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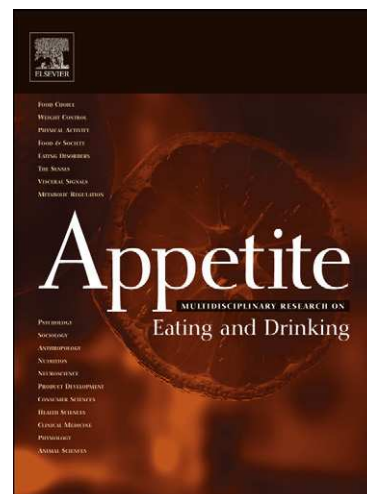
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1 **Consistency of metabolic responses and appetite sensations**
2 **under postabsorptive and postprandial conditions**

3
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15

16 **Abstract**

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

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

35 Introduction

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

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

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

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

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

81 2011; Rosen, Ostman, & Bjorck, 2011). Hence clarifying the day to day agreement in
82 metabolic and satiety responses to cereal-based breakfasts is warranted.

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

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

97

98

99 **Methods**

100

101 *Design*

102

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

111

112

113 *Subjects*

114

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

127

128 *Anthropometric measurements*

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

133

134 *Energy expenditure and substrate oxidation*

135

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

144

145 *Appetite sensations*

146

147 Paper based, 100 mm VAS were completed to determine appetite sensations.

148 Questions asked were used to determine hunger, fullness, satisfaction and prospective

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

151

152 *Test meal*

153

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

159

160 *Statistical analysis*

161

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

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

177

178

179 **Results**

180

181 *Energy expenditure and substrate oxidation*

182

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

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

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

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

198

199 *Subjective appetite ratings*

200

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

213

214 **Discussion**

215

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

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

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

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

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

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

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

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

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

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

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

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

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

315 postprandial episode were shown to be comparable to those reported previously over a
316 4.5 h period. Thus in physically active males, 2 h is enough time to detect differences in
317 metabolic (namely, energy expenditure and fat oxidation) and appetite responses to
318 breakfast meals within studies requiring a shorter time period of sampling such as pre-
319 load and exercise intervention studies. Typical errors indicate that a 211 kJ, 6.7 g and a
320 16.5 mm difference in postprandial energy expenditure, fat oxidation and AUC for
321 hunger would be a needed for an intervention to be considered meaningful for studies of
322 a similar design.

323

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406 **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

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

410 **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

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

413 **Figure Legends**

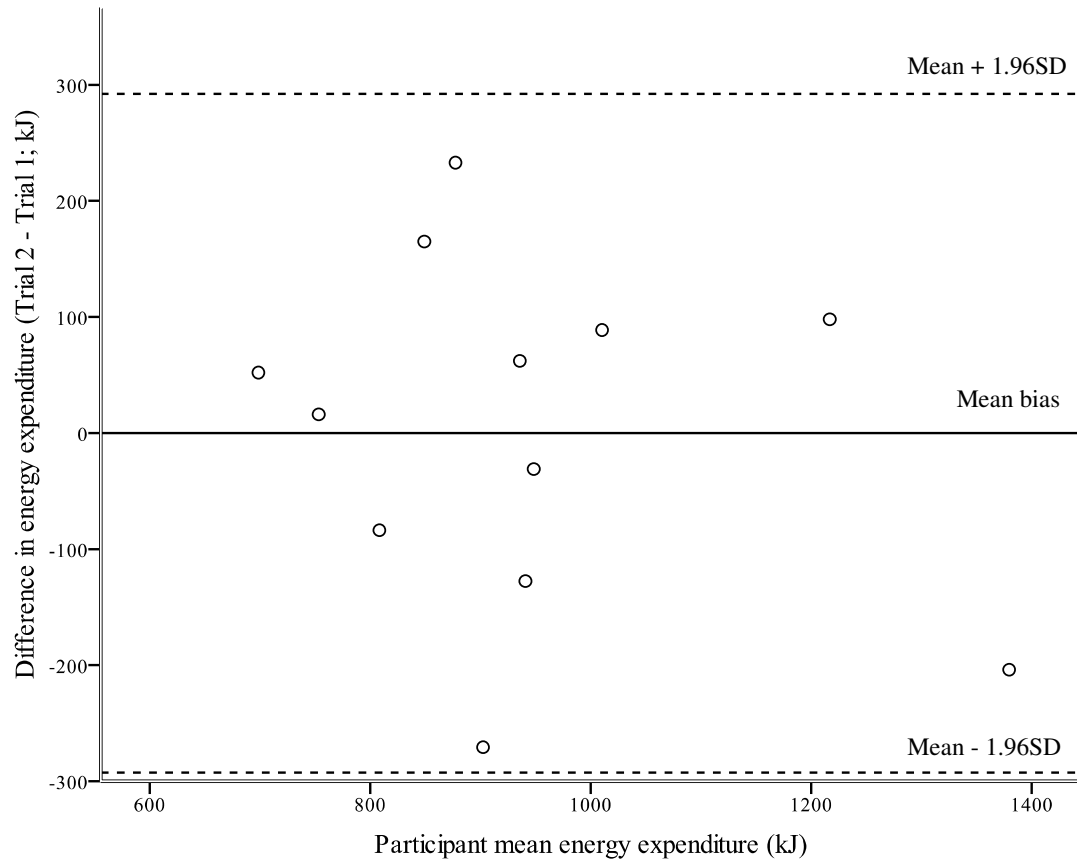
414 **Figure 1.** Bland and Altman plot for difference in energy expenditure over a 120 min
415 period following consumption of a cereal-based breakfast on two occasions.

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

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

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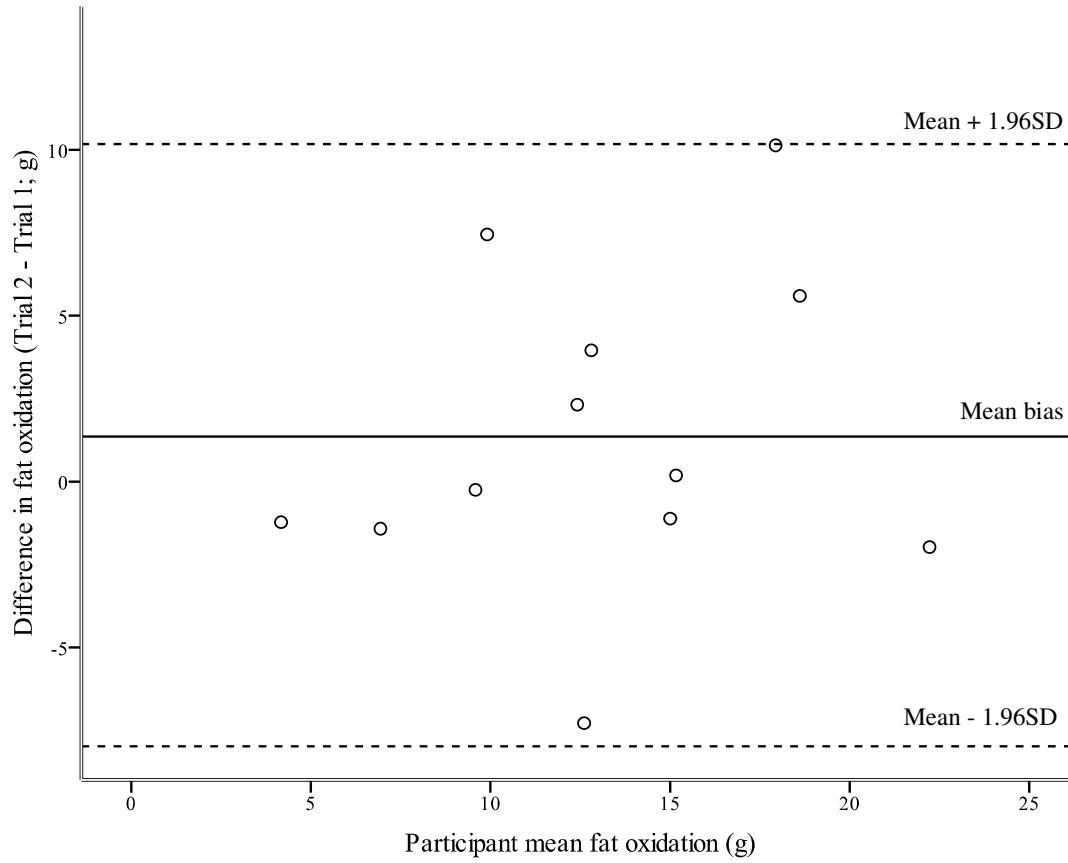
421

422 **Figure 1**

423

424 **Figure 2**

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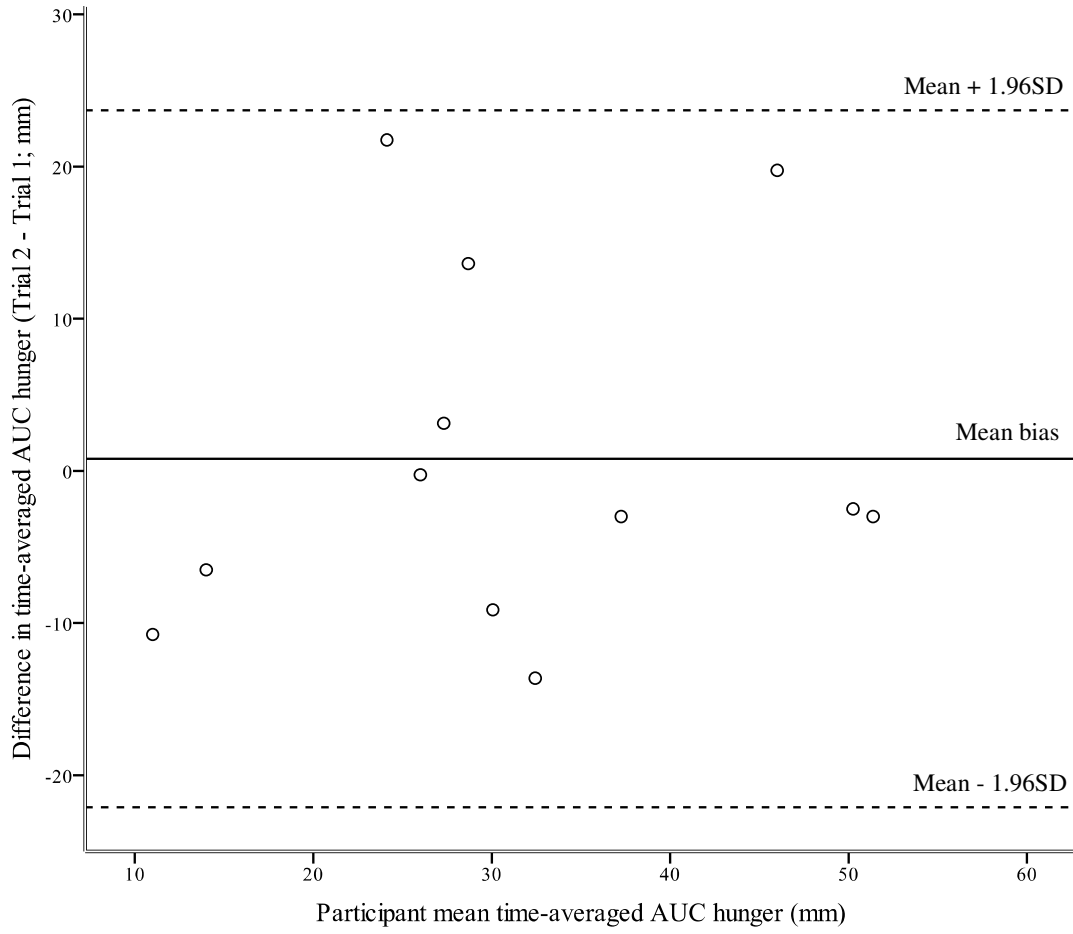


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427 **Figure 3**

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430 Highlights

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432 • Reliability of metabolic and appetite responses to breakfast were evaluated

433 • Indirect calorimetry estimated energy expenditure and fat oxidation

434 • Visual analogue scales determined subjective appetite sensations

435 • Reproducibility of energy expenditure was superior to fat oxidation

436 • Appetite responses were reliable to a similar extent as longer protocols

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