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# Consistency of metabolic responses and appetite sensations under postabsorptive and postprandial conditions

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## Abstract

The present study aimed to investigate the reliability of metabolic and subjective appetite responses under fasted conditions and following consumption of a cereal-based breakfast. Twelve healthy, physically active males completed two postabsorption (PA) and two postprandial (PP) trials in a randomised order. In PP trials a cereal based breakfast providing 1859 kJ of energy was consumed. Expired gas samples were used to estimate energy expenditure and fat oxidation and 100 mm visual analogue scales were used to determine appetite sensations at baseline and every 30 min for 120 min. Reliability was assessed using limits of agreement, coefficient of variation (CV), intraclass coefficient of correlation and 95% confidence limits of typical error. The limits of agreement and typical error were 292.0 and 105.5 kJ for total energy expenditure, 9.3 and 3.4 g for total fat oxidation and 22.9 and 8.3 mm for time-averaged AUC for hunger sensations, respectively over the 120 min period in the PP trial. The reliability of energy expenditure and appetite in the 2 h response to a cereal-based breakfast would suggest that an intervention requires a 211 kJ and 16.6 mm difference in total postprandial energy expenditure and time-averaged hunger AUC to be meaningful, fat oxidation would require a 6.7 g difference which may not be sensitive to most meal manipulations.


Key words: reproducibility; breakfast; energy expenditure; hunger, fat oxidation

**Introduction**

Consumption of a meal transiently augments energy expenditure carbohydrate oxidation and feelings of fullness, and suppresses fat oxidation, and feelings of hunger (Miles, Wong, Rumpler, & Conway, 1993; Piers, Soares, Makan, & Shetty, 1992; Stevenson, Astbury, Simpson, Taylor, & Macdonald, 2009; Weststrate et al., 1990). Both metabolic and appetitive responses to meals have implications for energy balance, particularly as in Western societies the majority of the day is spent in the postprandial state (De Castro, 1997). The duration of the postprandial period (the period after eating a meal before which all of the previous meal has been absorbed from the intestine) is dependent upon the energy and macronutrient content of the meal, but typically lasts between 6 and 12 hours (Compher, Frankenfield, Keim, & Roth-Yousey, 2006). The stage which follows absorption, but before the effects of prolonged fasting are underway, is known as the postabsorptive state.

The test-retest reproducibility of these measures is pertinent in order to be confident that an intervention or variable is the cause of a difference in a trial and not random variability or systematic bias (Atkinson & Nevill, 1998; Hopkins, 2000). Reliability can be defined as producing the same or similar result when a protocol is repeated a number of times (Atkinson & Nevill, 1998). It has been proposed that reliability should be assessed using a variety of statistical measures (Atkinson & Nevill, 1998) such as Bland and Altman limits of agreement (Bland & Altman, 1986), coefficient of variation (CV), intraclass coefficient of correlation (ICC) and 95% confidence limits of typical error. The inclusion of multiple analyses of reliability allows for interpretation of the components of reliability, comparison with similar studies using different analyses and is further justified due to a current lack of consensus on a primary method to ascertain reliability (Atkinson & Nevill, 2000; Hopkins, 2000).

Research on postprandial thermogenesis have concluded that a high test-retest reliability exists (Segal, Chun, Coronel, Cruz-Noori, & Santos, 1992) with a reliability coefficient of r = 0.932 ($P$<0.001), yet often the meal is in liquid form (Katch, Moorehead, Becque, & Rocchini, 1992; Piers et al., 1992; Segal et al., 1992). Some have investigated the reliability of thermogenesis following solid food consumption exhibiting relatively high CVs of 26-32% (Miles et al., 1993; Weststrate et al., 1990). The reliability of appetite visual analogue scales (VAS) have previously been assessed in response to a solid (Flint, Raben, Blundell, & Astrup, 2000) and liquid (Raben, Tagliabue, & Astrup, 1995) mixed meals. The CVs were shown to vary from 7-25%, with prior diet standardisation not improving the consistency. However, in the United Kingdom, around one-third of the population consume cereal-based breakfasts (Gibson & Gunn, 2011); recommended for numerous health benefits. To the current author's knowledge, the reliability of energy expenditure and appetite has not been assessed in response to a cereal and milk-based breakfast.

As the physical composition of a meal can influence metabolic and endocrine responses (Peracchi et al., 2000), then the reliability of metabolism is likely to be affected due to additional biological processes arising, each with an inherent variability. Moreover, the number of recent publications using cereal and milk based breakfasts with appetite and/or energy expenditure and fat oxidation as outcomes is considerable (Astbury, Taylor, & Macdonald, 2011; Isaksson et al., 2011; Ping-Delfos & Soares, 2011; Rosen, Ostman, & Bjorck, 2011). Hence clarifying the day to day agreement in metabolic and satiety responses to cereal-based breakfasts is warranted.

The measurement of the thermic effect of food is recommended to be performed over a 400 min period (Levine, 2005). Nonetheless, this may not be possible under complex study designs, particularly those following a more typical daily patterns of food consumption where between meal intervals are between 100 and 300 min (De Castro, 1997). This is particularly apparent in those combining metabolic and appetite measures, as the period of time following a preload can influence the relationship between appetite sensations and energy intake (Blundell et al., 2010). Therefore, studies may wish to abbreviate the postprandial preload period prior to an *ad libitum* meal. It is not known, however to what extent this shortened period would have on the reliability of the measurement of energy expenditure and appetite sensations following meal consumption.

Accordingly, the aim of the present study was to evaluate the reproducibility of whole body energy expenditure and substrate utilisation, along with appetite sensations in response to a typical breakfast.

## Methods

### *Design*

Participants attended the laboratory at 0730 h after a 10-14 h fast on four occasions. In a randomised order, each participant completed two postabsorption (PA; after a 10-14 h fast) and two postprandial (PP) trials. Food and fluid intake was matched for 24 h prior to all trials, and vigorous physical activity was prohibited. Following baseline measurements of energy expenditure, substrate metabolism and appetite sensations, a test meal was served (PP) or omitted (PA). Further measures were taken every 30 min for the following 120 min. Fluid intake was recorded on the first trial and replicated for subsequent trials.

*Subjects*

Twelve healthy, physically active males (age: 23.2 ± 4.3 y, stature: 178 ± 7 cm, mass: 77.2 ± 5.3 kg, BMI: 24.5 ± 2.0 kg/m$^2$, self-reported activity level: 4024 ± 3018 met-min/wk) were recruited from the student and staff population at Northumbria University and all participants completed the full protocol. Participants who self-reported as physically inactive, defined by less than 30 min of moderate activity, 5 times a week by the International Physical Activity Questionnaire (Craig et al., 2003) restrained eaters, defined by a score of >11 on the Three Factor Eating Questionnaire (Stunkard & Messick, 1985) or those with any metabolic disorders were omitted. The present study was conducted in accordance with the guidelines stated in the 1964 Declaration of Helsinki. Prior to recruitment, all participants provided informed written consent and the study was approved by the School of Life Sciences Ethics Committee at Northumbria University.

*Anthropometric measurements*

Body mass was determined to the nearest 0.1 kg using balance scales (Seca, Birmingham, UK) upon arrival to the laboratory, with participants wearing only light clothing. Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Birmingham, UK).

*Energy expenditure and substrate oxidation*

Energy expenditure was calculated by indirect calorimetry using an online gas analysis system (Metalyzer 3B, Cortex, Germany) calibrated using gases of known concentration and a 3 L syringe. Participants wore a facemask, were sat in an upright position at all times and following a 2 min stabilisation phase, 5 min samples of expired gas were obtained and averaged. Substrate oxidation was calculated with oxygen uptake and carbon dioxide production values using stoichiometric equations assuming protein oxidation to be negligible (Peronnet & Massicotte, 1991). Respiratory exchange ratio (RER) was averaged over the 120 min time-periods.

*Appetite sensations*

Paper based, 100 mm VAS were completed to determine appetite sensations. Questions asked were used to determine hunger, fullness, satisfaction and prospective food consumption. VAS ratings were double-measured by two researchers and means were taken where discrepancies occurred.

*Test meal*

The test meal consisted of 72 g quick cook porridge oats (Oatso Simple Golden Syrup, Quaker Oats, Reading, UK) with 360 ml semi-skimmed milk (Tesco, Dundee, UK). The porridge was cooked for 4 min at full power in a 1000 W microwave and was served after 10 min of cooling. The test meal was consumed within 10 min and provided 1859 kJ of energy (17% protein, 60% carbohydrate, 23% fat).

*Statistical analysis*

All data were calculated as mean ± SD. VAS ratings were calculated as time-averaged area under the curve (AUC) for postprandial and postabsorptive periods. Reliability was assessed using a variety of statistical techniques, with typical error taken as the primary assessment tool. Namely, mean difference, ICC, CV and typical error were employed for all variables (Atkinson & Nevill, 1998; Hopkins, 2000). ICCs were considered to show good reproducibility when $ICC \geq 0.8$, moderate reproducibility when $0.7 \leq ICC < 0.8$, and acceptable reproducibility when $0.6 \leq ICC < 0.7$. Energy expenditure, fat oxidation and hunger during the postprandial trials were assessed using Bland-Altman limits of agreement (Bland & Altman, 1986). Data were checked for heteroscedasticity such that the appropriate statistical techniques could be employed. To determine whether either BMI or physical activity levels affected the reliability of the variables, pearson product-moment correlation coefficients were used to determine relationships between CVs of metabolic and appetite responses, and BMI and physical activity level. Paired student's t tests were used to detect differences in mean values and CVs. Values were considered significant when $P<0.05$.

## Results

### *Energy expenditure and substrate oxidation*

Postprandial energy expenditure was higher than postabsorptive energy expenditure, yet CV and typical errors were similar (Table 1). A Bland-Altman plot for postprandial energy expenditure can be seen in Figure 1. Fat oxidation showed greater variation than energy expenditure at baseline and throughout both trials (CVs 20 and 8%, respectively). Postprandial fat oxidation is displayed as a Bland-Altman plot in Figure 2. Mean CVs were not significantly different for either energy expenditure or fat oxidation ($P=0.80$ and $P=0.12$, respectively) with the postprandial trial compared to the postabsorptive trial (Table 1).

Both carbohydrate oxidation and RER revealed similar typical errors and CVs under postabsorptive and postprandial conditions (Table 1).

Both postprandial and postabsorptive energy expenditure CVs showed positive relationships with BMI (r = 0.61 and 0.64, respectively; both $P<0.05$), but not with physical activity level (r = -0.13 and -0.21, respectively; both $P$>0.05) whereas neither postprandial, nor postabsorptive fat oxidation CVs showed significant relationships with either BMI or physical activity level (all $P$>0.05).

*Subjective appetite ratings*

CVs of baseline measures for hunger, fullness, satisfaction and prospective consumption were 21, 42, 43 and 19% respectively. During the postabsorptive trial, all ratings showed an improvement in reliability, yet fullness and satisfaction were less reproducible than hunger and prospective consumption (Table 2). However this was nullified somewhat under postprandial conditions (Table 2). Bland-Altman limits of agreement for the time-averaged, postprandial hunger AUC were ± 22.9 mm (Figure 3). Fullness and satisfaction time-averaged AUC CVs tended to be lower during the postprandial trial compared to the postabsorptive trial ($P$=0.077 and $P$=0.067, respectively). On the other hand, time-averaged AUC for hunger tended to be greater on the postprandial trial ($P$=0.069) and was significantly greater for prospective consumption ($P$=0.016). No significant relationships were determined between any appetite rating CVs and either BMI or physical activity level (all $P$>0.05).

## Discussion

The present study evaluated the consistency of metabolic and appetite responses under postabsorptive conditions and following the consumption of a cereal and milk-based breakfast. Energy expenditure and fat oxidation displayed typical errors of ~100 kJ and ~3 g respectively for the postprandial periods. Postprandial typical errors of time-averaged AUC for hunger and fullness were 8.26 and 10.29 mm, respectively.

Energy expenditure demonstrated reasonable reproducibility under 2 h of postabsorptive conditions, with an acceptable ICC and a CV of 8.6% (Table 1). Under postprandial conditions, the reliability of EE was slightly improved, with both correlation coefficients increasing and the CV and typical error remaining relatively constant. These correlations are lower than the r=0.932 presented by Segal et al. (1992) after consumption of a liquid meal. It may be that due to the meal in the present study being of a semi-solid consistency, the rate of consumption, gastric emptying and intestinal absorption add further locations where biological variation in the metabolism of the meal can persist. Indeed, the rate of eating can affect the glycaemic response, which is associated with postprandial thermogenesis (Segal et al., 1992). Also, others have demonstrated high variability in the thermic effect of solid meals (Miles et al., 1993). The CV (26%) demonstrated by Miles et al. is higher than that of the present study, which could be due to a less diet and exercise standardisation (12 h vs. 24 h prior to trials). The limits of agreement for EE correspond to 292 kJ (Figure 1), which although may be sensitive enough to detect a difference between groups of individuals, it is of substantial magnitude to question the sensitivity to detect subtle differences in meal composition.

The relationship shown between the CVs of EE and BMI suggests that the reliability of EE measurement is reduced as BMI is increased. An explanation for this is not readily available. Although a tentative suggestion is that the higher absolute EE seen with a higher BMI would affect the degree of variance. However, it should be noted that the relatively tight range of BMI in this study may limit the validity of this statistic.

When fasted, fat oxidation also displayed strong reproducibility with a good ICC, and reasonable CV (Table 1). However, these values did deteriorate to a degree during the postprandial trial (Table 1), though not to a significant extent with regards to the CV. To the author's best knowledge, this is the first study to exhibit the consistency of the fat oxidation response to a non-liquid meal. It appears that the fat oxidation response is comparable to, yet slightly less reliable than energy expenditure. Bland-Altman limits of agreement for FO were also relatively large at 9.3 g (Figure 2). This may mean that differences in an intervention are difficult to detect with this 2 h postprandial protocol. In a similar fashion to fat oxidation, the typical error for postprandial carbohydrate oxidation was substantial and a 13.9 g difference would be required by an intervention to be considered meaningful (Table 1). RER displayed tighter CVs (Table 1), and the typical error indicates that under both postabsorptive and postprandial conditions, a mean difference of 0.08 would be considered a meaningful difference. The CV for RER under postprandial conditions is similar to the 1.9% previously reported (Piers, Soares, Makan, & Shetty, 1992) during a basal metabolic rate measurement (under postabsorptive conditions).

At baseline, hunger and prospective consumption ratings provided a reasonable degree of consistency, in contrast to fullness and satisfaction, as demonstrated by high CVs. A similar pattern emerged during the postabsorptive trials (Table 2), where hunger and prospective consumption were more reliable than fullness and satisfaction, although all showed an improvement. This was probably due to the increase in the number of measures taken. Previous research has also shown reduced coefficients of repeatability (CR = 2 x SD) with mean postprandial measures versus fasting (Flint et al., 2000). It was suggested that as the number of time points increases, the reliability improves as individual outlying data points will be reduced in their impact. The former study had averaged ratings over a 4.5 h period, resulting in 10 data points. The present study demonstrates that the CV is improved after just 2 h (5 data points) to a level comparable to that found previously (Raben et al., 1995). Postabsorptive appetite ratings generally showed improved reliability compared to baseline (although the reliability of prospective consumption ratings weakened). In terms of CV, the pattern was reversed compared to postabsorptive conditions, whereby hunger and prospective consumption displayed higher CVs compared to fullness and satisfaction. A likely explanation for this is that hunger and prospective consumption ratings are high in the fasted state and are reduced following meal consumption. Fullness and satisfaction ratings respond in a converse fashion. Thus, lower values may be more susceptible to a greater variation as a percentage (CV) when absolute variation is similar. The limits of agreement (22.9 mm) for postprandial hunger AUC were similar to those reported previously (Flint et al., 2000) over a 4.5 h period (24 mm). This would suggest that there is no difference in the reliability of hunger ratings between a 2 h period of sampling (5 time points when sampled every 30 min) compared to a 4.5 h sampling epoch.

It is unsurprising that appetite ratings are less consistent than metabolic data, particularly in the postprandial state. The physiological processes involved in the consumption of the food are likely to influence appetite ratings, carrying with it the variation in digestion, absorption and metabolism. This adds to the variation in the other factors involved in appetite sensations from environmental and psychological stimuli (Stubbs et al., 2000).

Each statistical test of reliability possesses its own inherent limitations. It is beyond the scope of this paper to rigorously critique each statistical method in relation to one another, although it is useful to bear in mind the principle benefits and constraints of each method. The ICC is sensitive to systematic bias but requires heterogenous data and is not recommended as a solitary method (Atkinson & Nevill, 1998). The typical error and CVs represent 68% of the variance, yet CV depends on the magnitude of the measured values (Atkinson & Nevill, 1998). Limits of agreement represent 95% of the likely variance between measures in repeat tests. However, unlike typical error these can be influenced by sample size (Hopkins, 2000). This assortment of analyses not only allows for a more resolute picture of global reliability, but also facilitates the comparison with similar studies.

The condensed expired gas sampling periods used in the present study could be seen as a limitation, yet 5 min of stable measures have been deemed sufficient for best practise methods for the determination of energy expenditure (Compher et al., 2006). As this study suggests that fat oxidation is less reliable, then considerations may be made that a longer sampling period may be necessary for the determination of postprandial fat oxidation in future studies.

It is worthy to note that the participants of both the present study and that of Flint et al. (2000) were young healthy males of normal BMI. An interesting avenue for future research could be to investigate whether the reliability remains at a similar echelon when studying different populations (females, children, overweight and insulin resistant).

In conclusion, the reliability of the measurement of energy expenditure in response to a cereal and milk based breakfast is reasonable when taken over a 2 h period. Fat oxidation following breakfast was slightly less consistent and may not be as sensitive to interventions. The reproducibility of appetite sensations over a 2 h postprandial episode were shown to be comparable to those reported previously over a 4.5 h period. Thus in physically active males, 2 h is enough time to detect differences in metabolic (namely, energy expenditure and fat oxidation) and appetite responses to breakfast meals within studies requiring a shorter time period of sampling such as pre-load and exercise intervention studies. Typical errors indicate that a 211 kJ, 6.7 g and a 16.5 mm difference in postprandial energy expenditure, fat oxidation and AUC for hunger would be a needed for an intervention to be considered meaningful for studies of a similar design.

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**Table 1.** Reliability of metabolic variables over 120 min postabsorptive and postprandial periods

| | Postabsorptive | | | | Postprandial | | | |
|---|---|---|---|---|---|---|---|---|
| | TEE (kJ) | TFO (g) | TCO (g) | RER | TEE (kJ) | TFO (g) | TCO (g) | RER |
| Trial 1 | | | | | | | | |
| Mean | 843 | 15.8 | 16.4 | 0.78 | 943 | 12.4 | 26.1 | 0.84 |
| SD | 162 | 6.0 | 8.6 | 0.04 | 222 | 5.1 | 7.8 | 0.04 |
| Trial 2 | | | | | | | | |
| Mean | 851 | 16.6 | 15.5 | 0.77 | 943 | 13.8 | 24.8 | 0.83 |
| SD | 155 | 5.8 | 6.7 | 0.06 | 186 | 6.1 | 9.2 | 0.06 |
| Mean difference | 7.9 | 0.75 | -0.89 | -0.01 | 0.13 | 1.36 | 1.30 | -0.01 |
| 95% CI | -78.1, 93.8 | -1.53, 3.03 | -6.66, 4.88 | -0.04, 0.02 | -94.93, 94.67 | -1.67, 4.39 | -6.41, 3.80 | -0.04, 0.01 |
| ICC | 0.68 | 0.84 | 0.18 | 0.37 | 0.77 | 0.68 | 0.37 | 0.45 |
| 95% CI | 0.20, 0.90 | 0.55, 0.95 | -0.37, 0.64 | -0.13, 0.72 | 0.38, 0.93 | 0.21, 0.90 | -0.13, 0.72 | -0.03, 0.76 |
| CV (%) | 8.6 | 11.5 | 27.3 | 3.9 | 8.9 | 20.0 | 26.3 | 3.8 |
| Typical error | 95.7 | 2.54 | 7.04 | 0.04 | 105.5 | 3.37 | 6.96 | 0.04 |
| 95% CI | 67.8, 162.5 | 1.80, 4.31 | 5.14, 11.59 | 0.03, 0.06 | 74.7, 179.1 | 2.39, 5.73 | 5.20, 10.79 | 0.03, 0.06 |

SD, standard deviation; ICC, intra-class correlation coefficient; CV, coefficient of variation; TEE, total energy expenditure; TFO, total fat oxidation; TCO, total carbohydrate oxidation; RER, respiratory exchange ratio.

**Table 2**. Reliability of appetite AUC over 120 min postabsorptive and postprandial periods.

| | Postabsorptive | | | | Postprandial | | | |
|---|---|---|---|---|---|---|---|---|
| | Hunger | Fullness | Satisfaction | Prospective Consumption | Hunger | Fullness | Satisfaction | Prospective Consumption |
| Trial 1 | | | | | | | | |
| Mean | 64.4 | 22.2 | 23.5 | 71.0 | 31.1 | 66.3 | 62.8 | 36.6 |
| SD | 14.2 | 5.8 | 6.6 | 10.5 | 13.2 | 11.5 | 11.9 | 16.8 |
| Trial 2 | | | | | | | | |
| Mean | 62.5 | 24.1 | 26.9 | 67.7 | 31.9 | 60.8 | 62.7 | 40.5 |
| SD | 19.3 | 10.9 | 11.7 | 14.7 | 15.0 | 15.9 | 14.4 | 19.3 |
| Mean difference | -1.93 | 1.98 | 3.33 | -3.32 | 0.79 | -5.50 | -0.03 | 3.93 |
| 95% CI | -8.95, 5.10 | -3.34, 7.29 | -1.56, 8.23 | -9.73, 3.09 | -6.63, 8.22 | -14.75, 3.75 | -8.14, 8.08 | -7.39, 15.24 |
| ICC | 0.82 | 0.59 | 0.71 | 0.73 | 0.70 | 0.49 | 0.58 | 0.56 |
| 95% CI | 0.49, 0.94 | 0.05, 0.86 | 0.26, 0.91 | 0.30, 0.9 | 0.24, 0.90 | -0.08, 0.82 | 0.03, 0.86 | 0.01, 0.85 |
| CV (%) | 12.8 | 23.7 | 21.2 | 9.5 | 25.2 | 14.3 | 11.3 | 28.3 |
| Typical error | 7.82 | 5.92 | 5.45 | 7.13 | 8.26 | 10.29 | 9.02 | 12.59 |
| 95% CI | 5.54, 13.28 | 4.19, 10.04 | 3.86, 9.25 | 5.05, 12.11 | 5.85, 14.03 | 7.29, 17.48 | 6.39, 15.32 | 8.92, 21.38 |

SD, standard deviation; ICC, intra-class correlation coefficient; CV, coefficient of variation; AUC, area under the curve.

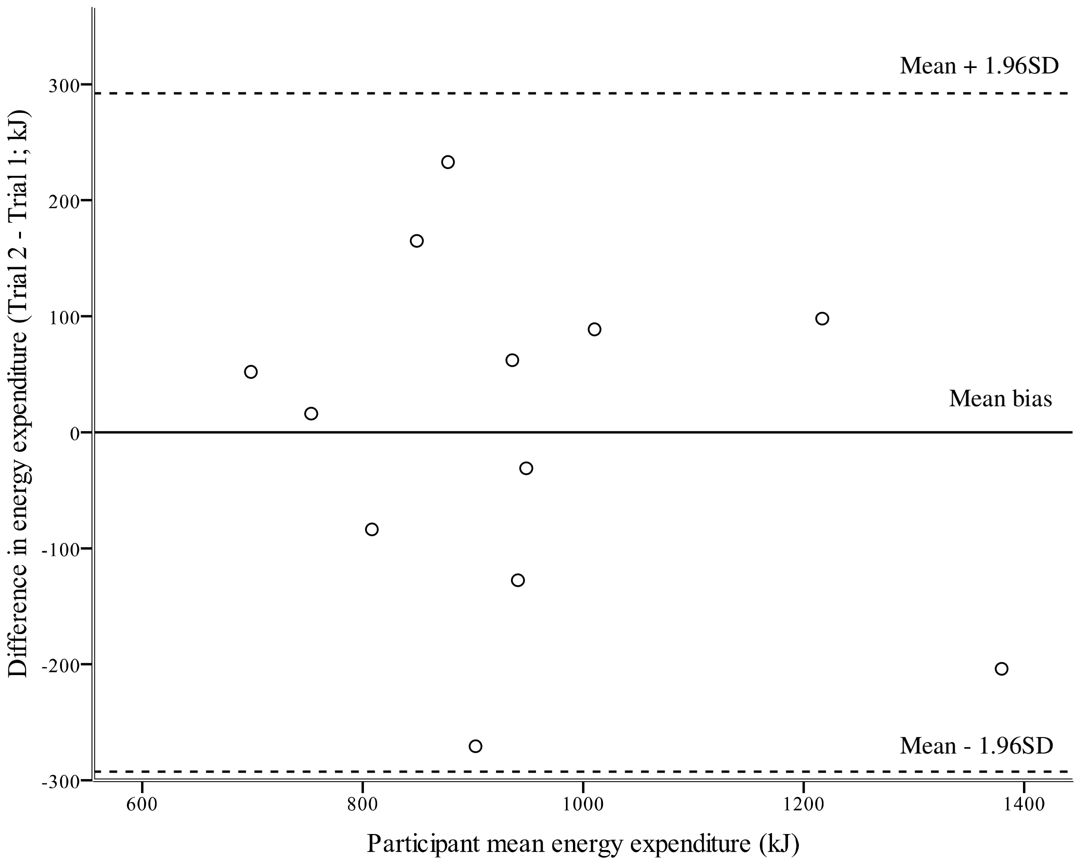
**Figure Legends**

**Figure 1.** Bland and Altman plot for difference in energy expenditure over a 120 min period following consumption of a cereal-based breakfast on two occasions.

**Figure 2.** Bland and Altman plot for total fat oxidation over a 120 min period following consumption of a cereal-based breakfast on two occasions.

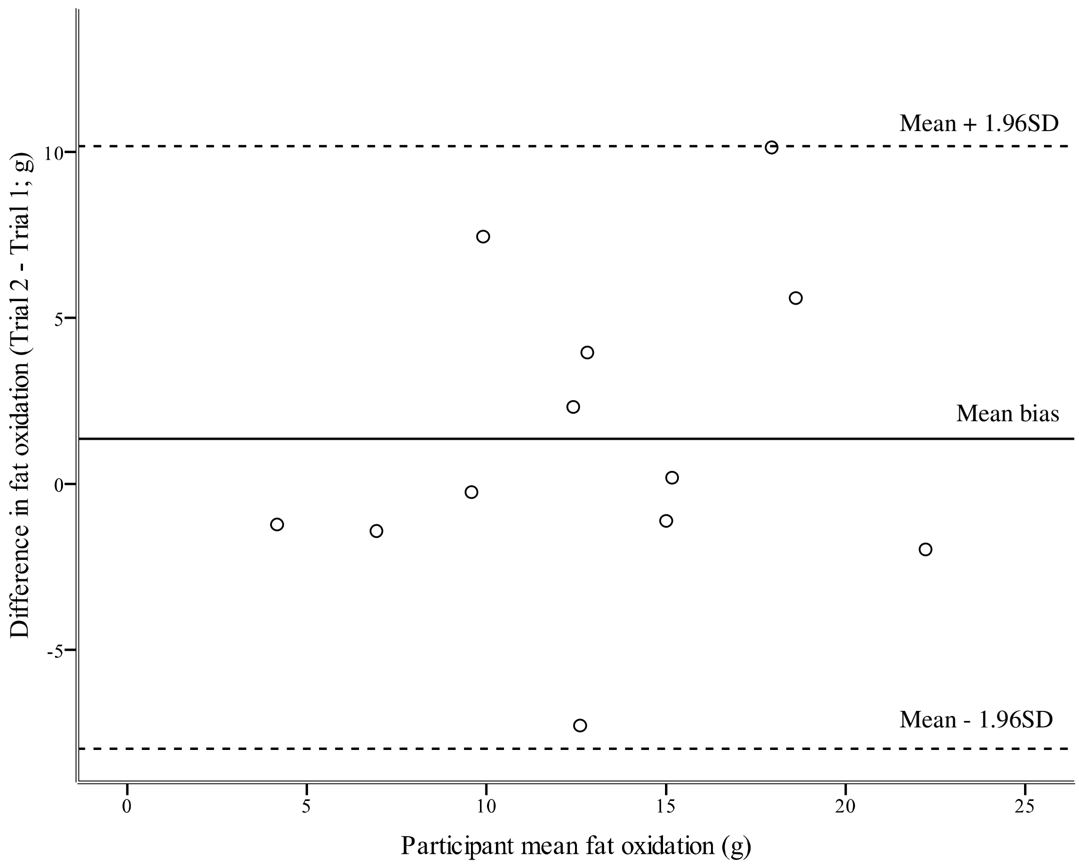
**Figure 3.** Bland and Altman plot for time-averaged AUC for hunger over a 120 min period following consumption of a cereal-based breakfast on two occasions. AUC, area under the curve.

421
422 **Figure 1**



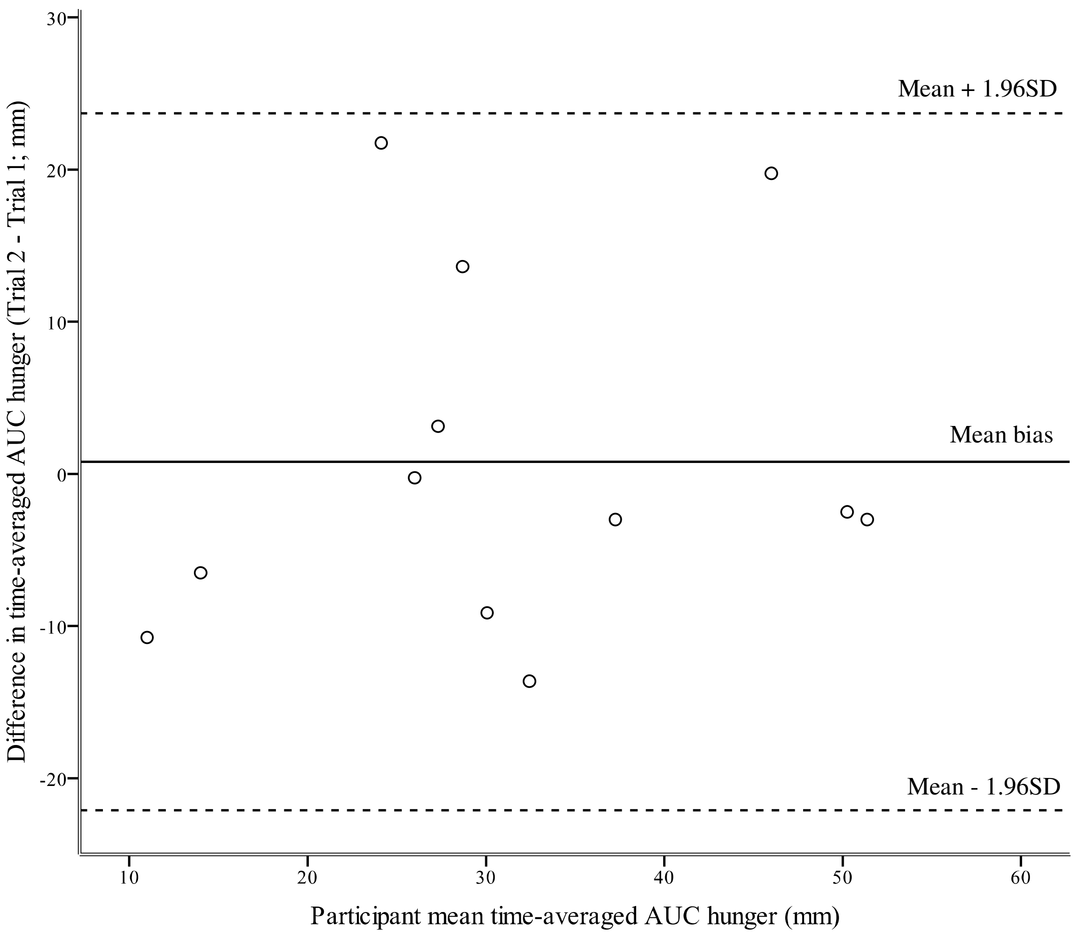
423
424 **Figure 2**

425



426
427 **Figure 3**

428



Highlights

- Reliability of metabolic and appetite responses to breakfast were evaluated
- Indirect calorimetry estimated energy expenditure and fat oxidation
- Visual analogue scales determined subjective appetite sensations
- Reproducibility of energy expenditure was superior to fat oxidation
- Appetite responses were reliable to a similar extent as longer protocols