newcastle upon Tyne, NE1 8ST, UK.

***Corresponding author**

Javier Gonzalez

Brain, Performance and Nutrition Research Centre, School of Life Sciences, Northumbria University, Northumberland Building, Newcastle upon Tyne, NE1 8ST, UK

Tel: +44 (0)191 243 7012; Fax: +44 (0)191 243 7012;

Email: javier.gonzalez@northumbria.ac.uk

Running title: Integrated effects of breakfast and exercise

Key words: Appetite; Fasted; Glycaemia; Fat oxidation

Abstract

This study examined the impact of breakfast and exercise on postprandial metabolism, appetite and macronutrient balance. Twelve (blood variables n = 11) physically active males completed four trials in a randomised, crossover design comprising a continued overnight fast followed by rest (FR), a continued overnight fast followed by exercise (FE), breakfast consumption (1859 kJ) followed by rest (BR), and breakfast consumption followed by exercise (BE). Exercise was continuous moderate-intensity running (expending ~2.9 MJ). The equivalent time was spent sitting during resting trials. A test drink (1500 kJ) was ingested on all trials followed 90 min later by an *ad libitum* lunch. The difference between the BR and FR trial in blood glucose time-averaged area under the curve following test drink consumption approached significance (BR: 4.33 ± 0.14 vs. FR 4.75 ± 0.16 mmol/l; $P = 0.08$), was not different between FR and FE (FE: 4.77 ± 0.14 mmol/l; $P = 0.65$) but was greater in BE (BE: 4.97 ± 0.13 mmol/l) vs. BR ($P = 0.012$). Appetite following the test drink was reduced with BR vs. FR ($P = 0.006$) and with BE vs. FE ($P = 0.029$). Following lunch, the most positive energy balance was observed with BR and least positive with FE. Regardless of breakfast, acute exercise produced a less positive energy balance following *ad libitum* lunch consumption. Energy and fat balance is further reduced with breakfast omission. Breakfast improved the overall appetite responses to foods consumed later in the day, but abrogated the appetite-suppressive effect of exercise.

Introduction

Regular breakfast consumption has been inversely associated with body mass index[1], yet it is not clear whether this association is due to differences in energy expenditure, metabolism or energy intake. Although the ostensible benefits of regular breakfast consumption could be due to improved diet composition with breakfast cereals[1], rather than meal pattern *per se*, acute consumption of breakfast can enhance glucose tolerance, insulin sensitivity and subjective and physiological satiety responses to a test drink[2].

A recent position statement concluded that more research is required in regular exercisers with regards to meal pattern, metabolism and appetite regulation[3] as research in exercising individuals in this area is sparse. However this population do use diet/exercise strategies such as training in the fasted state to control body fat/mass and improve metabolic adaptations to training[4]. Exercise attenuates adverse dietary outcomes such as fat-induced glucose intolerance[5] and the nutritional state in which exercise is performed can modulate the magnitude of these improvements[5]. Exercise in the fasted state results in a greater reliance on fat as a substrate[6] and has led to its use as a tool to reduce body fat by athletes[4]. Training in the fasted state also leads to enhanced fat transporter protein mRNA content[5], mitochondrial enzyme activity and maximal aerobic capacity[7], making exercise in the fasted state an attractive proposition for both recreational and elite athletes. On the other hand, high carbohydrate availability during exercise training may result in improved body composition, as gains in fat free mass are amplified whilst fat loss is similar[8]. Hence, although there is a suggestion that exercise in the fasted state can maximise some benefits already associated with exercise, ensuing effects on appetite and metabolism are not entirely clear.

The regulation of acute energy balance involves (not exclusively) the exposure and sensitivity to the circulating hormonal and metabolic milieu[9], which underscores the importance of determining these changes concomitant with measuring energy balance. Exercise training improves glucose tolerance[5], yet acute exercise effects are less lucid[10-13]. Muscle glucose uptake is increased after exercise[14], as assessed in rat hind limb muscle. However, both this method and the most commonly used technique for assessing insulin sensitivity in humans (the euglycaemic-hyperinsulinaemic clamp) possess some caveats. Firstly, they ignore the gastrointestinal response to food ingestion. Direct contact of nutrients with L-cells in the intestine stimulates secretion of glucagon-like peptide 1 (GLP-1) which potentiates insulin secretion and sensitivity and reduces food intake[9]. GLP-1 exists in two active forms; in humans, the primary circulating form is $GLP\text{-}1_{7\text{-}36}$[9]. Acute exercise has been shown to increase GLP-1 concentrations in the fed state[15]. Therefore,

GLP-1 may be an important mediator in the acute regulation of energy homeostasis regarding breakfast consumption and exercise.

Secondly, provision of nutrients other than glucose can influence glucose tolerance and insulin sensitivity. Protein, for example, stimulates insulin and/or incretin hormone secretion[16]. Flavoured-milk providing mixed-macronutrients is an increasingly consumed post-exercise drink due to its recovery enhancing potential[17]. Therefore, assessing the whole-body metabolic and endocrine response to an orally-ingested mixed-nutrient load provides more applicable findings to regular exercisers. Acute exercise can transiently suppress hunger[15, 18] possibly via changes in appetite-related hormones[15, 18, 19]. Subsequent relative energy intake is usually also reduced[18, 19]. The influence of nutritional status on appetite regulation and energy intake following exercise is not entirely understood. Of the studies investigating appetite responses to fasted vs. fed exercise, one used a high fat (70%) meal[20] which is not representative of a typical breakfast, and another compared meal-exercise sequence rather than omission of breakfast *per se*[21].

Accordingly, the aim of the current investigation was to explore the interaction of breakfast consumption and exercise on the metabolic, endocrine and appetite responses to a commonly consumed post-exercise drink, and to assess subsequent energy intake and macronutrient balance in physically active males.

Materials and Methods

Participants

Following completion of informed written consent, twelve healthy males were recruited from the student and staff population at Northumbria University between December 2010 and April 2011. All participants completed the entire study. Participants self-reported as physically inactive, defined by less than 30 min of moderate activity, 5 times a week by the International Physical Activity Questionnaire[22], restrained eaters, defined by a score of >11 on the Three Factor Eating Questionnaire[23] or those with any metabolic disorders or on medications were omitted. The protocol was approved by the School of Life Sciences Ethics Committee at Northumbria University.

Preliminary measurements

Participants undertook preliminary tests to establish 1) the relationship between oxygen uptake and running speed on a flat treadmill (Woodway ELG, Woodway, Waukesha, WI, USA) using a 4

stage, 16-min test, and 2) their VO_{2peak} using an incremental treadmill test whereby the gradient was increased by 1 %/min to exhaustion as previously described in detail[24]. The duration of the exercise period in main trials was calculated from submaximal oxygen uptake and carbon dioxide values in order to expend 2.9 MJ (693 kcal) whilst running as a speed estimated to elicit 60% VO_{2peak}. This value was chosen to equate to approximately 1 h on average whilst maintaining similar energy expenditure across participants. On the same day, participants were familiarised with the visual analogue scales (VAS) to assess subjective appetite sensations in main trials and it was verbally confirmed that participants did not have any particular disliking to foods contained in the test meals.

Experimental design

All participants completed four trials in a randomised (performed by J.T.G with a statistical package), crossover design separated by ≥ 7 d consisting of breakfast omission, where the overnight fast was extended at rest (FR), breakfast consumption and rest (BR), fasted exercise (FE), and breakfast consumption and exercise (BE). By necessity of the design (food intake and exercise), the intervention was not blinded. All trials were performed under similar laboratory conditions (ambient temperature, humidity and pressure; all $P > 0.05$; data not shown). Food and fluid diaries were kept for the day preceding the first trial and participants were instructed to replicate this for all subsequent trials. Alcohol, caffeine and vigorous activity were prohibited for 24 h prior to trials.

On trial days, participants arrived in the laboratory at 0730 after a 10-14 h fast and a cannula was inserted into an antecubital vein for blood sampling. After baseline samples of expired gas and VAS, on B trials participants consumed a porridge breakfast. On F trials, participants were permitted to consume water only, which was consumed *ad libitum* on the first exercise and non-exercise trials and water consumption was replicated for the following exercise and non-exercise trials, respectively (Figure 1). Following 120 min of rest, during E trials participants ran on a treadmill at (mean ± SEM) 61.1 ± 0.6% VO_{2peak} for 59 ± 2 min based on the *a priori* estimated energy expenditure. Treadmill speed was adjusted accordingly on the first trial to obtain the appropriate VO_2. Changes in speed were noted for duplication on subsequent exercise trials. On R trials, participants rested for the equivalent amount of time as the exercise trials.

Within 20 min of exercise termination, participants ingested a chocolate milk test drink. Following a 90 min postprandial period, a homogenous *ad libitum* test lunch was provided. Participants were provided with an initial 430 g (3694 kJ; 882 kcal) portion of the test meal, which was replaced upon completion. The test meal was terminated when the participant instructed that they felt

"comfortably full". Participants were constantly reminded to follow this instruction and were always presented with fresh, warmed portions before participant-induced termination to ensure that the end of a portion was not the reason for meal termination. Remaining food was then removed and weighed out of sight of the participants to determine energy intake.

Anthropometric measurements

Body mass was determined to the nearest 0.1 kg using balance scales (Seca, Birmingham, UK) upon arrival at the laboratory, immediately prior to and following exercise, where participants wore only light clothing. Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Birmingham, UK).

Test meals

The breakfast consisted of 72 g oats (Oatso Simple Golden Syrup, Quaker Oats, Reading, UK) and 360 ml semi-skimmed milk (Tesco, Dundee, UK) and provided 1859 kJ (444 kcal; 17% protein, 60% carbohydrate and 23% fat). The test drink was 500 ml of chocolate milk (Yazoo, Campina Ltd, West Sussex, UK) and contained 1500 kJ of energy (358; 18% protein, 63% carbohydrate and 19% fat). The test lunch comprised pasta (Tesco, Dundee, UK) tomato sauce (Tesco, Dundee, UK), cheddar cheese (Tesco, Dundee, UK) and olive oil (Tesco, Dundee, UK) and provided 859 kJ per 100 g (205 kcal; 14% protein, 52% carbohydrate and 34% fat).

Blood sampling and analysis

10 ml blood samples were collected at baseline, immediately prior to, and following exercise (or the equivalent points in resting trials), at 15, 30, 50, 70 and 90 min following consumption of the test drink (immediately prior to the test meal). All samples were obtained whilst participants were seated upright to control for postural changes in plasma volume. Additional 5 ml samples were collected at 5, 10, 20 and 25 min following test drink ingestion where blood glucose was determined immediately by a glucose analyzer (Biosen C_line, EKF Diagnostics, Magdeberg, Germany). Of the 10 ml samples, a 20 µl capillary tube was filled with whole blood to determine blood glucose concentrations, 4 ml was dispensed into an EDTA vacutainer containing 100 µl aprotinin and immediately centrifuged at 3000 rpm and 4°C for 10 min. Plasma was stored for later determination of GLP-$1_{7\text{-}36}$ using an immunoassay (Phoenix Pharmaceuticals Inc., Burlingame, CA). Remaining whole blood from 10 ml samples was allowed to stand for 30 min in a non-anticoagulant tube before being centrifuged at 3000 rpm and 4°C for 10 min. Aliquots of serum were then stored for later determination of non-esterified fatty acid (NEFA; WAKO Diagnostics,

Richmond, VA) and insulin (DIAsource ImmunoAssays S.A., Nivelles, Belgium) concentrations in duplicate. All plasma/serum was stored at -80°C. The intra-assay coefficients of variation were 5.6% and 7.2% for NEFA and insulin, respectively. Inter-assay coefficients of variation were 8.1%, 3.6% and 18.5% for NEFA, insulin and GLP-1_{7-36}, respectively. In order to reduce the inter-assay variation, samples from each participant were analysed during the same run where possible. It was decided that it was unnecessary to adjust analyte concentrations to account for plasma volume changes as exercise of a similar and greater intensity and duration does not result in changes in plasma volume[15, 25].

Energy expenditure and substrate oxidation

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 2 min stabilisation phase, 5 min samples were obtained and averaged at baseline, every 30 min after breakfast consumption (or equivalent time in breakfast omission trials), and at 5, 15, 30, 50 70 and 90 min following consumption of the test drink. 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.

Substrate metabolism was calculated assuming negligible protein oxidation, with oxygen consumption and carbon dioxide production values using stoichiometric equations and was adjusted during exercise to account for the contribution of glycogen to metabolism[26]:

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

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

$$\text{Rate of carbohydrate oxidation during exercise (g/min)} = (4.210 \times VCO_2) - (2.962 \times VO_2)$$

(VO_2 and VCO_2 are L/min)

Energy expenditure was calculated based on fat, 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[26].

Subjective ratings

Paper based, 100 mm VAS were completed at baseline, prior to and immediately following breakfast and every 30 min thereafter until exercise (or equivalent time points in breakfast omission trials), further VAS were completed immediately following exercise and after test drink consumption, and at 30 min intervals thereafter. A final VAS was completed following termination of the test meal. Questions asked were used to determine hunger, fullness, satisfaction and prospective food consumption. An overall appetite score was calculated by the following formula as previously used[27]:

$$\text{Overall appetite} = [\text{hunger} + \text{prospective food consumption} + (100 - \text{fullness}) + (100 - \text{satisfaction})] / 4$$

Statistical analysis

Due to difficulties with blood collection, data for GLP-$1_{7\text{-}36}$ are presented from 10 participants and, for all other blood analytes, from 11 participants. Post-consumption of the test drink, glucose, insulin, GLP-$1_{7\text{-}36}$ and NEFA concentrations and appetite sensations were converted into area under the curve (AUC) using the trapezoidal rule. As indices of insulin secretion and sensitivity, post-test drink serum insulin AUC to blood glucose AUC ratio ($AUC_{INS/GLU}$) and Matsuda insulin sensitivity index ($ISI_{Matsuda}$) were calculated as previously described[28, 29]. Unless otherwise stated, all data are presented as mean ± SEM. One-way, repeated measures ANOVA were used to determine differences at baseline, between all AUC values and total fat and carbohydrate oxidation and energy expenditure between trials. Two-way repeated measures ANOVA (trial x time) were used to detect differences for all variables and following a significant interaction effect, simple main effects analyses were employed. This approached allowed for a comparison between the 4 conditions (FR, FE, BR and BE) across time to determine the most appropriate diet/exercise strategy. Holm-Bonferroni step-wise post-hoc test was utilised to determine the location of the variance and all *P* values reported have already been adjusted for multiple-comparisons. Differences were considered significant at $P < 0.05$.

Results

The participants' age, height, body mass, BMI and peak oxygen uptake (V_{O2peak}) were (mean ± SD) 23.2 ± 4.3 years, 178.0 ± 7.0 cm, 77.2 ± 5.3 kg, 24.5 ± 2.0 kg/m^2 and 53.1 ± 5.5 ml/kg/min, respectively.

Blood glucose

Blood glucose concentration displayed a trial x time interaction effect (Figure 2A; $P < 0.001$). Breakfast consumption reduced time to reach peak blood glucose concentration following test drink ingestion by 10 and 4 min during rest and exercise trials, respectively ($P = 0.012$ and $P = 0.02$, respectively). Peak blood glucose concentration was unaffected by breakfast consumption during resting trials (FR: 5.95 ± 0.20, BR: 5.75 ± 0.14 mmol/l; $P = 0.20$). No difference was observed in peak, nor time to peak blood glucose concentrations with FR vs. FE ($P = 0.73$ and $P = 0.28$, respectively). However, with BE, blood glucose concentration reached 6.66 ± 0.24 mmol/l; significantly greater than FE (5.89 ± 0.17 mmol/l; $P = 0.06$) and BR ($P = 0.030$). The difference between the BR and FR trial in AUC for blood glucose approached statistical significance (Figure 2B; $P = 0.09$), was not significantly different between FR and FE ($P = 0.65$), but was greater with BE vs. BR ($P = 0.012$).

Serum insulin

A trial x time interaction effect was observed for serum insulin concentrations ($P < 0.001$), where peak concentrations occurred at 37 ± 3 min in the FR trial, and the delay compared to BR (29 ± 1 min; $P = 0.09$) and FE (30 ± 4 min; $P = 0.10$) approached statistical significance. Serum insulin concentrations rose after test-drink consumption (Figure 3A) to a similar peak between trials (FR: 682 ± 71, BR: 607 ± 46, FE: 570 ± 72, BE: 586 ± 64 pmol/l; $P = 0.21$). The greater AUC for serum insulin with FR vs. all other trials approached statistical significance (Figure 3B; $P = 0.07$, $P = 0.12$ and $P = 0.09$ vs. BR, FE and BE, respectively).

Indices of insulin secretion and sensitivity

The $AUC_{INS/GLU}$ was similar between FR and BR (82 ± 7 and 80 ± 6 pmol/l·mmol/l^{-1}; $P = 0.45$), but was reduced by exercise compared to FR (FE: 70 ± 7 and BE: 67 ± 6 pmol/l·mmol/l^{-1}; $P = 0.03$ and 0.04 for FE and BE, respectively. $ISI_{Matsuda}$ was similar between trials (12 ± 4, 12 ± 4, 12 ± 4 and 13 ± 5 au, for FR, BR, FE and BE respectively; all $P > 0.05$).

Serum NEFA

Test-drink consumption transiently suppressed NEFA concentrations and a significant trial x time interaction effect was observed (Figure 4A; $P < 0.001$). The time at which the nadir of NEFA concentrations were reached was delayed in the FR trial (81 ± 3 min) compared to all other trials (BR: 65 ± 3 min, $P = 0.019$; FE: 57 ± 3 min, $P < 0.001$; BE: 55 ± 6 min, $P = 0.007$). The AUC for BR was lower than that of FR and BE (Figure 4B; $P = 0.019$ and $P = 0.004$, respectively).

Plasma GLP-1$_{7\text{-}36}$

There was no trial x time interaction effect or main effects of trial on GLP-1$_{7\text{-}36}$ concentrations (Figure 5A; both $P > 0.05$). There was also no difference in AUC (Figure 5B), peak or time to peak GLP-1$_{7\text{-}36}$ concentrations ($P = 0.17$, $P = 0.27$ and $P = 0.45$, respectively).

Energy intake, metabolism and balance

Energy expenditure, fat oxidation and carbohydrate oxidation did not differ at baseline ($P = 0.43$, $P = 0.13$ and $P = 0.57$, respectively).

In the breakfast postprandial period, energy expenditure was not significantly different between trials (Table 1). Less fat and more carbohydrate was utilised during the breakfast postprandial period in B trials vs. F trials (Table 1; $P = 0.005$ and $P < 0.001$, respectively).

The exercise bout lasted 59 ± 2 min and mean oxygen uptake was similar between FE and BE during this period (2.52 ± 0.11 and 2.50 ± 0.11 l/min; $P = 0.54$). In spite of the equivalent amount of external work performed, exercise increased energy expenditure more during B trials (3279 ± 50 kJ) compared to during F trials (2627 ± 43 kJ; $P < 0.01$). Breakfast consumption reduced the reliance on fat as a substrate and subsequently raised carbohydrate metabolism in the exercise period. An effect which was independent of exercise/rest (Table 1). This resulted in similar carbohydrate balance (intake minus oxidation) post-exercise between FE and BE, in spite of a large difference in carbohydrate balance prior to exercise (pre-exercise: -17 ± 2 and 43 ± 2 g, $P < 0.001$; post-exercise: -108 ± 7 and -102 ± 8 g, $P = 0.38$ for FE and BE respectively). Following consumption of the test drink, energy expenditure and fat oxidation were greater in both exercise trials compared to rest, yet carbohydrate oxidation was similar (Table 1).

There was no detectable difference in *ad libitum* energy intake at lunch (Figure 6; $P = 0.78$). Hence, when energy intake from the breakfast and the test drink are taken into consideration, breakfast trials produced a greater total energy intake (Figure 6; $P < 0.001$). The variation in the compensation of energy intake to account for the increase in energy expenditure (energy intake on

exercise trials minus energy intake on resting trials) ranged from -1916 to 3749 kJ (-458 to 895 kcal) on the fast trials and from -1447 to 3683 kJ (-346 to 880 kcal) during breakfast trials. Seven individuals consumed less on FE vs. FR, four individuals partially compensated for exercise, consuming more than on FE vs. FR but not enough to overcome the exercise-induced energy expenditure. Only one participant overcompensated for exercise consuming more than the exercise-induced energy expenditure on FE vs. FR. On breakfast trials, six individuals consumed less on BE vs. BR, five partially compensated and only one overcompensated for the exercise-induced energy expenditure. No significant relationship was present between the compensation on fast days and the compensation on breakfast days (r = -0.07, $P > 0.05$).

Energy balance post-lunch was most positive with BR and least positive with FE (Figure 7). There was no detectable difference in carbohydrate balance when breakfast was omitted vs. consumed, although the difference at rest approached significance (FR vs. BR, $P = 0.06$; FE vs. BE, $P = 0.95$; Figure 7). Yet, fat balance was significantly different between all trials apart from FR vs. BE, albeit with BE a reduction which approached statistical significance was observed ($P = 0.06$).

Subjective ratings

Feelings of hunger during the exercise period were suppressed with FE vs. FR ($P = 0.015$) and BE vs. BR ($P = 0.016$). This was still the case immediately post-exercise with FE vs. FR ($P = 0.002$), yet, with BE vs. BR, there was no detectable difference ($P = 0.45$). FE also reduced ratings of prospective consumption during and after exercise vs. FR ($P = 0.028$ and $P = 0.032$, respectively), whereas BE did not significantly affect prospective consumption ratings compared to BR ($P = 0.67$ and $P = 0.15$, respectively). Overall appetite rating showed similar findings (Figure 8A) where the change from pre- to during the exercise period was significantly different between the FR trial and the FE trial (2 ± 1 vs. -11 ± 4; $P = 0.048$) but not between the BR and BE trials (6 ± 2 vs. 0 ± 4; P = 0.21).

Breakfast did not influence hunger immediately pre-lunch during exercise trials ($P = 0.11$) but did reduce hunger in resting trials ($P = 0.006$). The same pattern was observed with prospective consumption (FR vs. BR: $P = 0.005$; BR vs. FE: $P = 0.005$; FE vs. BE: $P = 0.10$). However, immediately prior to lunch, overall appetite was suppressed on the BR trial compared to both F trials ($P = 0.001$ and $P = 0.005$, for rest and exercise, respectively; Figure 8B).

There was no detectable difference in AUC for hunger between exercise and rest ($P = 0.47$ and P=0.71 for FR vs. FE and BR vs. BE, respectively). The AUC for overall appetite following

314 consumption of the test drink was greater in the FR trial vs. the BR trial (Table 2; $P = 0.006$) and
315 this pattern was still apparent although was attenuated when exercise was performed (Table 2; $P =$
316 0.029). Similar patterns were shown for hunger and prospective consumption AUC and mirrored by
317 fullness and satisfaction AUC (Table 2).

318

Discussion

This study attempted to examine the cumulative effects of breakfast consumption and exercise on the metabolic and appetite responses to foods consumed later in the day and on subsequent energy and macronutrient balance. The main findings were that acute breakfast consumption is likely to reduce postprandial glycaemia and insulinaemia at rest. Acute exercise did not affect glucose tolerance when breakfast was omitted, but reduced glucose tolerance when breakfast was consumed; the pertinence of this chronically should be noted with caution, given the benefits of exercise training. Exercise in the fasted state led to a greater transitory reduction in appetite compared to exercise in the fed state. Energy and fat balance were least positive following exercise in the fasted state.

Acute breakfast consumption has been shown to improve glucose tolerance[2]. The present findings in physically active males somewhat support the previous data, although the effect may be more trivial in these aerobically fit individuals with magnitude-based inferences[30] indicating 41 and 59 % likelihoods of beneficial and negligible effects respectively on glucose tolerance. This could be due to subjects in the present study being regular exercisers and therefore displaying better basal glucose tolerance[5]. Lower fasting blood glucose concentrations (~4.5 vs. ~4.8 mmol/l), support this proposition. Lower NEFA exposure prior to consumption of the test drink in BR compared to FR is a possible cause of the potential improvement in glucose tolerance, as prolonged NEFA elevations reduce insulin-stimulated glucose disposal by inhibiting insulin signalling[31]. The (non-significant) increase in insulinaemia and delay in peak insulin concentrations do support this proposition.

Muscle contraction stimulates insulin-independent glucose uptake[14], and thus explains why glucose uptake is augmented following an acute bout of exercise in spite of increased NEFA concentrations which were present in the FE and BE trials. Increased glucose uptake is a well-established observation at the muscle[14] and whole-body level[32]. Thus, based on insulin clamp studies it may seem surprising that there was no difference in glucose tolerance between the fasted rest and exercise trials but this does in fact corroborate studies using oral glucose tolerance tests. Until present, studies in healthy participants have shown either decreases[10, 11, 33-37], or no difference[12, 13, 38] in glucose tolerance following acute endurance exercise. Those displaying no difference were either performed in the fasted state[13, 38], or glucose tolerance was assessed more than 2 h after exercise[12]. The present study is the first to demonstrate that when nutrients are ingested immediately post-exercise, the effect on acute postprandial glucose kinetics may depend

upon the nutritional state (fasted or fed) prior to exercise. It may be the accrual of this acute effect which contributes to the attenuated improvements in glucose tolerance seen by exercise training when carbohydrate availability is high[5].

Regarding the effects of exercise when fasted, endurance exercise increases the rate of appearance of endogenous glucose[37]. Therefore, the increase in muscle glucose uptake after exercise[14] (affecting rate of disappearance) could ostensibly be offset by the increase in splanchnic glucose output (affecting rate of appearance) and hence result in an increase in flux, but no difference in the systemic concentrations of glucose with exercise compared to rest when fasted. Although future studies are needed, to address whether this is indeed the mechanism at play.

Food consumption prior to exercise also increases splanchnic blood flow during exercise[6]. As mesenteric blood flow is positively associated with intestinal glucose absorption[39], speculation may be made that the increase in blood flow (from breakfast consumption), combined with increased passive absorption (from exercise), results in the greater peak blood glucose concentration with BE compared to FE. However, recent evidence associates the increase in intestinal absorption with *reduced* gut blood flow occurring during intense exercise and may result in intestinal damage[40], indicating faster entry of glucose into the circulation when gut blood flow is reduced [which occurs when exercising fasted compared to fed[6]]. This adds confusion to the previous conjecture, as the putative increase in splanchnic blood flow in BE would result in less intestinal cell damage and reduced passive absorption leading to a lower blood glucose AUC (assuming that endogenous glucose production and glucose disappearance remain constant; which can be presumed due to similar carbohydrate balance post-exercise and thus similar whole-body glycogen concentrations).

The present study used an exercise intensity which was lower (61% VO_{2peak} vs. 70% of maximum power output) than that of van Wijck *et al.*[40]. At lower intensities (55% VO_{2peak}), the exercise-induced reduction in splanchnic blood flow is abolished[6]. This makes it tempting to presume that other factors such as heat or mechanical stresses, or changes in hormone concentrations contribute to the increase in intestinal glucose absorption following exercise[41]. Another factor at play could be reductions in insulin sensitivity of non-exercised (upper limb) muscle following exercise[42]. Clearly, this area has great scope for future work, pertinent to the understanding of the impact of food intake and exercise on subsequent whole body glucose tolerance.

The $AUC_{INS/GLU}$ was lower in both exercise trials compared to FR, whereas $ISI_{Matsuda}$ was similar between trials, suggesting that postprandial insulin secretion is reduced immediately following

383 exercise, but insulin sensitivity is unaffected[28, 29]. This strengthens the assumption that the change
384 in glucose kinetics seen in the present study is due to a difference in the glucose rate of appearance.

385 The finding that GLP-1_{7-36} concentrations were not different between trials is in accordance with the
386 proposition that glucose entered the circulation via passive absorption. Intravenous infusion of
387 glucose mirroring the plasma glucose profile to oral ingestion does not augment GLP-1
388 concentrations[43]. Therefore as GLP-1_{7-36} concentrations were not different between trials, this
389 provides support for elevated glucose appearance from passive absorption, as greater GLP-1_{7-36}
390 secretion would not occur. GLP-1_{7-36} is also a potent incretin hormone, stimulating insulin secretion
391 and also suppressing appetite[9]. Thus, as GLP-1_{7-36} did not differ between trials, it would seem that
392 other factors are playing a role in enhanced insulin action and appetite suppression with breakfast
393 consumption. Although it should be noted that GLP-1_{7-36} may interact with neurons expressed
394 locally in L-cells, prior to being rapidly degraded upon entry into the circulation where its clearance
395 can exceed cardiac output 2-3 times[44]. Hence, GLP-1_{7-36} can still influence appetite in spite of no
396 detectable rise in plasma concentrations.

397 There was evidence of delayed suppression of NEFA following consumption of the test drink in the
398 FR trial compared to the BR trial, suggestive of metabolic inflexibility, again associated with
399 insulin resistance. Exercise uncoupled the link between breakfast, NEFA and insulin concentrations
400 whereby, in both the FE and BE trials, insulin and NEFA concentrations were similar prior to and
401 following consumption of the test drink. Increased NEFA availability during and following exercise
402 is required to support higher rates of fat oxidation by skeletal muscle as carbohydrate is used to
403 replenish glycogen stores[11]. As such, NEFA flux is raised, and, as insulin-resisting effects of
404 NEFA on muscle seem to be time dependent[31], turnover may be more important than NEFA
405 concentrations for insulin sensitivity.

406 Exercise transiently suppressed hunger and overall appetite. This is a common phenomenon [15, 18,
407 45], yet less is known about the effect of nutritional status on the ability of exercise to influence
408 appetite. The present study found that, compared to rest, exercise suppressed hunger, and overall
409 appetite, to a greater extent when fasted compared to the fed state (~17% vs. ~9%, respectively).
410 Nevertheless it should be noted that appetite was higher in the fasting state prior to exercise. To our
411 knowledge this is the first crossover study to demonstrate the effect of exercise in fasted and fed
412 conditions on appetite sensations compared to resting trials in the equivalent nutritional state.

413 Harmonious with preceding research[15, 18] the exercise-induced suppression of appetite was
414 abolished within 30 min of exercise termination and appetite was subsequently similar between

exercise and rest trials until lunch. Breakfast consumption, however, reduced overall appetite following test drink consumption by ~17% and ~14% in the rest and exercise trials, respectively. Despite a 10% reduction in appetite ratings with breakfast consumption, no detectable difference in energy intake between trials was observed at lunch. This occurred regardless of the additional 1859 kJ consumed with breakfast and ~2423 kJ expended during exercise. Subsequently, energy intake was higher on breakfast trials. Observational data corroborates the present findings with daily energy intake increased in regular breakfast consumers compared to omitters[1]. Yet when BMI was measured, it was still inversely associated with breakfast consumption[1], suggesting it may be increased energy expenditure and improved metabolic responses to food consumption that result in better weight maintenance.

The outcome that exercise did not influence subsequent energy intake is in accord with most of the prior research in this area, although some have found an increase in immediate energy intake[46]. It may be that individual variation exists, whereby some individuals drive to eat following exercise is dominated by hedonic processes[47]. This leads to a divergence of those who compensate for extra energy expenditure by increasing intake and non-compensators who fail to increase intake in the face of an increase in expenditure. In the present study, the range of compensation for exercise-induced energy expenditure was large (5665 kJ separated the individual who over compensated, and the individual who under-compensated, the greatest). This variation in the compensation of energy expenditure is likely to account for the variation seen in body fat changes with an exercise intervention [reviewed by Caudwell *et al.*[48]]. It is interesting to note that there was no significant relationship between the degree of compensation to exercise on fasted trials and breakfast trials, suggesting that those who over-compensate during exercise in one nutritional state (ie. the fasted/fed state) may not overcompensate in the opposing circumstance. Another possibility is that exercise energy expenditure is gradually compensated for by energy intake which is likely to require a period of weeks, and even then is not likely to be fully compensated for [49].

The higher total energy intake with breakfast trials and the exercise induced energy expenditure led to energy balance being most positive on the BR trial, and least positive on the FE trial. BE resulted in a ~1110 kJ reduction in energy balance compared to FR. When taken in concert with the similar appetite sensations to resting trials, exercise may provide a more attractive option for restricting energy availability compared to omitting breakfast. Interestingly, in spite of differing quantities of carbohydrate and fat oxidized with all trials, carbohydrate balance was remarkably similar between FE and BE whereas fat balance was 3-fold more positive with BE. Although this may not be as clear at rest as the difference between FR and BR in carbohydrate balance did approach a

448 statistically significant difference (Figure 7) but was higher than exercise trials. At least in the short
449 term, the regulation of carbohydrate stores is more tightly regulated than fat stores[19]. The findings
450 of this study add that consumption/omission of breakfast will not alter carbohydrate balance,
451 whereas exercise can reduce carbohydrate balance.

452 The increased energy expenditure observed during exercise with breakfast consumption was
453 provided by a higher rate of carbohydrate oxidation, this has previously been reported[50-52] and may
454 be magnified during running due to the weight-bearing component[53]. The relevance of this with
455 respect to energy balance was however, trivial, as energy balance was lower in the FE trial
456 compared to the BE trial.

457 This controlled experimental study involved the provision of a popular breakfast food consumed
458 prior to a bout of exercise or rest in physically active males, with a structure similar to the eating
459 patterns in western society. It could be viewed that a caveat with the present study is that the
460 participants were physically active and that a sedentary population would benefit more from
461 exercise/diet-induced improvements in metabolism and appetite. However, those who regularly
462 perform exercise still utilise energy/carbohydrate restriction in order to regulate body
463 composition[4]. Therefore the results are pertinent to these populations yet it would undoubtedly be
464 of virtue to investigate these responses in other populations (females, sedentary and obese) to
465 extrapolate findings to a wider population. Moreover, future work should examine whether there is
466 a difference in energy intake in subsequently consumed meals over a longer duration.
467 It is also of merit to recognize that the environmental conditions were similar between trials which
468 is important, due to the potential effect of environmental temperature on appetite and energy
469 intake[46].
470 The findings of the present investigation suggest that in an acute setting, energy intake from
471 breakfast, and energy expenditure from exercise are not compensated for at lunch. Consequently,
472 energy balance was most positive following breakfast and rest and least positive following breakfast
473 omission and exercise. When exercise is performed, it may be more pertinent to omit breakfast if a
474 negative fat balance is desirable, although the findings of this study are unable to predict the longer-
475 term outcomes of energy and fat balance due to the single-meal design, and as such this conclusion
476 should be interpreted with caution.
477 This study aimed to explore the effect of breakfast and exercise on the metabolic and appetite
478 responses to subsequent food consumption. The findings indicate that breakfast ingestion may
479 improve the metabolic and appetite responses to subsequently consumed foods when sedentary.
480 When breakfast is consumed, subsequent postprandial glycaemia is higher following exercise, yet

481 care should be applied to the interpretation for chronic effects, as exercise training almost always
482 confers a benefit for glucose tolerance and insulin sensitivity. Exercise also resulted in an
483 ephemeral reduction in appetite, which is greater when performed fasted.
484
485

486 **Acknowledgements**

487 We gratefully thank the volunteers for their participation and A.Wilde for technical assistance. This
488 project received no external funding. J.T.G. and E.J.S. designed the study, J.T.G. and R.C.V.
489 performed the data collection, and all authors contributed to data analysis and interpretation and
490 writing of the manuscript. The authors declare no conflicts of interest.

491

References

1. Cho S, Dietrich M, Brown CJ *et al.* (2003) The effect of breakfast type on total daily energy intake and body mass index: results from the Third National Health and Nutrition Examination Survey (NHANES III). *J Am Coll Nutr* **22**, 296-302.

2. Astbury NM, Taylor MA & Macdonald IA (2011) Breakfast consumption affects appetite, energy intake, and the metabolic and endocrine responses to foods consumed later in the day in male habitual breakfast eaters. *J Nutr* **141**, 1381-1389.

3. La Bounty PM, Campbell BI, Wilson J *et al.* (2011) International Society of Sports Nutrition position stand: meal frequency. *J Int Soc Sports Nutr* **8**, 4.

4. Morton JP, Robertson C, Sutton L *et al.* (2010) Making the weight: a case study from professional boxing. *Int J Sport Nutr Exerc Metab* **20**, 80-85.

5. Van Proeyen K, Szlufcik K, Nielens H *et al.* (2010) Training in the fasted state improves glucose tolerance during fat-rich diet. *J Physiol* **588**, 4289-4302.

6. Enevoldsen LH, Simonsen L, Macdonald IA *et al.* (2004) The combined effects of exercise and food intake on adipose tissue and splanchnic metabolism. *J Physiol* **561**, 871-882.

7. Stannard SR, Buckley AJ, Edge JA *et al.* (2010) Adaptations to skeletal muscle with endurance exercise training in the acutely fed versus overnight-fasted state. *J Sci Med Sport* **13**, 465-469.

8. Nybo L, Pedersen K, Christensen B *et al.* (2009) Impact of carbohydrate supplementation during endurance training on glycogen storage and performance. *Acta Physiol (Oxf)* **197**, 117-127.

9. Suzuki K, Simpson KA, Minnion JS *et al.* (2010) The role of gut hormones and the hypothalamus in appetite regulation. *Endocr J* **57**, 359-372.

10. Folch N, Peronnet F, Massicotte D *et al.* (2001) Metabolic response to small and large 13C-labelled pasta meals following rest or exercise in man. *Br J Nutr* **85**, 671-680.

11. Folch N, Peronnet F, Massicotte D *et al.* (2003) Metabolic response to a large starch meal after rest and exercise: comparison between men and women. *Eur J Clin Nutr* **57**, 1107-1115.

517 12. Venables MC, Shaw CS, Jeukendrup AE *et al.* (2007) Effect of acute exercise on glucose
518 tolerance following post-exercise feeding. *Eur J Appl Physiol* **100**, 711-717.
519 13. Long W, 3rd, Wells K, Englert V *et al.* (2008) Does prior acute exercise affect postexercise
520 substrate oxidation in response to a high carbohydrate meal? *Nutr Metab (Lond)* **5**, 2.
521 14. Goodyear LJ, King PA, Hirshman MF *et al.* (1990) Contractile activity increases plasma
522 membrane glucose transporters in absence of insulin. *Am J Physiol* **258**, E667-672.
523 15. Martins C, Morgan LM, Bloom SR *et al.* (2007) Effects of exercise on gut peptides, energy
524 intake and appetite. *Journal of Endocrinology* **193**, 251-258.
525 16. Frid AH, Nilsson M, Holst JJ *et al.* (2005) Effect of whey on blood glucose and insulin
526 responses to composite breakfast and lunch meals in type 2 diabetic subjects. *Am J Clin Nutr* **82**,
527 69-75.
528 17. Thomas K, Morris P & Stevenson E (2009) Improved endurance capacity following chocolate
529 milk consumption compared with 2 commercially available sport drinks. *Applied Physiology,*
530 *Nutrition and Metabolism* **34**, 78-82.
531 18. King JA, Miyashita M, Wasse LK *et al.* (2010) Influence of prolonged treadmill running on
532 appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite* **54**, 492-498.
533 19. Burton FL, Malkova D, Caslake MJ *et al.* (2010) Substrate metabolism, appetite and feeding
534 behaviour under low and high energy turnover conditions in overweight women. *Br J Nutr* **104**,
535 1249-1259.
536 20. Cheng MH, Bushnell D, Cannon DT *et al.* (2009) Appetite regulation via exercise prior or
537 subsequent to high-fat meal consumption. *Appetite* **52**, 193-198.
538 21. Borer KT, Wuorinen E, Chao C *et al.* (2005) Exercise energy expenditure is not consciously
539 detected due to oro-gastric, not metabolic, basis of hunger sensation. *Appetite* **45**, 177-181.
540 22. Craig CL, Marshall AL, Sjostrom M *et al.* (2003) International physical activity questionnaire:
541 12-country reliability and validity. *Med Sci Sports Exerc* **35**, 1381-1395.

542 23. Stunkard AJ & Messick S (1985) The three-factor eating questionnaire to measure dietary
543 restraint, disinhibition and hunger. *J Psychosom Res* **29**, 71-83.
544 24. Williams C, Nute MG, Broadbank L *et al.* (1990) Influence of fluid intake on endurance
545 running performance. A comparison between water, glucose and fructose solutions. *European*
546 *Journal of Applied Physiology and Occupational Physiology* **60**, 112-119.
547 25. Burns SF, Broom DR, Miyashita M *et al.* (2007) A single session of treadmill running has no
548 effect on plasma total ghrelin concentrations. *Journal of Sports Sciences* **25**, 635-642.
549 26. Jeukendrup AE & Wallis GA (2005) Measurement of substrate oxidation during exercise by
550 means of gas exchange measurements. *International Journal of Sports Medicine* **26 Suppl 1**, S28-
551 37.
552 27. Anderson GH, Catherine NL, Woodend DM *et al.* (2002) Inverse association between the effect
553 of carbohydrates on blood glucose and subsequent short-term food intake in young men. *Am J Clin*
554 *Nutr* **76**, 1023-1030.
555 28. Retnakaran R, Shen S, Hanley AJ *et al.* (2008) Hyperbolic relationship between insulin
556 secretion and sensitivity on oral glucose tolerance test. *Obesity* **16**, 1901-1907.
557 29. Matsuda M & DeFronzo RA (1999) Insulin sensitivity indices obtained from oral glucose
558 tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* **22**, 1462-1470.
559 30. Batterham AM & Hopkins WG (2006) Making meaningful inferences about magnitudes.
560 *International journal of sports physiology and performance* **1**, 50-57.
561 31. Hirabara SM, Silveira LR, Abdulkader F *et al.* (2007) Time-dependent effects of fatty acids on
562 skeletal muscle metabolism. *J Cell Physiol* **210**, 7-15.
563 32. Mikines KJ, Sonne B, Farrell PA *et al.* (1988) Effect of physical exercise on sensitivity and
564 responsiveness to insulin in humans. *Am J Physiol* **254**, E248-259.
565 33. O'Connor AM, Pola S, Ward BM *et al.* (2006) The gastroenteroinsular response to glucose
566 ingestion during postexercise recovery. *Am J Physiol Endocrinol Metab* **290**, E1155-1161.

567 34. King DS, Baldus PJ, Sharp RL *et al.* (1995) Time course for exercise-induced alterations in
568 insulin action and glucose tolerance in middle-aged people. *J Appl Physiol* **78**, 17-22.
569 35. Krzentowski G, Pirnay F, Luyckx AS *et al.* (1982) Metabolic adaptations in post-exercise
570 recovery. *Clin Physiol* **2**, 277-288.
571 36. Pestell RG, Ward GM, Galvin P *et al.* (1993) Impaired glucose tolerance after endurance
572 exercise is associated with reduced insulin secretion rather than altered insulin sensitivity.
573 *Metabolism* **42**, 277-282.
574 37. Rose AJ, Howlett K, King DS *et al.* (2001) Effect of prior exercise on glucose metabolism in
575 trained men. *Am J Physiol Endocrinol Metab* **281**, E766-771.
576 38. Englert V, Wells K, Long W *et al.* (2006) Effect of acute prior exercise on glycemic and
577 insulinemic indices. *J Am Coll Nutr* **25**, 195-202.
578 39. Williams JH, Jr., Mager M & Jacobson ED (1964) Relationship of Mesenteric Blood Flow to
579 Intestinal Absorption of Carbohydrates. *J Lab Clin Med* **63**, 853-863.
580 40. van Wijck K, Lenaerts K, van Loon LJ *et al.* (2011) Exercise-induced splanchnic hypoperfusion
581 results in gut dysfunction in healthy men. *PLoS One* **6**, e22366.
582 41. Lambert GP (2009) Stress-induced gastrointestinal barrier dysfunction and its inflammatory
583 effects. *J Anim Sci* **87**, E101-108.
584 42. Devlin JT, Barlow J & Horton ES (1989) Whole body and regional fuel metabolism during
585 early postexercise recovery. *The American journal of physiology* **256**, E167-172.
586 43. Gutniak M, Orskov C, Holst JJ *et al.* (1992) Antidiabetogenic effect of glucagon-like peptide-1
587 (7-36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* **326**, 1316-1322.
588 44. Holst JJ & Deacon CF (2005) Glucagon-like peptide-1 mediates the therapeutic actions of DPP-
589 IV inhibitors. *Diabetologia* **48**, 612-615.

590 45. Deighton K, Zahra JC & Stensel DJ (2012) Appetite, energy intake and resting metabolic
591 responses to 60 min treadmill running performed in a fasted versus a postprandial state. *Appetite* **58**,
592 946-954.
593 46. Shorten AL, Wallman KE & Guelfi KJ (2009) Acute effect of environmental temperature
594 during exercise on subsequent energy intake in active men. *Am J Clin Nutr* **90**, 1215-1221.
595 47. Finlayson G, Bryant E, Blundell JE *et al.* (2009) Acute compensatory eating following exercise
596 is associated with implicit hedonic wanting for food. *Physiol Behav* **97**, 62-67.
597 48. Caudwell P, Gibbons C, Hopkins M *et al.* (2011) The influence of physical activity on appetite
598 control: an experimental system to understand the relationship between exercise-induced energy
599 expenditure and energy intake. *The Proceedings of the Nutrition Society* **70**, 171-180.
600 49. Blundell JE, Stubbs RJ, Hughes DA *et al.* (2003) Cross talk between physical activity and
601 appetite control: does physical activity stimulate appetite? *The Proceedings of the Nutrition Society*
602 **62**, 651-661.
603 50. Miller DS, Mumford P & Stock MJ (1967) Gluttony: 2. Thermogenesis in Overeating Man. *Am*
604 *J Clin Nutr* **20**, 1223-1229.
605 51. Miller DS & Wise A (1975) Exercise and dietary-induced thermogenesis. *Lancet* **305**, 1290.
606 52. Welle S (1984) Metabolic responses to a meal during rest and low-intensity exercise. *Am J Clin*
607 *Nutr* **40**, 990-994.
608 53. Taboga P, Lazzer S, Fessehatsion R *et al.* (2012) Energetics and mechanics of running men: the
609 influence of body mass. *Eur J Appl Physiol*.

610

611

612 **Figure Legends**

613 **Figure 1.** Schematic representation of trials. VO_{2peak}, peak oxygen consumption.
614

615 **Figure 2.** (a) Blood glucose concentration in response to test drink consumption in the FR (open
616 circles), BR (closed circles), FE (open triangles) and BE (closed triangles) trials. BL, baseline; PE,
617 pre-exercise; EX; exercise; a, FE different to BR; b, FR different to FE; c, FR different to BE; d,
618 BR different to FE; e, BR different to BE; f, FE different to BE ($P < 0.05$). (b) Time-averaged blood
619 glucose area under the curve following test-drink consumption. Bars with different superscript
620 letters are significantly different from one another ($P < 0.05$).

621

622 **Figure 3.** (a) Serum insulin concentration in response to test drink consumption in the FR (open
623 circles), BR (closed circles), FE (open triangles) and BE (closed triangles) trials. BL, baseline; PE,
624 pre-exercise; EX; exercise; a, FE different to BR; b, FR different to FE; c, FR different to BE; d,
625 BR different to FE; e, BR different to BE; f, FE different to BE ($P < 0.05$). (b) Time-averaged
626 serum insulin area under the curve following test-drink consumption.

627

628 **Figure 4.** (a) Serum non-esterified fatty acid (NEFA) concentration in response to test drink
629 consumption in the FR (open circles), BR (closed circles), FE (open triangles) and BE (closed
630 triangles) trials. BL, baseline; PE, pre-exercise; EX; exercise; a, FE different to BR; b, FR different
631 to FE; c, FR different to BE; d, BR different to FE; e, BR different to BE; f, FE different to BE ($P <$
632 0.05). (b) Time-averaged serum NEFA area under the curve following test-drink consumption. Bars
633 with different superscript letters are significantly different from one another ($P < 0.05$).

634

635 **Figure 5.** (a) Plasma Glucagon like peptide-$1_{7\text{-}36}$ (GLP-$1_{7\text{-}36}$) concentration in response to test drink
636 consumption in the FR (open circles), BR (closed circles), FE (open triangles) and BE (closed
637 triangles) trials. BL, baseline; PE, pre-exercise; EX; exercise. (b) Time-averaged GLP-$1_{7\text{-}36}$ area
638 under the curve following test-drink consumption.

639

640 **Figure 6.** Energy intake. Energy intake at lunch (black bars) and throughout the whole trial (white
641 bars). Bars with different superscript letters are significantly different from one another ($P < 0.05$).

642

643 **Figure 7.** Substrate balance. Carbohydrate (black bars), fat (white bars) and energy (black and
644 white bars combined) balance at the end of the trial. Bars with different superscript letters are
645 significantly different from one another ($P < 0.05$).

646 **Figure 8.** Overall appetite. Overall appetite sensations during the breakfast postprandial and
647 exercise periods (a) and following test drink consumption (b) in the FR (open circles), BR (closed
648 circles), FE (open triangles) and BE (closed triangles) trials. BL, baseline; EX, exercise; DE, during
649 exercise; EE, end of exercise; PL, post-lunch; a, FE different to BR; b, FR different to FE; c, FR
650 different to BE; d, BR different to FE; e, BR different to BE; f, FE different to BE ($P < 0.05$).
651
652

653 **Tables**

654 **Table 1.** Energy expenditure and substrate metabolism during the breakfast postprandial period,
655 exercise or the equivalent rest period, and the recovery period following test drink consumption.

656

	Breakfast Period (120 min)			Exercise Period (~60 min)			Recovery Period (90 min)		
Trial	EE (kJ)	FO (g)	CO (g)	EE (kJ)	FO (g)	CO (g)	EE (kJ)	FO (g)	CO (g)
FR									
Mean	919	17.4	13.5	377	7.3	5.0	754	12.6	15.5
SEM	90	1.9	2.8	25	0.8	0.9	4	1.6	2.0
BR									
Mean	922	12.4[a]	26.6[a]	376	5.9[a]	8.6[a]	775	11.1	20.5
SEM	61	1.5	2.5	20	0.8	1.1	47	1.2	2.1
FE									
Mean	875	15.0	16.8[b]	3003[a,b]	35.3[a,b]	91.7[a,b]	831[a]	15.3[b]	13.2
SEM	46	1.4	1.8	43	3.1	7.0	37	1.2	1.8
BE									
Mean	946	13.8[a]	24.3a	3655[a,b,c]	29.3[a,b,c]	144.6[a,b,c]	832[a]	14.7[b]	14.9
SEM	60	1.8	2.4	47	3.2	7.6	37	1.5	2.2

657 F, fasting; R, rest; B, breakfast consumption; E, exercise; EE, energy expenditure; FO, fat
658 oxidation; CO, carbohydrate oxidation. [a], different from FR; [b], different from BR, [c], different from
659 FE ($P < 0.05$).

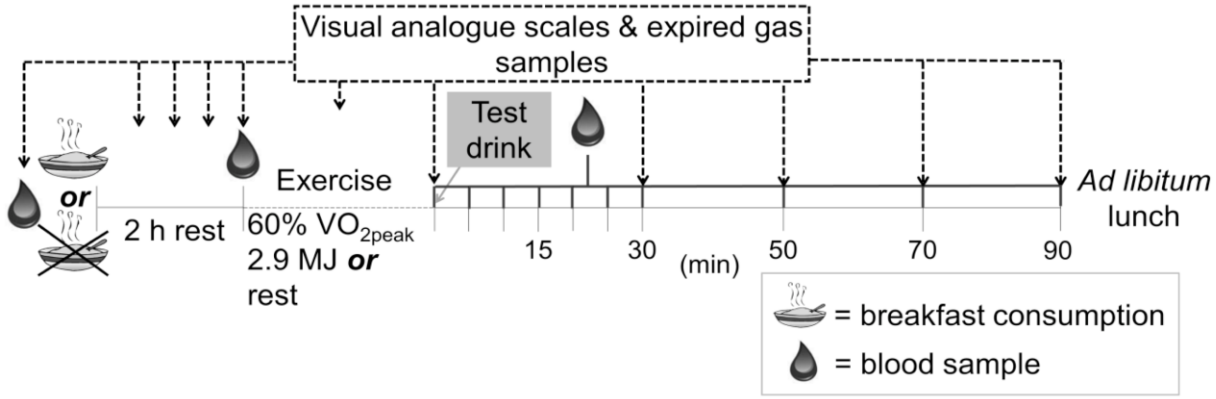
660

661

662 **Table 2.** Time-averaged area under the curve values for subjective appetite responses to
663 consumption of the test drink.

Trial	Hunger (mm)	Fullness (mm)	Satisfaction (mm)	Prospective consumption (mm)	Overall Appetite (mm)
FR					
Mean	65	30	27	72	70
SEM	4	4	2	3	2
BR					
Mean	54[a]	40	40[a]	58[a]	58[a]
SEM	4	4	3	4	3
FE					
Mean	63	28[b]	29[b]	68[b]	67[b]
SEM	3	4	3	4	3
BE					
Mean	55	40	40[a,c]	62[a]	59[a,c]
SEM	4	4	3	4	4

664 F, fasting; R, rest; B, breakfast consumption; E, exercise. [a], different from FR; [b], different from BR,
665 [c], different from FE ($P < 0.05$).
666



667 **Figure 1**

668

669

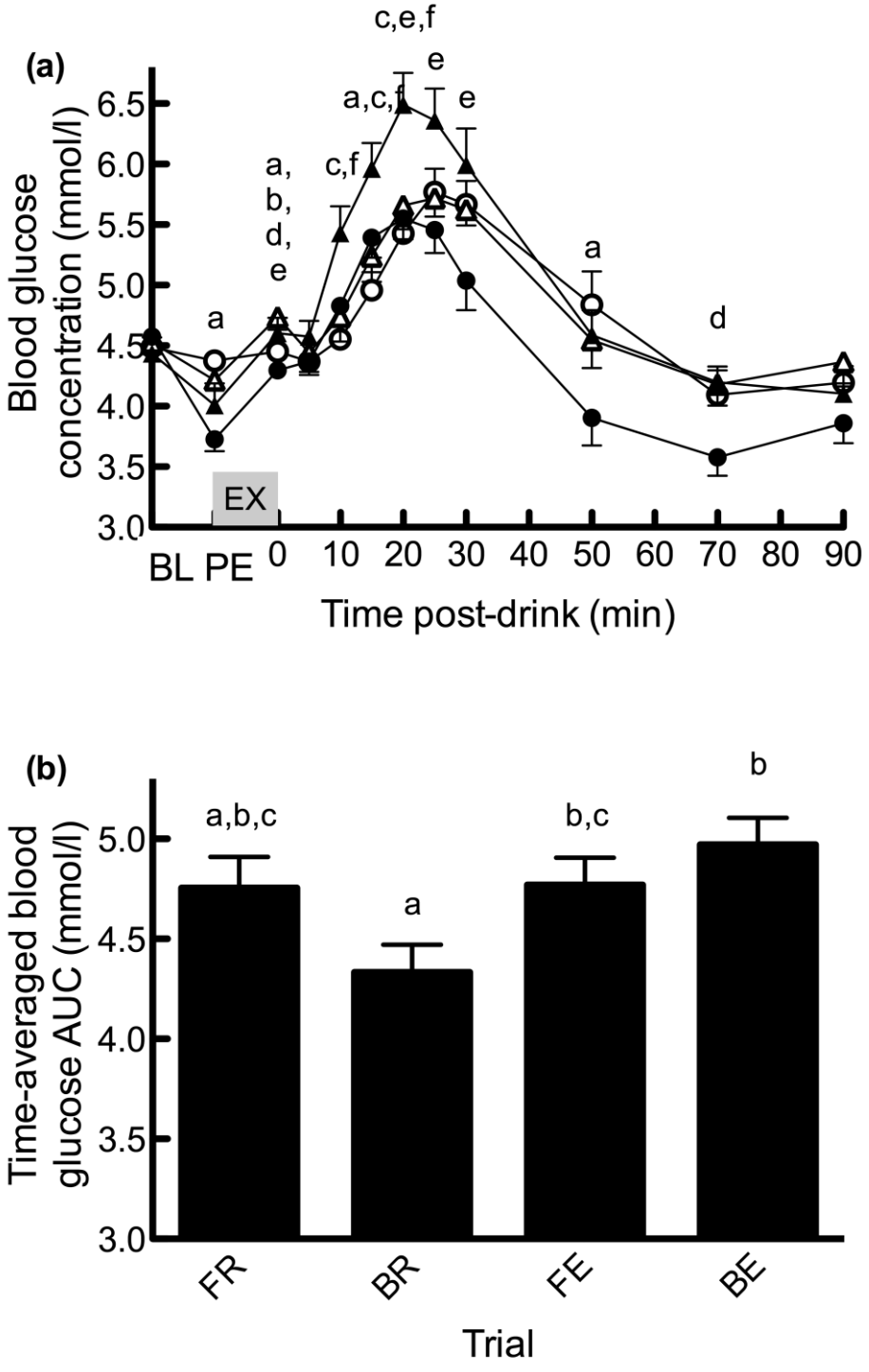
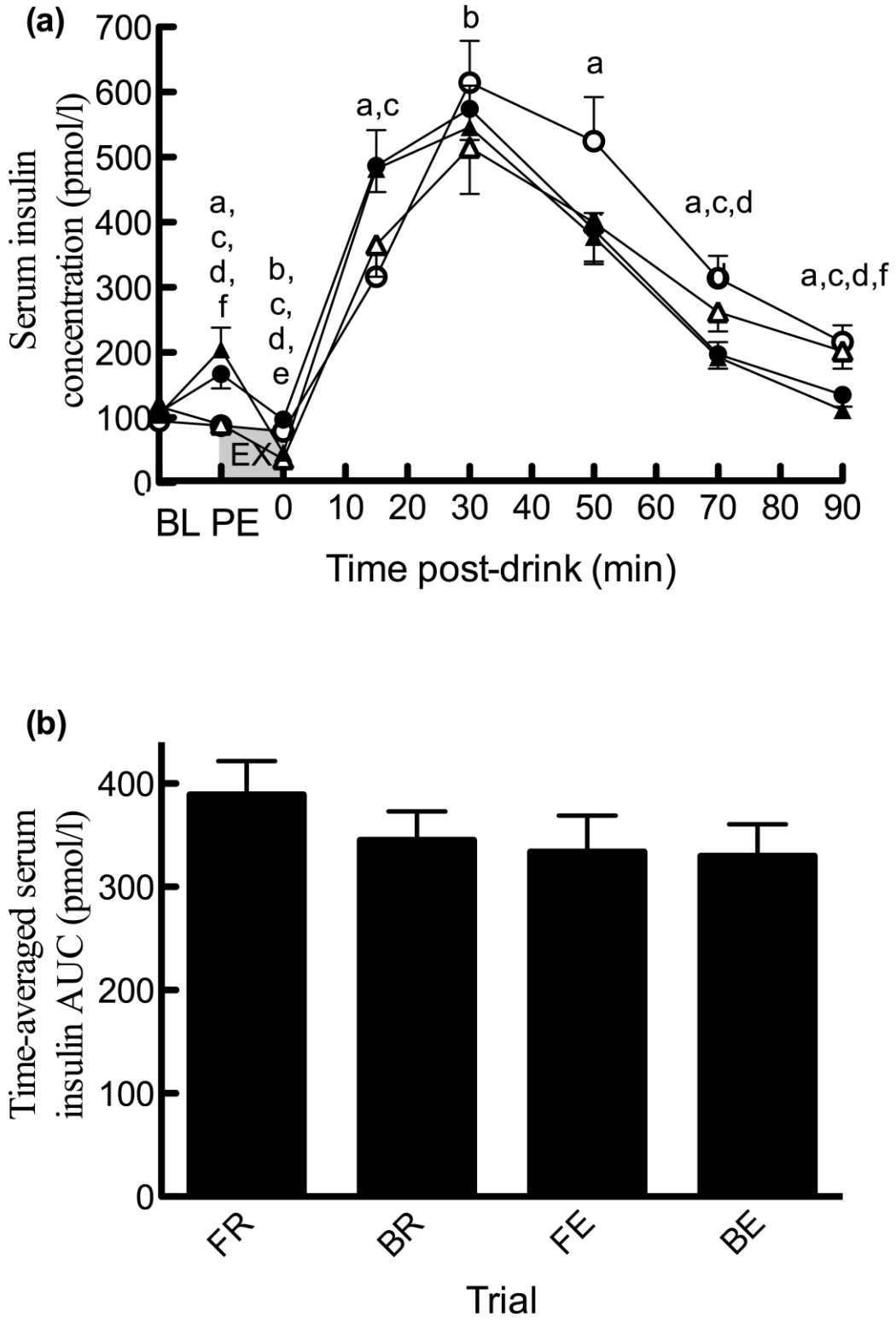

(a)
Blood glucose concentration (mmol/l)
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
EX
BL PE
0
10
20
30
40
50
60
70
80
90
Time post-drink (min)
a
a, b, d, e
c,f
a,c,f
c,e,f
e
e
a
d
(b)
Time-averaged blood glucose AUC (mmol/l)
5.0
4.5
4.0
3.5
3.0
a,b,c
a
b,c
b
FR
BR
FE
BE
Trial

Figure 2



674 **Figure 3**

675

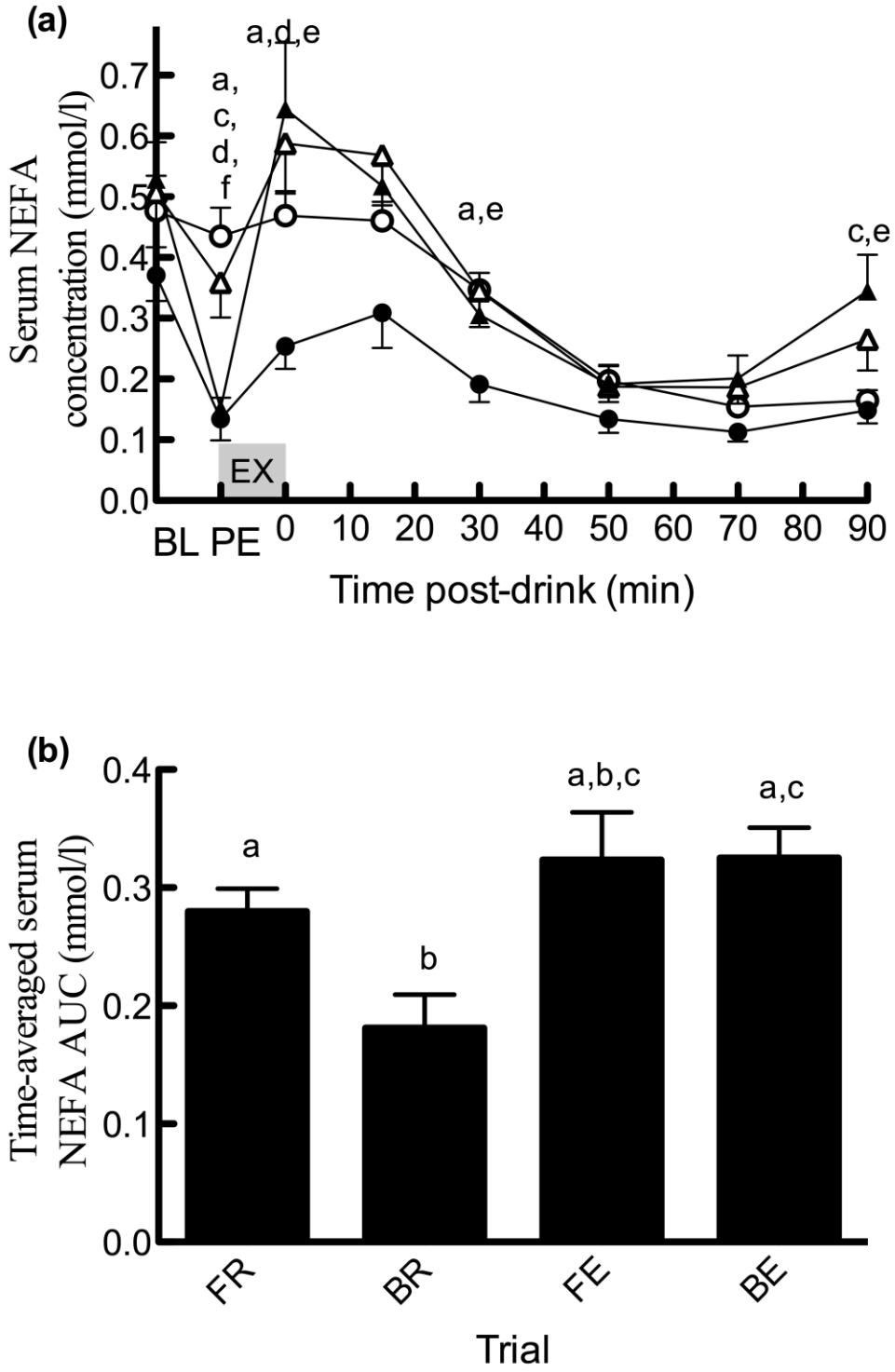

(a)
Serum NEFA concentration (mmol/l)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
a,
c,
d,
f
a,d,e
a,e
c,e
EX
BL PE
0
10
20
30
40
50
60
70
80
90
Time post-drink (min)
(b)
Time-averaged serum NEFA AUC (mmol/l)
0.0
0.1
0.2
0.3
0.4
a
b
a,b,c
a,c
FR
BR
FE
BE
Trial

Figure 4

676

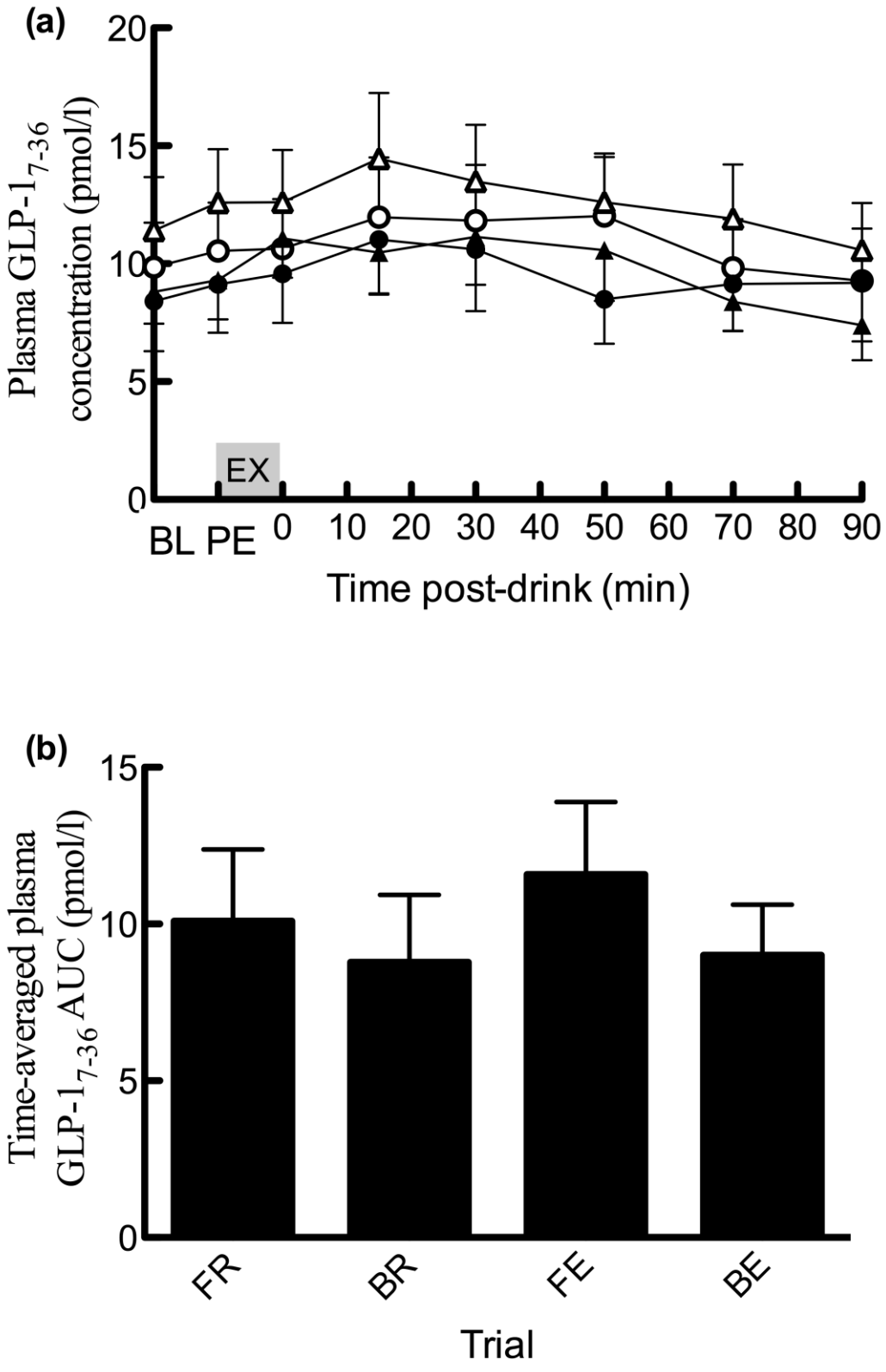
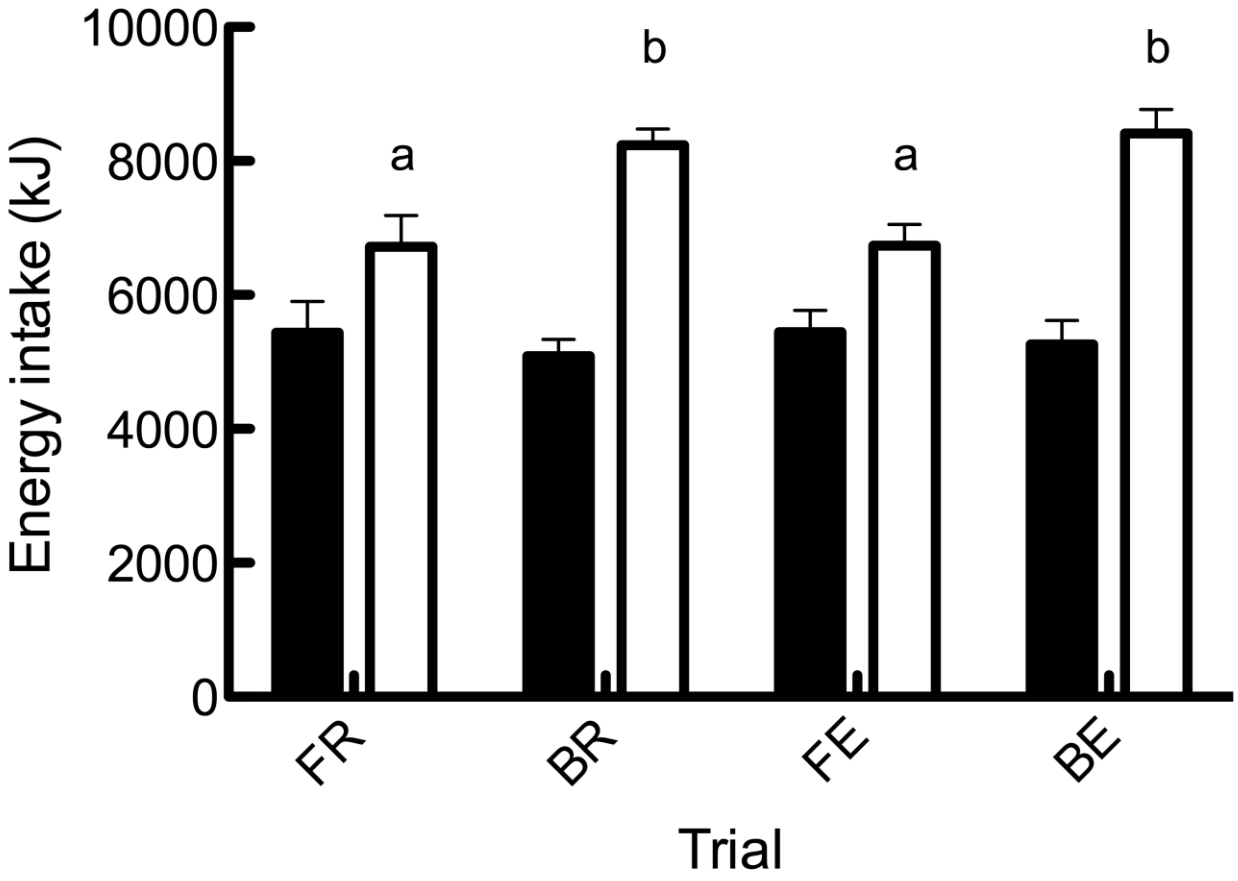
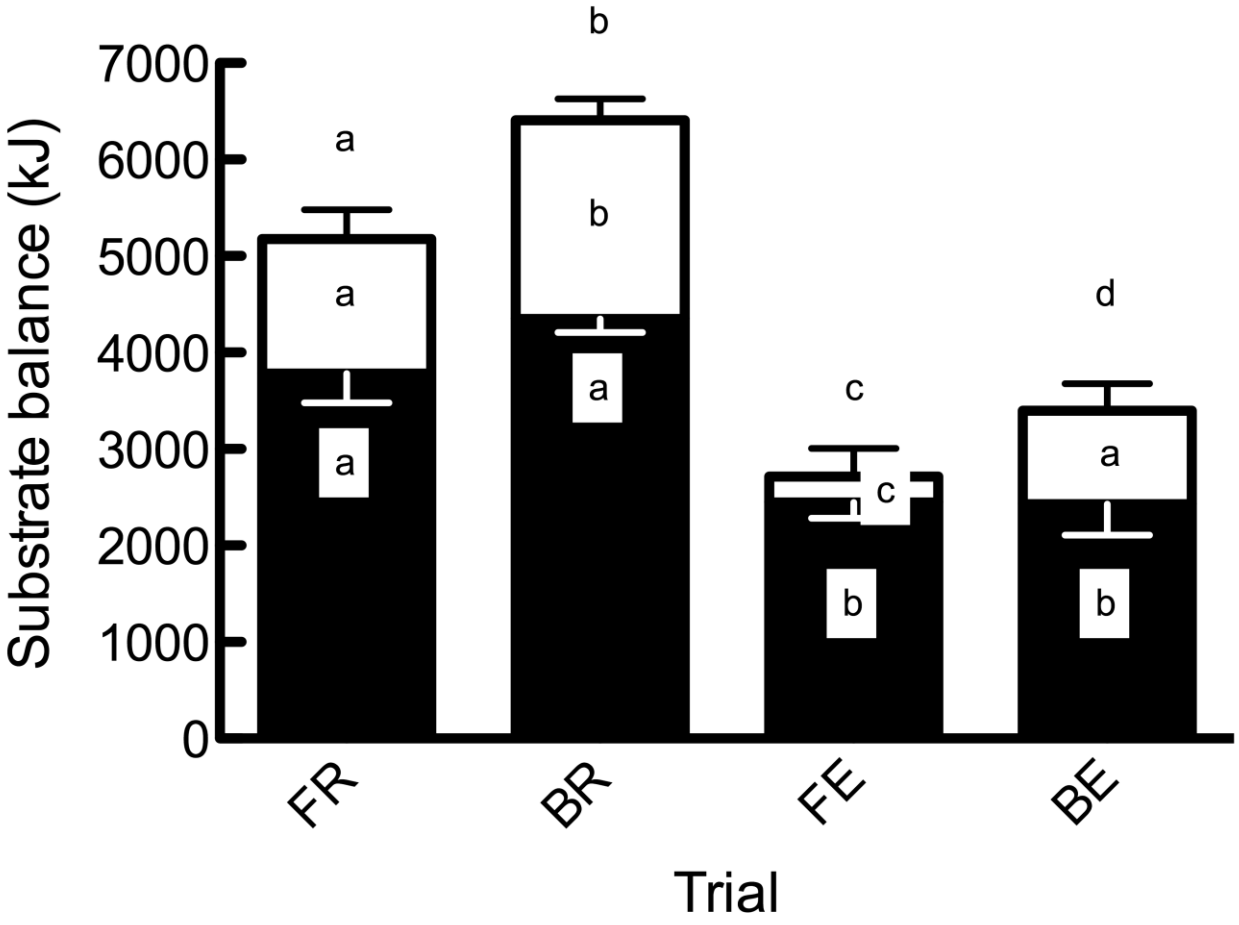

(a)
Plasma GLP-1 7-36 concentration (pmol/l)
20
15
10
5
0
EX
BL PE
0
10
20
30
40
50
60
70
80
90
Time post-drink (min)
(b)
Time-averaged plasma GLP-1 7-36 AUC (pmol/l)
15
10
5
0
FR
BR
FE
BE
Trial

Figure 5

677

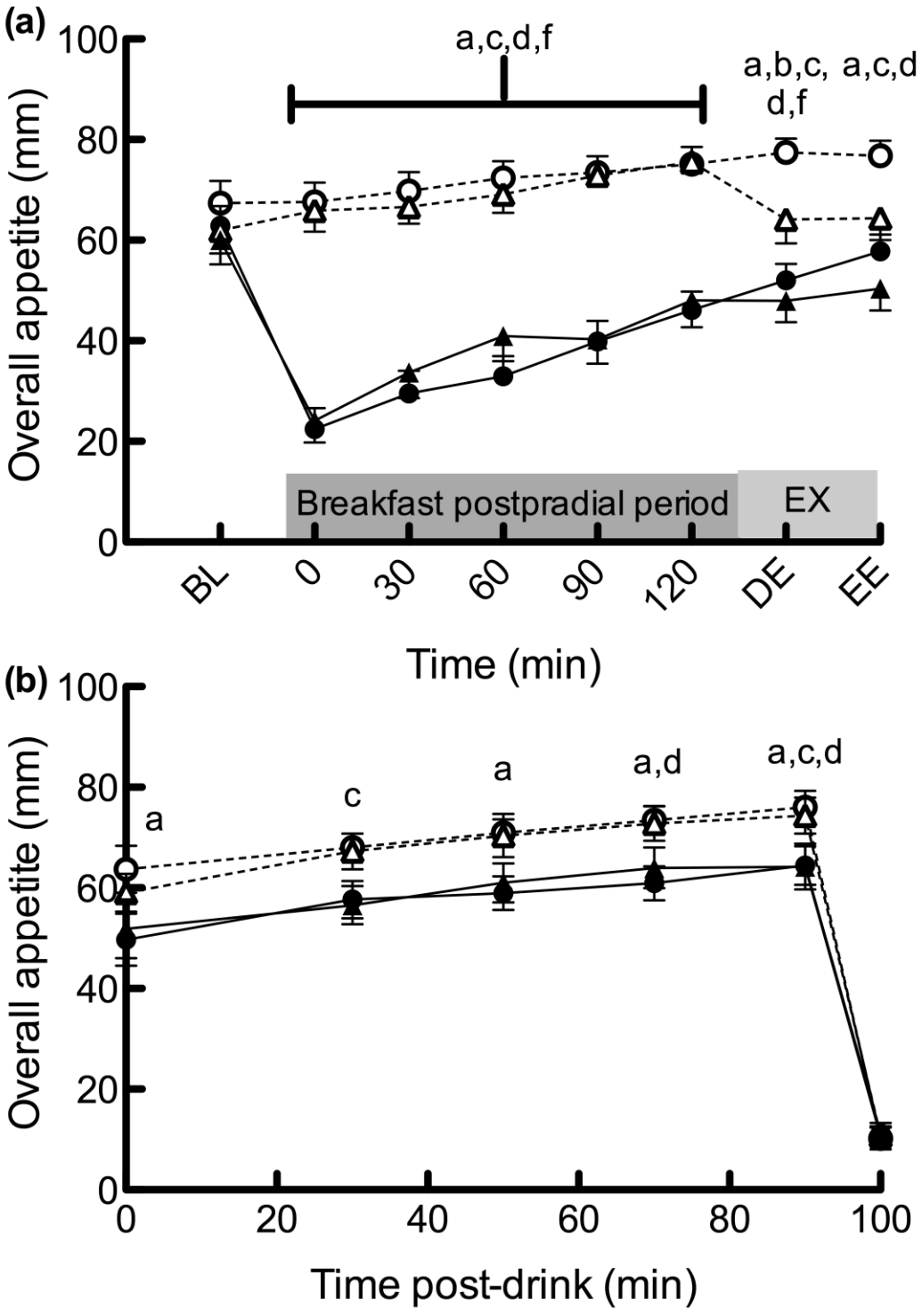

10000
8000
6000
4000
2000
0
Energy intake (kJ)
a
b
a
b
FR
BR
FE
BE
Trial

678
679


Substrate balance (kJ)
7000
6000
5000
4000
3000
2000
1000
0
a
a
a
b
b
a
c
c
b
d
a
b
FR
BR
FE
BE
Trial

680

681


(a)
Overall appetite (mm)
100
80
60
40
20
0
a,c,d,f
a,b,c, a,c,d
d,f
Breakfast postpradial period
EX
BL
0
30
60
90
120
DE
EE
Time (min)
(b)
Overall appetite (mm)
a
c
a
a,d
a,c,d
0
20
40
60
80
100
Time post-drink (min)

682