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1 **Consistency of metabolic responses and appetite sensations**
2 **under postabsorptive and postprandial conditions**

3
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17 **Abstract**

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

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

36

37 **Introduction**

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

50 The test-retest reproducibility of these measures is pertinent in order to be
51 confident that an intervention or variable is the cause of a difference in a trial and not
52 random variability or systematic bias (Atkinson & Nevill, 1998; Hopkins, 2000).
53 Reliability can be defined as producing the same or similar result when a protocol is
54 repeated a number of times (Atkinson & Nevill, 1998). It has been proposed that
55 reliability should be assessed using a variety of statistical measures (Atkinson & Nevill,
56 1998) such as Bland and Altman limits of agreement (Bland & Altman, 1986),
57 coefficient of variation (CV), intraclass coefficient of correlation (ICC) and 95%
58 confidence limits of typical error. The inclusion of multiple analyses of reliability
59 allows for interpretation of the components of reliability, comparison with similar

60 studies using different analyses and is further justified due to a current lack of
61 consensus on a primary method to ascertain reliability (Atkinson & Nevill, 2000;
62 Hopkins, 2000).

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

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

83 2011; Rosen, Ostman, & Bjorck, 2011). Hence clarifying the day to day agreement in
84 metabolic and satiety responses to cereal-based breakfasts is warranted.

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

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

99

100

101 **Methods**

102

103 *Design*

104

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

113

114

115 *Subjects*

116

117 Twelve healthy, physically active males (age: 23.2 ± 4.3 y, stature: 178 ± 7 cm,
118 mass: 77.2 ± 5.3 kg, BMI: 24.5 ± 2.0 kg/m², self-reported activity level: 4024 ± 3018
119 met-min/wk) were recruited from the student and staff population at Northumbria
120 University and all participants completed the full protocol. Participants who self-
121 reported as physically inactive, defined by less than 30 min of moderate activity, 5
122 times a week by the International Physical Activity Questionnaire (Craig et al., 2003)
123 restrained eaters, defined by a score of >11 on the Three Factor Eating Questionnaire
124 (Stunkard & Messick, 1985) or those with any metabolic disorders were omitted. The
125 present study was conducted in accordance with the guidelines stated in the 1964
126 Declaration of Helsinki. Prior to recruitment, all participants provided informed written
127 consent and the study was approved by the School of Life Sciences Ethics Committee at
128 Northumbria University.

129

130 *Anthropometric measurements*

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

135

136 *Energy expenditure and substrate oxidation*

137

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

146

147 *Appetite sensations*

148

149 Paper based, 100 mm VAS were completed to determine appetite sensations.
150 Questions asked were used to determine hunger, fullness, satisfaction and prospective

151 food consumption. VAS ratings were double-measured by two researchers and means
152 were taken where discrepancies occurred.

153

154 *Test meal*

155

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

161

162 *Statistical analysis*

163

164 All data were calculated as mean \pm SD. VAS ratings were calculated as time-
165 averaged area under the curve (AUC) for postprandial and postabsorptive periods.
166 Reliability was assessed using a variety of statistical techniques, with typical error taken
167 as the primary assessment tool. Namely, mean difference, ICC, CV and typical error
168 were employed for all variables (Atkinson & Nevill, 1998; Hopkins, 2000). ICCs were
169 considered to show good reproducibility when $ICC \geq 0.8$, moderate reproducibility when
170 $0.7 \leq ICC < 0.8$, and acceptable reproducibility when $0.6 \leq ICC < 0.7$. Energy expenditure,
171 fat oxidation and hunger during the postprandial trials were assessed using Bland-
172 Altman limits of agreement (Bland & Altman, 1986). Data were checked for
173 heteroscedasticity such that the appropriate statistical techniques could be employed. To

174 determine whether either BMI or physical activity levels affected the reliability of the
175 variables, pearson product-moment correlation coefficients were used to determine
176 relationships between CVs of metabolic and appetite responses, and BMI and physical
177 activity level. Paired student's t tests were used to detect differences in mean values and
178 CVs. Values were considered significant when $P < 0.05$.

179

180

181 **Results**

182

183 *Energy expenditure and substrate oxidation*

184

185 Postprandial energy expenditure was higher than postabsorptive energy
186 expenditure, yet CV and typical errors were similar (Table 1). A Bland-Altman plot for
187 postprandial energy expenditure can be seen in Figure 1. Fat oxidation showed greater
188 variation than energy expenditure at baseline and throughout both trials (CVs 20 and
189 8%, respectively). Postprandial fat oxidation is displayed as a Bland-Altman plot in
190 Figure 2. Mean CVs were not significantly different for either energy expenditure or fat
191 oxidation ($P=0.80$ and $P=0.12$, respectively) with the postprandial trial compared to the
192 postabsorptive trial (Table 1).

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

195 Both postprandial and postabsorptive energy expenditure CVs showed positive
196 relationships with BMI ($r = 0.61$ and 0.64 , respectively; both $P < 0.05$), but not with

197 physical activity level ($r = -0.13$ and -0.21 , respectively; both $P > 0.05$) whereas neither
198 postprandial, nor postabsorptive fat oxidation CVs showed significant relationships with
199 either BMI or physical activity level (all $P > 0.05$).

200

201 *Subjective appetite ratings*

202

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

215

216 **Discussion**

217

218 The present study evaluated the consistency of metabolic and appetite responses
219 under postabsorptive conditions and following the consumption of a cereal and milk-
220 based breakfast. Energy expenditure and fat oxidation displayed typical errors of ~ 100

221 kJ and ~3 g respectively for the postprandial periods. Postprandial typical errors of
222 time-averaged AUC for hunger and fullness were 8.26 and 10.29 mm, respectively.

223 Energy expenditure demonstrated reasonable reproducibility under 2 h of
224 postabsorptive conditions, with an acceptable ICC and a CV of 8.6% (Table 1). Under
225 postprandial conditions, the reliability of EE was slightly improved, with both
226 correlation coefficients increasing and the CV and typical error remaining relatively
227 constant. These correlations are lower than the $r=0.932$ presented by Segal et al. (1992)
228 after consumption of a liquid meal. It may be that due to the meal in the present study
229 being of a semi-solid consistency, the rate of consumption, gastric emptying and
230 intestinal absorption add further locations where biological variation in the metabolism
231 of the meal can persist. Indeed, the rate of eating can affect the glycaemic response,
232 which is associated with postprandial thermogenesis (Segal et al., 1992). Also, others
233 have demonstrated high variability in the thermic effect of solid meals (Miles et al.,
234 1993). The CV (26%) demonstrated by Miles et al. is higher than that of the present
235 study, which could be due to a less diet and exercise standardisation (12 h vs. 24 h prior
236 to trials). The limits of agreement for EE correspond to 292 kJ (Figure 1), which
237 although may be sensitive enough to detect a difference between groups of individuals,
238 it is of substantial magnitude to question the sensitivity to detect subtle differences in
239 meal composition.

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

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

261 At baseline, hunger and prospective consumption ratings provided a reasonable
262 degree of consistency, in contrast to fullness and satisfaction, as demonstrated by high
263 CVs. A similar pattern emerged during the postabsorptive trials (Table 2), where hunger
264 and prospective consumption were more reliable than fullness and satisfaction, although
265 all showed an improvement. This was probably due to the increase in the number of
266 measures taken. Previous research has also shown reduced coefficients of repeatability
267 ($CR = 2 \times SD$) with mean postprandial measures versus fasting (Flint et al., 2000). It
268 was suggested that as the number of time points increases, the reliability improves as

269 individual outlying data points will be reduced in their impact. The former study had
270 averaged ratings over a 4.5 h period, resulting in 10 data points. The present study
271 demonstrates that the CV is improved after just 2 h (5 data points) to a level comparable
272 to that found previously (Raben et al., 1995). Postabsorptive appetite ratings generally
273 showed improved reliability compared to baseline (although the reliability of
274 prospective consumption ratings weakened). In terms of CV, the pattern was reversed
275 compared to postabsorptive conditions, whereby hunger and prospective consumption
276 displayed higher CVs compared to fullness and satisfaction. A likely explanation for
277 this is that hunger and prospective consumption ratings are high in the fasted state and
278 are reduced following meal consumption. Fullness and satisfaction ratings respond in a
279 converse fashion. Thus, lower values may be more susceptible to a greater variation as a
280 percentage (CV) when absolute variation is similar. The limits of agreement (22.9 mm)
281 for postprandial hunger AUC were similar to those reported previously (Flint et al.,
282 2000) over a 4.5 h period (24 mm). This would suggest that there is no difference in the
283 reliability of hunger ratings between a 2 h period of sampling (5 time points when
284 sampled every 30 min) compared to a 4.5 h sampling epoch.

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

291 Each statistical test of reliability possesses its own inherent limitations. It is
292 beyond the scope of this paper to rigorously critique each statistical method in relation

293 to one another, although it is useful to bear in mind the principle benefits and
294 constraints of each method. The ICC is sensitive to systematic bias but requires
295 heterogenous data and is not recommended as a solitary method (Atkinson & Nevill,
296 1998). The typical error and CVs represent 68% of the variance, yet CV depends on the
297 magnitude of the measured values (Atkinson & Nevill, 1998). Limits of agreement
298 represent 95% of the likely variance between measures in repeat tests. However, unlike
299 typical error these can be influenced by sample size (Hopkins, 2000). This assortment of
300 analyses not only allows for a more resolute picture of global reliability, but also
301 facilitates the comparison with similar studies.

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

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

313 In conclusion, the reliability of the measurement of energy expenditure in
314 response to a cereal and milk based breakfast is reasonable when taken over a 2 h
315 period. Fat oxidation following breakfast was slightly less consistent and may not be as
316 sensitive to interventions. The reproducibility of appetite sensations over a 2 h

317 postprandial episode were shown to be comparable to those reported previously over a
318 4.5 h period. Thus in physically active males, 2 h is enough time to detect differences in
319 metabolic (namely, energy expenditure and fat oxidation) and appetite responses to
320 breakfast meals within studies requiring a shorter time period of sampling such as pre-
321 load and exercise intervention studies. Typical errors indicate that a 211 kJ, 6.7 g and a
322 16.5 mm difference in postprandial energy expenditure, fat oxidation and AUC for
323 hunger would be a needed for an intervention to be considered meaningful for studies of
324 a similar design.

325

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- 406
- 407
- 408

409 **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

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

413

414 **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

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

417

418 **Figure Legends**

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

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

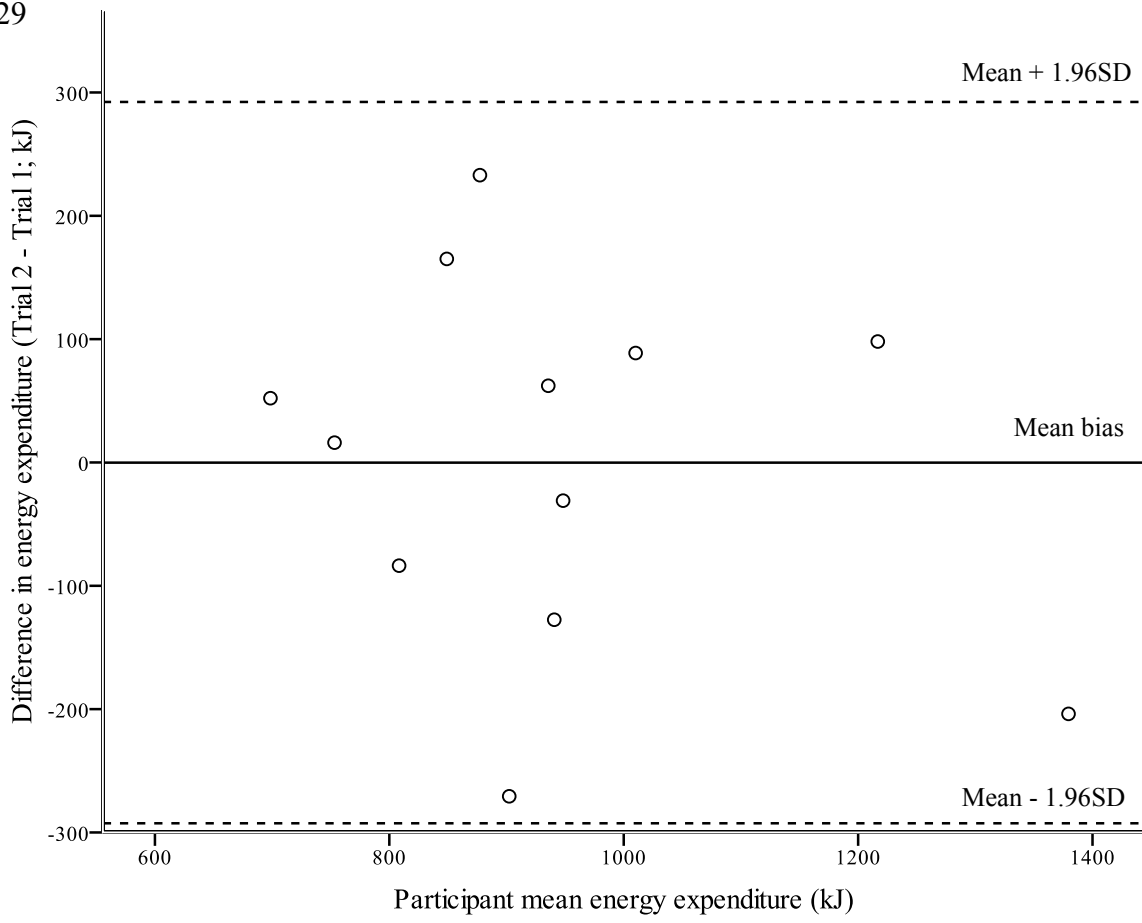
423 **Figure 3.** Bland and Altman plot for time-averaged AUC for hunger over a 120 min
424 period following consumption of a cereal-based breakfast on two occasions. AUC, area
425 under the curve.

426

427

428 **Figure 1**

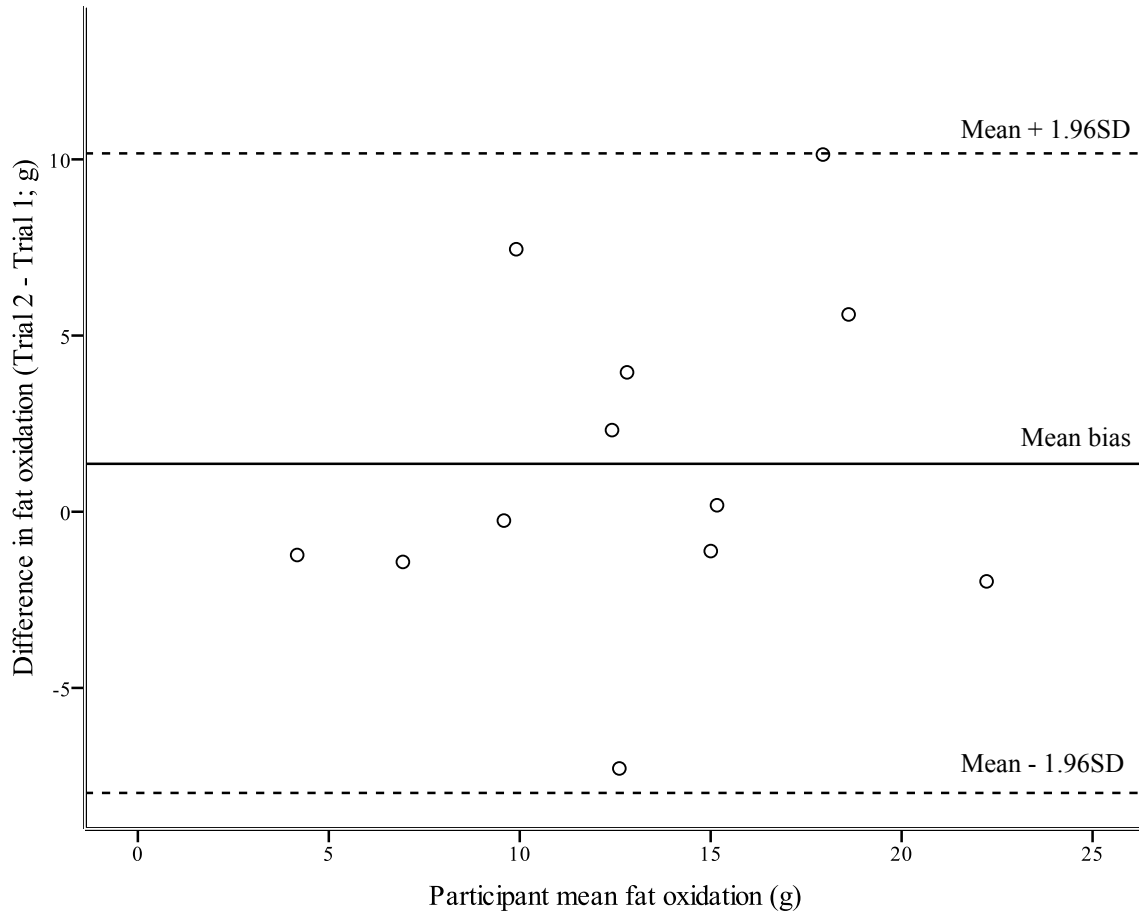
429



430

431 **Figure 2**

432



434

435 **Figure 3**

436

