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1 **Individual variation in hunger, energy intake and ghrelin responses to acute exercise**

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34 **ABSTRACT**

35 **Purpose:** To characterise the immediate and extended impact of acute exercise on hunger,
36 energy intake and circulating acylated ghrelin concentrations using a large dataset of
37 homogenous experimental trials; and to describe the variation in responses between
38 individuals. **Methods:** Data from 17 of our group's experimental crossover trials were
39 aggregated yielding a total sample of 192 young, healthy, males. In these studies, single bouts
40 of moderate to high-intensity aerobic exercise ($69 \pm 5\%$ VO_2 peak; mean \pm SD) were completed
41 with detailed participant assessments occurring during and for several hours post-exercise.
42 Mean hunger ratings were determined during ($n = 178$) and after ($n = 118$) exercise from visual
43 analogue scales completed at 30 min intervals whilst *ad libitum* energy intake was measured
44 within the first hour after exercise ($n = 60$) and at multiple meals ($n = 128$) during the remainder
45 of trials. Venous concentrations of acylated ghrelin were determined at strategic time points
46 during ($n = 118$) and after ($n = 89$) exercise. **Results:** At group-level, exercise transiently
47 suppressed hunger ($P < 0.010$; Cohen's $d = 0.77$) but did not affect energy intake. Acylated
48 ghrelin was suppressed during exercise ($P < 0.001$; Cohen's $d = 0.10$) and remained
49 significantly lower than control (no exercise) afterwards ($P < 0.024$; Cohen's $d = 0.61$).
50 Between participants, there were notable differences in responses however a large proportion
51 of this spread lay within the boundaries of normal variation associated with biological and
52 technical assessment error. **Conclusion:** In young men, acute exercise suppresses hunger and
53 circulating acylated ghrelin concentrations with notable diversity between individuals. Care
54 must be taken to distinguish true inter-individual variation from random differences within
55 normal limits.

56

57 **KEY WORDS:** Physical activity, Energy balance, Appetite, Variation

58

59 **INTRODUCTION**

60 The interaction between exercise, appetite and food intake has received widespread scientific
61 attention within recent years given the direct relevance for energy balance and weight control
62 (4). Emergent from this body of research is a consensus that single bouts of moderate- to high-
63 intensity exercise transiently suppress appetite but have no influence on *ad libitum* energy
64 intake (10,33). Energy homeostasis therefore seems insensitive to acute energy deficits
65 imposed by exercise; with more prolonged or repeated perturbations necessary to induce partial
66 compensatory responses (36,39). In association with this line of research has been a related
67 interest in seeking to understand the mechanisms underpinning appetite control and
68 perturbations in energy balance resulting from exercise and dietary interventions. Notably, the
69 responses of several gut peptides to exercise (acylated ghrelin, peptide YY₃₋₃₆, glucagon-like-
70 peptide-1, cholecystokinin) have been scrutinised as possible modulators of appetite and food
71 intake (34). The most consistent finding from these investigations is that exercise transiently
72 alters the circulating concentrations of these hormones in directions associated with suppressed
73 appetite; however, circulating concentrations are typically not different from control at 30 to
74 60 min post-exercise (10).

75

76 With a growing emphasis within biomedical science on ‘precision medicine’ (2) recent
77 research has sought to characterise the individual variability in appetite and energy intake
78 responses to exercise (13, 18, 20, 27). The primary question addressed within these studies is
79 whether some individuals are more or less likely to compensate for energy expended during
80 exercise by increasing post-exercise energy intake. The implication of this inquiry is that
81 exercise may be less useful for weight management in ‘compensators’ compared with ‘non-
82 compensators’. Unfortunately, to date, the studies which have examined this issue are limited
83 by small sample sizes and the failure to appreciate the importance of internal sources of

84 variation (technical error and biological variation) (1). Additional research is therefore needed
85 to provide greater insight within this area of research.

86

87 Over the last 15 years our research group has conducted many experimental exercise
88 interventions examining the effects of acute exercise on appetite, *ad libitum* energy intake and
89 appetite-regulatory hormones. Given the uniqueness of acylated ghrelin as the only circulating
90 hormone known to stimulate appetite and promote positive energy balance (9,40), our research
91 has maintained a central focus on the interaction between exercise, appetite, *ad libitum* energy
92 intake and acylated ghrelin. Usefully, the experimental designs (randomised cross-over trials
93 with exercise and control trials), participants (lean, young, healthy, males) and exercise
94 protocols (aerobic moderate- to high-intensity exercise) utilised within these studies have been
95 remarkably similar. This similarity permits the aggregation of data which provides enhanced
96 power to investigate experimental intervention effects and to interrogate associations between
97 key variables. Uniquely, in this context, this large dataset also provides a novel opportunity to
98 comprehensively explore the variability in appetite and *ad libitum* energy intake responses to
99 exercise between individuals.

100

101 The primary aims of this study were two-fold. Firstly, using our large, pooled dataset of
102 experimental trials, we sought to characterise the immediate (during and shortly after exercise)
103 and extended (several hours post-exercise) impact of acute exercise on perceived hunger, *ad*
104 *libitum* energy intake and circulating concentrations of acylated ghrelin. Secondly, with precise
105 consideration of the day-to-day biological and technical error inherent within outcome
106 measurements, we sought to determine the individual variation in hunger, *ad libitum* energy
107 intake and circulating acylated ghrelin responses, both during and in the hours after a single
108 bout of exercise. To achieve this second aim we have collected new data to determine the day-

109 to-day variation (with no intervention) in hunger, circulating acylated ghrelin and energy intake
110 (during *ad libitum* feeding) in young, healthy males. The findings reported in this manuscript
111 provide novel insights concerning the interaction between exercise, appetite control and energy
112 homeostasis.

113

114 **METHODS**

115 **Research studies and participants**

116 The data described in this manuscript were derived from 17 studies (16 published in peer
117 reviewed scientific journals; one currently in press) which were conducted between 2004 and
118 2014 in the exercise physiology laboratory led by Professor David Stensel at Loughborough
119 University, UK. All included studies received ethical approval from the institutional ethical
120 advisory board and written informed consent was obtained from all participants before any trial
121 procedures commenced. Each trial included within this pooled analysis was an acute
122 randomised-crossover trial with participants having completed paired exercise (see detail
123 below) and control (resting within the laboratory) trials. The key features of each study in this
124 pooled investigation are described in tables within the accompanying Supplementary Digital
125 Content (1 – 8). In all of the studies the participants ($n = 192$ in total) were young ((mean \pm
126 SD) 22.3 ± 2.7 years), lean (BMI 23.4 ± 2.2 kg/m²), recreationally active ($\dot{V}O_2$ peak ($n = 178$)
127 57.8 ± 8.2 mL/kg/min) males who were metabolically healthy. All of the participants were
128 weight stable (< 2.5 kg change in body weight) for at least three months before experimental
129 trials.

130

131 **Exercise protocol characteristics**

132 The exercise stimuli imposed within the studies included in this pooled analysis were
133 homogenous; in all instances being characterised as a single bout of moderate- to high-intensity

134 aerobic exercise. In all trials, exercise was conducted within a controlled laboratory setting
135 with participants exercising under the direct supervision of study experimenters. In all except
136 one study (which involved an acute bout of swimming), the mode of exercise completed was
137 treadmill running or ergometer cycling with indirect calorimetry (Douglas bags) used to
138 monitor exercise intensity and determine energy expenditure and substrate oxidation (15).
139 Across exercise trials the intensity of exercise ranged from 56 to 83 percent of $\dot{V}O_2$ peak with
140 a mean intensity of $69 \pm 5\%$. The duration of each acute exercise bout ranged from 30 to 90
141 min (30 min, two studies; 60 min, 11 studies; 90 min, four studies).

142

143 **Anthropometry and standardisation**

144 Body mass and stature were determined using standard techniques with participants wearing
145 light clothing. Body composition (fat mass and fat-free mass) was determined using skin-fold
146 measurements (triceps, bicep, subscapular, suprailiac) and the published equations of Durnin
147 and Womersley (12) and Siri (35). Participants' age, stature and body mass was used to
148 estimate resting metabolic rate as described by Mifflin et al. (31). Participants refrained from
149 consuming alcohol, caffeine and participating in structured exercise for 24-48 h before main
150 experimental trials and during this period dietary intake was standardised using weighed food
151 records. Participants' last meal was consumed before study days on the prior evening (no later
152 than 22:00) and all main trials commenced the following morning after an overnight fast.
153 Participants maintained their habitual diet between trials in all experiments.

154

155 **Hunger analyses**

156 The primary analyses of interest in this study relating to hunger were: 1) individual variation
157 in fasting hunger ($n = 192$); 2) the immediate (during exercise, $n = 178$) and prolonged (up to
158 8 h post-exercise, $n = 118$) effects of exercise on perceived hunger. In each of the studies

159 included within these analyses participants reported their perceived hunger at intervals of 30
160 min using pen and paper based 100 mm visual analogue scales (14). The impact of exercise on
161 hunger was assessed by comparing mean hunger ratings calculated during and after exercise
162 with paired values calculated on each participant's control trial. In the post-exercise hunger
163 analysis mean hunger scores were calculated from data available until the end of trials or until
164 the occurrence of a buffet meal (when standardised appetite scores were no longer comparable).
165 The reproducibility of fasting perceived hunger was determined from baseline hunger ratings
166 at the start of paired exercise and control trials. Individual variation in hunger responses during
167 and after exercise were calculated by subtracting mean hunger ratings calculated during control
168 trials from mean hunger ratings observed during the same periods within exercise trials. For all
169 post-exercise analyses, hunger ratings obtained within the first 30 min after exercise was
170 excluded to eliminate any latent impact of the exercise bout.

171

172 In order to examine the individual variation in hunger responses during and after exercise we
173 compared each participant's response with our new data ($n = 15$ young, healthy males)
174 regarding the variation in hunger ratings across one hour (most common duration of exercise
175 in the present analyses) ($1 \text{ h: } \pm 30 \text{ mm; } 17.2\%$) and over an extended duration ($2.5 \text{ h: } \pm 20 \text{ mm;}$
176 13.8%) with no intervention.

177

178 **Energy intake analyses**

179 The primary analyses of interest relating to exercise and *ad libitum* energy intake were: 1) the
180 impact of acute exercise on energy intake at the first meal consumed shortly after exercise
181 (within 60 min) ($n = 60$); 2) the impact of acute exercise on energy intake across several hours
182 post-exercise (range 5 - 9 h) ($n = 128$). In each of the studies included within these analyses,
183 *ad libitum* energy intake was determined from buffet-style meals whereby participants had

184 access to a range of foods for a discrete period of time (30 mins) which was identical on paired
185 exercise and control trials. In all trials, participants were instructed to eat until ‘comfortably
186 full and satisfied’ and that additional food was available if desired. All meals were consumed
187 in isolation so that social factors did not influence eating behaviour. Variation in energy intake
188 responses to exercise was determined by subtracting each participant’s energy intake during
189 the control trial from their intake during paired exercise trials. Within the analyses examining
190 the delayed effects of exercise on energy intake, data was included only if participants had
191 remained in the laboratory during the entire period of observation. Additionally, data was only
192 assessed from meals consumed on the same day as exercise i.e. data was not included from
193 energy intake assessments conducted on the day after exercise (which occurred in three studies
194 identified within this paper).

195

196 Because the natural day-to-day variability in energy intake is highly dependent on the
197 participants studied and the format of *ad libitum* meal provision (i.e. homogenous meal versus
198 buffet meal and types of foods available at laboratory meals), we conducted a new study to
199 characterise the variation in *ad libitum* energy intake across two meals (breakfast and lunch)
200 when using a buffet meal (24) (Appendix 1) and participant cohort ($n = 18$; healthy, lean males)
201 identical to that utilised within the studies described in the present manuscript. In this setting
202 we found that the co-efficient of repeatability and intra-subject variation at breakfast was \pm
203 1937 kJ and 18.9%. Furthermore, when energy intake at breakfast was combined with a buffet
204 lunch, together, the corresponding repeatability values were 2138 kJ and 8.9%. These
205 boundaries of variation were used to determine the boundaries of ‘true variation’ in energy
206 intake responses in the present investigation.

207

208

209 **Acylated ghrelin analyses**

210 The primary analyses of interest relating to acylated ghrelin were: 1) the immediate (during
211 exercise, $n = 118$) and prolonged (up to 8 h post-exercise; $n = 89$) effects of acute exercise on
212 circulating acylated ghrelin concentrations; 2) day-to-day variation in fasting circulating
213 acylated ghrelin concentrations ($n = 138$). In each of the studies included within these analyses
214 circulating concentrations of acylated ghrelin were determined from venous blood samples
215 taken by venepuncture (fasting measurement in one study) or cannulas (16 studies) positioned
216 in antecubital veins. Across all studies, plasma acylated ghrelin concentrations were
217 determined using the same enzyme-linked immune-sorbent assay (SPI-BIO, Montigny le
218 Brettoneux, France) which has demonstrated good intra-assay (typically 6-8%) variation in our
219 laboratory. Importantly, identical sampling pre- and post-treatment was performed across all
220 studies as detailed previously (6). Variation in circulating acylated ghrelin responses to exercise
221 was determined by subtracting the plasma acylated ghrelin AUC during the period of interest
222 within the control trial (exercise period and post-exercise period) from the corresponding
223 period during the exercise trial. These data were then expressed as a percentage difference with
224 positive values indicating an increase in circulating acylated ghrelin in response to exercise
225 (and vice-versa). Acylated ghrelin data was expressed as percentage difference, rather than
226 absolute values (as per our hunger and energy intake data), due to variation in absolute acylated
227 ghrelin values obtained across our data (most likely related to antibody variation with ELISA
228 kits over time). To determine the day-to-day variability in circulating acylated ghrelin
229 concentrations over an extended period, we collected new data whereby circulating acylated
230 ghrelin concentrations were determined from six samples over a 2.5 h period on two separate
231 days with no intervention ($n = 15$ healthy, young males). With diet and physical activity
232 standardised in the prior 24 h, across a period of 1 h (the median exercise duration in the present
233 analysis), the co-efficient of repeatability and intra-subject variation for circulating acylated

234 ghrelin was ± 46 pg/mL and 17.2%, respectively. Over a longer period of 2.5 h the
235 corresponding values were ± 38 pg/mL/h and 14.4%.

236

237 **Statistical analyses**

238 Data was analysed using the Statistical Package for the Social Sciences (SPSS) software
239 version 22.0 (IBM SPSS, Inc., Chicago, IL). Area under the curve (AUC) was calculated for
240 plasma acylated ghrelin using the trapezoidal method. Repeated measures analysis of
241 covariance (ANCOVA) were used to assess differences in hunger (fasting and mean values),
242 energy intake and circulating acylated ghrelin (fasting and AUC) between paired control and
243 exercise trials. Study was included as a covariate for all analyses whilst additional covariates
244 were added if they correlated significantly with dependent variables. In effect, age and fat mass
245 were included as additional covariates in the fasting hunger analyses whilst fat mass was
246 included as a covariate in the post-exercise hunger analyses. Variation in fasting hunger ratings
247 and circulating acylated ghrelin concentrations were expressed as the co-efficient of intra-
248 subject variation ($CV_{\text{intra}} = \text{SDd} / (m\sqrt{2})$) and co-efficient of repeatability ($CR = 2 \times \text{SD}$) as
249 described by Horner et al (21). The Person product-moment correlation co-efficient was used
250 to examine relationships between key variables with the correlations interpreted as small (0.1),
251 medium (0.3), and large (0.5) (8). Within the correlation analyses exact participant numbers
252 are stated in parenthesis when this deviates from the number included within the main outcome
253 analysis. Effect sizes were calculated to determine the magnitude of statistical effects using
254 Cohen's *d* which adopts the following values to represent small (0.2), medium (0.5) and large
255 (0.8) effects (8). All data are presented as mean \pm standard deviation. Statistical significance
256 was identified if $P < 0.05$.

257

258 **RESULTS**

259 **Hunger responses**

260 Data describing paired fasting hunger scores at the beginning of an exercise and control trial
261 was available for 192 participants (see table; Supplementary Digital Content 1). There was no
262 significant difference in fasting hunger scores between trials (exercise 59 ± 23 mm; control 56
263 ± 24 mm; $P = 0.929$; $d = 0.13$). The intra-subject variation in fasting hunger between paired
264 exercise and control trials was 38% with a co-efficient of repeatability of ± 44 mm. Fasting
265 hunger was strongly correlated between each participant's main trials ($r = 0.557$, $P < 0.001$).
266 Mean fasting hunger scores were positively associated with fat-free mass ($n = 165$; $r = 0.213$;
267 $P = 0.006$) and age ($r = 0.143$; $P = 0.048$) and inversely related to fat mass ($n = 165$; $r = -0.213$;
268 $P = 0.006$). Mean fasting hunger was not related to weight ($r = -0.032$; $P = 0.662$), BMI ($r = -$
269 0.045 ; $P = 0.537$), $\dot{V}O_2$ peak ($n = 178$; $r = -0.057$; $P = 0.450$) or estimated resting metabolic
270 rate ($r = -0.039$; $P = 0.591$).

271

272 The tables in Supplementary Digital Content 2 and 3 identify the specific studies, along with
273 their associated characteristics, which were pooled to obtain data regarding hunger responses
274 during ($n = 178$) and after ($n = 118$) exercise. Mean hunger ratings during exercise were
275 significantly lower compared with paired hunger ratings during control trials (exercise 41 ± 26
276 mm; control 61 ± 22 mm; $P = 0.010$; $d = 0.77$). Figure 1a shows each participant's net individual
277 hunger response during exercise (difference between exercise and control) and demonstrates
278 the wide range of responses observed (-94 to $+73$ mm). Notably, 79% ($n = 140$) of participants
279 demonstrated suppressed hunger during exercise whilst 19% ($n = 34$) documented an increase
280 (2% showed no difference between control and exercise trials). Importantly, however, when
281 considering the natural variation in hunger assessment with no intervention (± 30 mm over one
282 hour) it can be seen that 37% ($n = 65$) of participants' hunger was suppressed to an extent
283 greater than the boundaries of normal variation whilst 3% ($n = 5$) demonstrated an increase.

284 The remaining 60% ($n = 108$) lay within this boundary. Further scrutiny of these data revealed
285 a weak inverse relationship between percent carbohydrate oxidation during exercise and mean
286 hunger ($n = 152$; $r = -0.177$; $P = 0.030$). There were no relationships between mean hunger
287 during exercise and fat oxidation ($n = 152$; $r = 0.079$; $P = 0.332$), exercise intensity ($n = 162$;
288 $r = -0.100$; $P = 0.204$), energy expenditure ($n = 162$; $r = -0.105$; $P = 0.182$) or $\dot{V}O_2$ peak ($n =$
289 164 ; $r = -0.088$; $P = 0.260$).

290

291

Insert figure 1 here

292

293 Hunger responses after exercise were analysed using data collected up until the end of trials,
294 or until the provision of an *ad libitum* meal (range 3-8 h post-exercise). There was no significant
295 difference in mean hunger ratings after exercise between the paired exercise (44 ± 17 mm) and
296 control trials (44 ± 18 mm) ($P = 0.142$; $d = 0.01$). Figure 1b shows the aggregate of each
297 participant's post-exercise mean hunger responses which varied widely (-52 to $+30$ mm). Fifty
298 percent ($n = 59$) of participants reported lower mean post-exercise hunger whilst 47% ($n = 56$)
299 demonstrated higher mean post-exercise hunger (3% reported no difference between trials).
300 Importantly, when normal variation is considered, 90% ($n = 106$) of participants' responses lay
301 within the boundaries of normal variation with 4% ($n = 5$) demonstrating higher mean hunger
302 after exercise and 6% ($n = 7$) reporting lower. Within these studies, we detected a small
303 significant correlation between post-exercise hunger and fat oxidation during exercise ($n = 106$;
304 $r = -0.247$; $P = 0.011$). No relationships were found between mean post-exercise hunger and
305 carbohydrate oxidation ($n = 106$; $r = -0.011$; $P = 0.911$), age ($n = 118$; $r = -0.062$; $P = 0.504$),
306 BMI ($n = 118$; $r = -0.055$; $P = 0.552$), weight ($n = 118$; $r = 0.032$; $P = 0.730$), fat-free mass (n
307 $= 107$; $r = -0.081$; $P = 0.404$), fat mass ($n = 107$; $r = 0.082$; $P = 0.402$), energy expenditure (n
308 $= 116$; $r = 0.162$; $P = 0.082$) or exercise intensity ($n = 116$; $r = 0.108$; $P = 0.250$).

309

310 **Energy intake responses**

311 Data was pooled from five of our previous research studies ($n = 60$) to explore the diversity of
312 *ad libitum* energy intake responses at one meal provided within 60 min after a single bout of
313 moderate- to high-intensity aerobic exercise. The table within Supplementary Digital Content
314 4 describes the characteristics of the individual studies included. As a group, there was no
315 significant difference in energy intake between paired exercise and control trials (exercise 5899
316 ± 1778 kJ; control 5770 ± 1966 kJ) ($P = 0.977$; $d = 0.10$) with energy intake between trials
317 showing a strong positive correlation ($P < 0.001$; $r = 0.688$). Figure 2a shows that on a crude
318 individual basis there was a range of responses observed (-5005 to $+4389$ kJ) with 55% ($n =$
319 33) of participants consuming more and 45% ($n = 27$) consuming less after exercise.
320 Importantly though, when these data are compared against the natural variation in *ad libitum*
321 energy intake at one meal with no intervention (± 1937 kJ; 18.9%) it is apparent that 85% ($n =$
322 51) of participants exhibited responses within this boundary of normal variation. Seven percent
323 of participants ($n = 4$) documented reduced post-exercise energy intake beyond this boundary
324 whilst 8% ($n = 5$) showed an increase above this boundary.

325

326

327

Insert figure 2 here

328

329 In this cohort there was no relationship between post-exercise energy intake and prior energy
330 expenditure ($r = 0.054$; $P = 0.720$), exercise intensity ($r = 0.029$; $P = 0.850$), carbohydrate ($r =$
331 0.113 ; $P = 0.454$) or fat oxidation ($r = -0.049$; $P = 0.746$) ($n = 46$). Hunger ratings immediately
332 before the first post-exercise meals were lower after exercise, likely reflecting a delayed
333 appetite suppressive effect (exercise 59 ± 28 mm; control 64 ± 23 mm; $P = 0.006$; $d = 0.36$).

334 Despite this, pre-meal hunger did not correlate with subsequent energy intake at the first post-
335 exercise meal in the control ($r = 0.158$; $P = 0.229$) or exercise trials ($r = -0.019$; $P = 0.886$) (n
336 $= 60$).

337

338 To examine the influence of acute exercise on food intake over the course of entire laboratory
339 trial days, including multiple *ad libitum* meals in some instances, data from a further six studies
340 were pooled ($n = 128$) (see table; Supplementary Digital Content 5). Three of the 11 studies
341 provided data from two *ad libitum* meals, the remainder utilised one meal (which was provided
342 > 1 h post-exercise). As a group, there was no significant difference in energy intake between
343 paired exercise and control trials (exercise 9694 ± 5468 kJ; control 9498 ± 5435 kJ; $P = 0.481$;
344 $d = 0.11$) with responses between trials showing a strong positive correlation ($P < 0.001$; $r =$
345 0.949). Figure 2b shows that on a crude individual basis there was a range of responses
346 observed; 59% ($n = 75$) of participants consumed more and 41% ($n = 53$) consumed less after
347 exercise. Importantly though, when these data are compared against the natural variation in *ad*
348 *libitum* energy intake from multiple meals with no intervention (± 2138 kJ; 8.9%), it is apparent
349 that 81% ($n = 105$) of participants exhibited responses within this boundary of normal variation
350 (Figure 2b). Nine percent ($n = 11$) of participants documented reduced post-exercise energy
351 intake beyond this boundary whilst 10% ($n = 12$) showed an increase. Across the control ($r =$
352 0.592) and exercise trials ($r = 0.623$) *ad libitum* energy intake was associated with hunger
353 ratings (both $P < 0.001$) determined after exercise (or the equivalent time period on the control
354 trial).

355

356

357 **Acylated ghrelin responses**

358 Data describing paired fasting acylated ghrelin plasma concentrations was available for 141
359 participants (see table; Supplementary Digital Content 6). Two outliers were identified and
360 removed from these analyses because the difference between paired samples was 4.5 and 10.5
361 fold greater than the standard deviation of differences between paired samples for the cohort
362 (± 31 pg/mL). One additional outlier was removed because their mean fasting plasma acylated
363 ghrelin values were 7.7 times greater than the group mean (949 pg/mL vs. 123 pg/mL). With
364 these outliers removed ($n = 138$), fasting acylated ghrelin plasma concentrations did not differ
365 between the control (125 ± 109 pg/mL) and exercise (121 ± 100 pg/mL) trials ($P = 0.638$, $d =$
366 0.12). The coefficient of repeatability and intra-subject variation between samples was ± 63
367 pg/mL and 19.2%, respectively. There were no significant correlations between mean fasting
368 acylated ghrelin and hunger ($r = -0.004$; $P = 0.959$), BMI ($r = -0.093$; $P = 0.275$), weight ($r =$
369 -0.091 ; $P = 0.288$), age ($r = -0.015$; $P = 0.860$), estimated resting metabolic rate ($r = -0.073$; P
370 $= 0.392$), fat-free mass ($n = 114$; $r = 0.092$; $P = 0.331$) or fat mass ($n = 114$; $r = -0.092$; $P =$
371 0.331).

372

373 Acylated ghrelin responses during exercise were examined using data derived from 12 studies
374 ($n = 118$, see table in Supplementary Digital Content 7). In eight studies the duration of exercise
375 was 60 min (80 participants); in three studies it was 90 min (30 participants) and in one study
376 it was 30 min (eight participants). As a group, the circulating acylated ghrelin AUC was 24%
377 lower during exercise (99 ± 94 pg/mL/hour) compared with control (131 ± 106 pg/mL/hour)
378 ($P < 0.001$; $d = 1.0$). Figure 3a shows the wide variation in acylated ghrelin responses to
379 exercise with 89% ($n = 105$) of participants exhibiting lower values on their exercise trial while
380 11% ($n = 13$) demonstrated higher values after exercise. Notably, when comparing these
381 responses to the natural variation in acylated ghrelin measurement over this period ($\pm 17.2\%$,
382 obtained from our new data) it can be seen that 27% ($n = 32$) of participants demonstrate

383 responses which fall within this normal range, with 66% ($n = 78$) and 7% ($n = 8$) showing a
384 suppression and increase beyond of this range, respectively. No significant correlations were
385 found between acylated ghrelin concentrations during exercise and exercise intensity ($r = -$
386 0.111 ; $P = 0.251$) or carbohydrate oxidation ($r = 0.122$; $P = 0.223$). Fat oxidation during
387 exercise was positively associated with acylated ghrelin concentrations ($r = 0.286$; $P = 0.004$).

388

389

Insert figure 3 here

390

391 The prolonged effects of exercise on circulating acylated ghrelin concentrations were assessed
392 by comparing paired post-exercise acylated ghrelin AUC values across nine studies ($n = 89$,
393 see the table in Supplementary Digital Content 8). Plasma acylated ghrelin concentrations were
394 measured between 3-8 h after exercise. As a group, the post-exercise acylated ghrelin AUC
395 was 16% lower after exercise (108 ± 101 pg/mL/hour) compared to control (128 ± 120
396 pg/mL/hour) ($P = 0.024$; $d = 0.61$). Individually, Figure 3b shows that 74% ($n = 66$) of
397 participants demonstrated reduced levels of acylated ghrelin whilst 26% ($n = 23$) showed an
398 increase after exercise. Notably, again, when comparing these responses with the natural
399 acylated ghrelin sampling variation seen across an extended period ($\pm 14.4\%$), 42% ($n = 37$) of
400 participants' responses were within the boundaries defined by this normal variation whilst 10%
401 ($n = 9$) and 48% ($n = 43$) of participants' responses were above and below this range,
402 respectively.

403

404

405

406 **DISCUSSION**

407 In this study we have pooled our research group's expansive data archive of acute experimental
408 research trials in an effort to provide novel insights regarding the interaction between exercise
409 and appetite regulation. Specifically, in this paper, the data from 17 of our group's previous
410 studies have been collated to interrogate interactions between exercise, hunger, *ad libitum*
411 energy intake and acylated ghrelin. Importantly, this large database of tightly controlled
412 experimental trials has enabled us to explore inter-subject variation in response to exercise
413 which is a key consideration in precision medicine and has begun to receive attention in energy
414 balance research (13,18,20,38). Our findings clarify and consolidate several previously
415 reported outcomes yet also provide new insights which have emerged from our unique
416 collection of data.

417

418 The hunger outcomes reported here are consistent with previous findings published within and
419 external to our laboratory which have shown that single bouts of moderate- to high-intensity
420 aerobic exercise transiently suppress hunger but have little impact in the hours afterwards
421 (22,23,25,26,29,30,37). Specifically, in our pool of 178 individuals, group-level analyses
422 showed that mean hunger perceptions are suppressed by approximately one-third during
423 exercise which represents a medium- to large-sized statistical effect. Interestingly, there was
424 marked variation in hunger responses which ranged from an extensive suppression to hunger
425 stimulation. Importantly though, even when we accounted for the natural day-to-day variation
426 in hunger assessment that occurs when using visual analogue scales, we saw that just over one-
427 third of the study sample reported suppressed hunger below this boundary of variation whilst
428 only a handful of individuals reported increased hunger above this level. The remainder of
429 participants' responses lay within the boundaries of normal variation and therefore it is
430 uncertain whether or not these responses represent true effects or random variation.

431

432 It is relevant to note that in our analyses we compared our hunger data to hunger variability
433 estimates derived from a sample of young, healthy males within our laboratory. We
434 purposefully chose to collect this new data so that our comparator values were derived from
435 the same population and under the same circumstances as per the experimental studies included
436 within this manuscript. Our variability estimates showed that mean hunger can vary by ± 30
437 mm over the course of one hour which was greater than with additional assessments over a
438 longer period of observation (2.5 h: ± 20 mm). Variability estimates for hunger ratings
439 calculated over extended durations have been published previously by others and which have
440 ranged ± 14 -24 mm (14,16,21,32). These values compare favourably with ours over an
441 extended period and support the validity of our comparisons. This new information shows that
442 despite a large amount of variability being apparent in short-term hunger assessments; exercise
443 is associated with a robust suppression of hunger for a large proportion of individuals.
444 Additional work is now needed to examine whether this effect of exercise is reproducible
445 across exposures within individuals and to identify the key moderating factors.

446

447 Our analyses of hunger responses in the hours after exercise demonstrated that single bouts of
448 moderate- to high-intensity aerobic exercise have no impact on hunger during the remainder of
449 the day thereafter for the majority of individuals. Again, this outcome is consistent with
450 previous findings and confirms that acute exercise-induced energy deficits do not create an
451 automatic drive to increase hunger (5). Notably, our data showed an even spread of net mean
452 hunger responses post-exercise; however, the vast majority of responses (90%) lay within
453 reported boundaries of normal variation. Consequently, our data shows that there is little
454 definitive variation in post-exercise hunger responses, with only 10% of individuals
455 demonstrating changes in post-exercise hunger outside of the normal variation boundaries. In

456 future studies it would be interesting to see whether these responses are consistent across
457 additional trials for this sub-set of individuals as opposed to representing random events.

458

459 Given the large number of fasting hunger ratings ($n = 192$) obtained at the beginning of the
460 paired control and exercise trials, we examined the variation between repeated assessments.

461 We identified a rather large variation in fasting hunger (38%, ± 44 mm) which is consistent
462 with results from previous studies. Specifically, in a sample of 12 active males, Gonzalez et al
463 (16) reported a 21% co-efficient of variation whilst in a similar population others have
464 calculated higher estimates (24-30%) (32). Furthermore, Horner et al (21) reported a higher
465 estimate in a sample of overweight and obese males (35%). Collectively, these data identify
466 the expected variation in fasting hunger ratings across repeated assessments in young, healthy
467 males and these data have implications for sample size calculations within experimental
468 research trials. Such high co-efficients of variation also support the measurement of hunger
469 perceptions at multiple time-points in response to an intervention rather than single fasted
470 values.

471

472 In our fasting hunger data we identified significant, albeit weak, correlations with fat-free mass
473 (positive) and fat mass (inverse). These findings support recent suggestions that fat-free mass
474 is a central driver of daily food intake (4) whilst adipose tissue may exert an inhibitory effect
475 on appetite and food intake in lean individuals (3). Homogeneity in our participants' body
476 composition may explain the lower strength of these associations in our cohort compared with
477 other published data (3). Alternatively, this discrepancy may be attributable to the correlational
478 rather than causal relationships between these variables.

479 In our analyses we also examined the impact of acute exercise on *ad libitum* energy intake at
480 buffet meals consumed within 60 min after exercise as well as at meals consumed over several

481 hours post-exercise. Consistent with previous data collected outside of our laboratory (25, 26,
482 28, 33), our pooled analysis showed that at group-level, energy intake was unaffected at meals
483 consumed within the first post-exercise hour. This outcome was apparent, despite hunger
484 ratings being significantly lower (8%) immediately before *ad libitum* meals following exercise.
485 Indeed, we actually found that 85% of participants' net energy intake responses (aggregate of
486 control and exercise values) lay within the boundaries of normal day-to-day variation, as
487 determined by our own repeatability experiment which was conducted with a similar
488 population and buffet meal. This is an important finding because it demonstrates that there is
489 actually very little true variation in *ad libitum* energy intake beyond the summated boundaries
490 of biological variation and technical measurement error. Previously, researchers have
491 attempted to categorise individual participants as 'compensators' or 'non-compensators' with
492 regards to the effect of exercise on energy intake based upon aggregated energy intake
493 responses after paired acute exercise and control trials (13,20). In these previous studies, it can
494 be seen however, that the net impact of exercise on energy intake is actually less than the natural
495 variation in energy intake from an *ad libitum* meal which has been defined as $\pm 1406-1477$ kJ
496 (9-12%) with *ad libitum* homogenous meals (17,21) and ± 1937 kJ (18.9%) with *ad libitum*
497 buffet meals (latter reported in this paper). Moreover, a recent study has elegantly demonstrated
498 that energy intake responses after exercise show a marked degree of inconsistency; collectively
499 meaning that individuals cannot reliably be classified as 'compensators' or 'non-compensators'
500 based upon their energy intake responses to acute exercise (38). Consequently, it is likely that
501 in our analyses, the 15% of participants who reported exercise-induced alterations in energy
502 intake beyond normal variation boundaries may not exhibit this same response if trials were
503 repeated.

504 In our energy intake analysis it is worth noting that the identified variability estimates for our
505 *ad libitum* buffet meals were considerably higher (± 1937 kJ, 18.9%) than previously reported

506 when homogenous meals are provided (17,21). This is most likely because a small change in
507 food selection with a buffet meal on one occasion can produce large differences in energy
508 intake across paired eating assessments. The implication of this is that for studies simply
509 concerned with intervention effects on *ad libitum* energy intake, rather than food selection, a
510 homogenous meal will reduce the variance in energy intake measurement and increase
511 statistical power.

512

513 Our analyses are the first to examine the variation in energy intake responses to multiple meals
514 over several hours after exercise. Again, our findings show that exercise had no impact on
515 energy intake across this extended period. Furthermore, the vast majority of variation in
516 responses once more lay within the boundaries of normal variation that we have determined
517 ourselves across two *ad libitum* buffet meals. Our results therefore confirm previous findings
518 demonstrating little impact of exercise on energy intake over extended periods (28) and
519 highlight the lack of true variability in responses.

520

521 In this manuscript we report the test-retest variability in circulating fasting acylated ghrelin
522 concentrations which has been calculated from a large sample of healthy males. We saw no
523 significant difference in fasting acylated ghrelin concentrations between paired trials. This
524 outcome supports the findings of Chandarana et al. (7) who also observed no differences in
525 fasting or postprandial plasma acylated ghrelin concentrations, with or without dietary
526 standardisation. Despite this, in our analyses, we identified a rather large variance in fasting
527 plasma concentrations (~19%) even with prior (24 h) dietary and physical activity
528 standardisation. This variance is composed of the technical error associated with the assay
529 measurement (typically 6-8% in our laboratory) and biological variation in ghrelin secretion
530 and clearance. For the participants in these analyses, dietary standardisation relied on

531 individuals accurately maintaining and subsequently following food diaries and it is possible
532 that biological error could be reduced if diet is standardised for a longer period, or if
533 participants are provided with all of their foods during the standardisation phase. Future
534 research should examine these methodological factors as it has direct relevance for appetite
535 and gut hormone assessment in experimental appetite-regulation research.

536

537 A recent meta-analysis of 18 datasets showed that acute exercise transiently suppresses
538 circulating concentrations of acylated ghrelin with a small (Cohen's d -0.2) effect size (34).
539 Half of the datasets from this analysis were from our laboratory and therefore it is unsurprising
540 that in the present analysis we identified a statistically large exercise-induced suppression of
541 circulating acylated ghrelin during exercise. The larger effect reported in our laboratory
542 compared with others is likely related to the characteristics of studies, particularly the exercise
543 intensity imposed, and also to variation in assays utilised. Importantly, our data shows that
544 circulating levels of acylated ghrelin are suppressed in response to acute exercise in the vast
545 majority of individuals examined. Of primary significance, in two-thirds of these cases the
546 reduction was beyond the boundaries of normal variation which we explicitly defined for the
547 purpose of this report. This finding highlights the consistency in the response to exercise yet
548 poses the question of why such robust changes were not seen in the remainder of the study
549 sample. Furthermore, the significance of this response is not fully understood and may be
550 unrelated to appetite given that acute changes in response to exercise have not been found to
551 be correlated consistently. In addition to this, although there have been many speculations (19),
552 the mechanism(s) responsible for the exercise related perturbation of acylated ghrelin remain
553 unclear.

554 In the present analysis we identified a statistically significant reduction in circulating acylated
555 ghrelin over the course of several hours post-exercise. This finding is interesting given that on

556 an individual study basis a prolonged reduction in circulating acylated ghrelin in the hours after
557 exercise has not been identified consistently. The substantially larger study sample used in this
558 pooled analysis was therefore necessary to identify this small statistical effect. Interestingly,
559 our data shows that this persistent effect of exercise can be seen robustly in almost half of
560 participants who exhibited suppressed ghrelin levels after exercise that were beyond the
561 calculated range associated with normal variation. Research is now needed to identify the
562 mechanisms producing this effect and to understand its physiological/metabolic significance.

563

564 The analyses in this paper have provided a novel insight regarding the interaction between
565 exercise, hunger, *ad libitum* energy intake and circulating acylated ghrelin. These analyses have
566 been made possible by the integration of over 10 years of experimental appetite research in our
567 laboratory using study protocols with a high degree of similarity. Our findings do however
568 have some limitations which should be recognised. The first important consideration is the
569 generalisability of our data. Because all of our participants were young, healthy men, we do
570 not know whether our findings would generalise to other populations such as women, children,
571 those who are inactive or obese. A second limitation of our data is that our homogenous sample
572 may have inhibited the ability to identify associations between key variables reported in this
573 paper. Thirdly, it is feasible that the energy intake response to exercise may differ between a
574 laboratory controlled environment and an ecologically valid social setting. However, the aim
575 of this study was to understand the physiological effects of exercise on appetite and energy
576 intake responses in a tightly controlled laboratory environment to control against other
577 confounding factors. Finally, it should be recognised that the studies included in the present
578 investigation involved acute exercise protocols that commenced either in the fasted state ($n =$
579 13) or after a breakfast snack ($n = 4$). Although our group have shown previously that appetite
580 and energy intake responses to acute exercise do not differ depending on feeding status (11),

581 there is the possibility that this factor could have interacted differently across the various
582 studies in our pooled analyses.

583

584 In conclusion, our large pooled dataset confirms that single bouts of moderate- to high-intensity
585 aerobic exercise transiently, yet robustly, suppress hunger but have no impact on *ad libitum*
586 energy intake across meals consumed on the day of exercise in healthy young men.
587 Additionally, our data shows that exercise robustly suppresses circulating concentrations of
588 acylated ghrelin which in this novel analyses was shown to remain suppressed for several hours
589 after exercise. Importantly, our findings underscore the necessity to consider normal day-to-
590 day variation in these outcomes when examining variability in responses between individuals.
591 Most notably, our research shows that in response to acute exercise, there is very little true
592 variation in post-exercise hunger and energy intake.

593

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598 necessarily those of the NHS, the NIHR or the Department of Health.

599

600 **CONFLICT OF INTEREST**

601 All authors declare that there are no conflicts of interest. The results of the present study do not
602 constitute endorsement by ACSM.

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749 **FIGURE LEGENDS**

750 **Figure 1:** mean hunger ratings (exercise minus control) obtained during (a, $n = 178$) and after
751 exercise (b, $n = 118$). Values above zero indicate increased hunger during or after exercise;
752 values below zero indicate reduced hunger. Horizontal lines represent zones of natural variation
753 across 1 h (1a: ± 30 mm) and 2.5 h (1b: ± 20 mm).

754

755 **Figure 2:** Energy intake (exercise minus control) at (a, $n = 60$) one meal consumed within 60
756 min post-exercise and (b, $n = 128$) at multiple meals after exercise. Each individual data point
757 represents the response for a single study participant. Values above zero indicate increased
758 energy intake after exercise; values below zero indicate reduced energy intake after exercise.
759 Horizontal lines represent zones of natural variation (2a ± 1937 kJ; 2b ± 2138 kJ).

760

761 **Figure 3:** circulating acylated ghrelin concentrations (exercise minus control) during (a, $n =$
762 118) and over several hours after (b, $n = 89$) exercise. Each individual data point represents the
763 response for a single study participant. Values above zero indicate increased acylated ghrelin
764 after exercise; values below zero indicate reduced acylated ghrelin after exercise. Horizontal
765 lines represent zones of natural variation (3a ± 17.2 %; 3b ± 14.4 %).

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774 **SUPPLEMENTAL DIGITAL CONTENT**

775 **SDC 1** (.doc file): studies included in the fasting hunger analyses ($n = 192$)

776

777 **SDC 2** (.doc file): Studies included in the analysis examining hunger responses during
778 exercise ($n = 178$)

779

780 **SDC 3** (.doc file): Studies included in the analysis examining hunger responses after exercise
781 ($n = 118$)

782

783 **SDC 4** (.doc file): Studies included in energy intake analysis at the first post-exercise meal (n
784 $= 60$)

785

786 **SDC 5** (.doc file): Studies included in energy intake analysis for all meals after exercise ($n =$
787 128)

788 **SDC 6** (.doc file): Studies included in fasting acylated ghrelin analysis ($n = 138$)

789

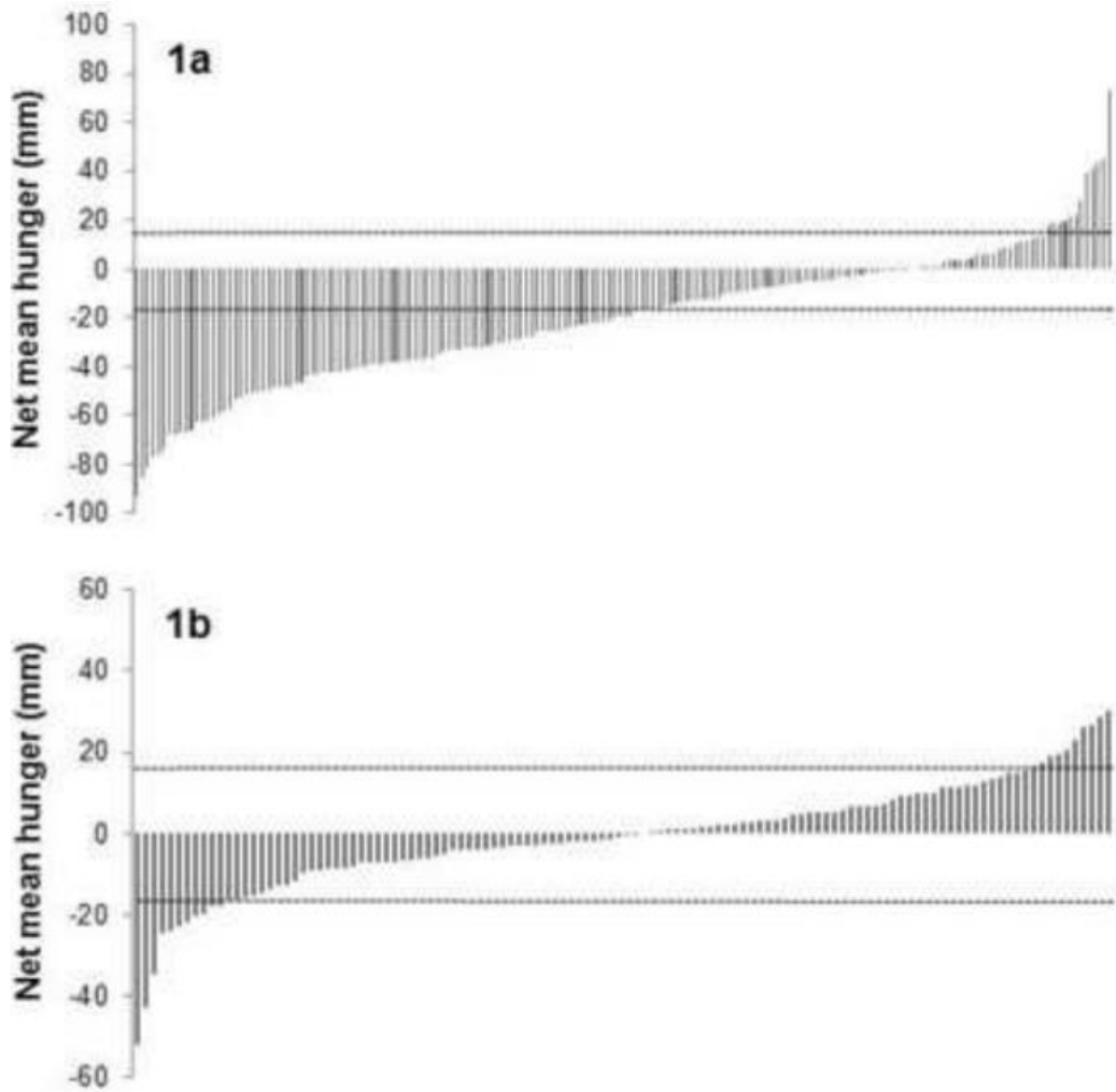
790 **SDC 7** (.doc file): Studies included in the analysis examining acylated ghrelin responses
791 during exercise ($n = 118$)

792

793 **SDC 8** (.doc file): Studies included in the analysis examining acylated ghrelin responses after
794 exercise ($n = 89$)

795

796 **Figure 1**

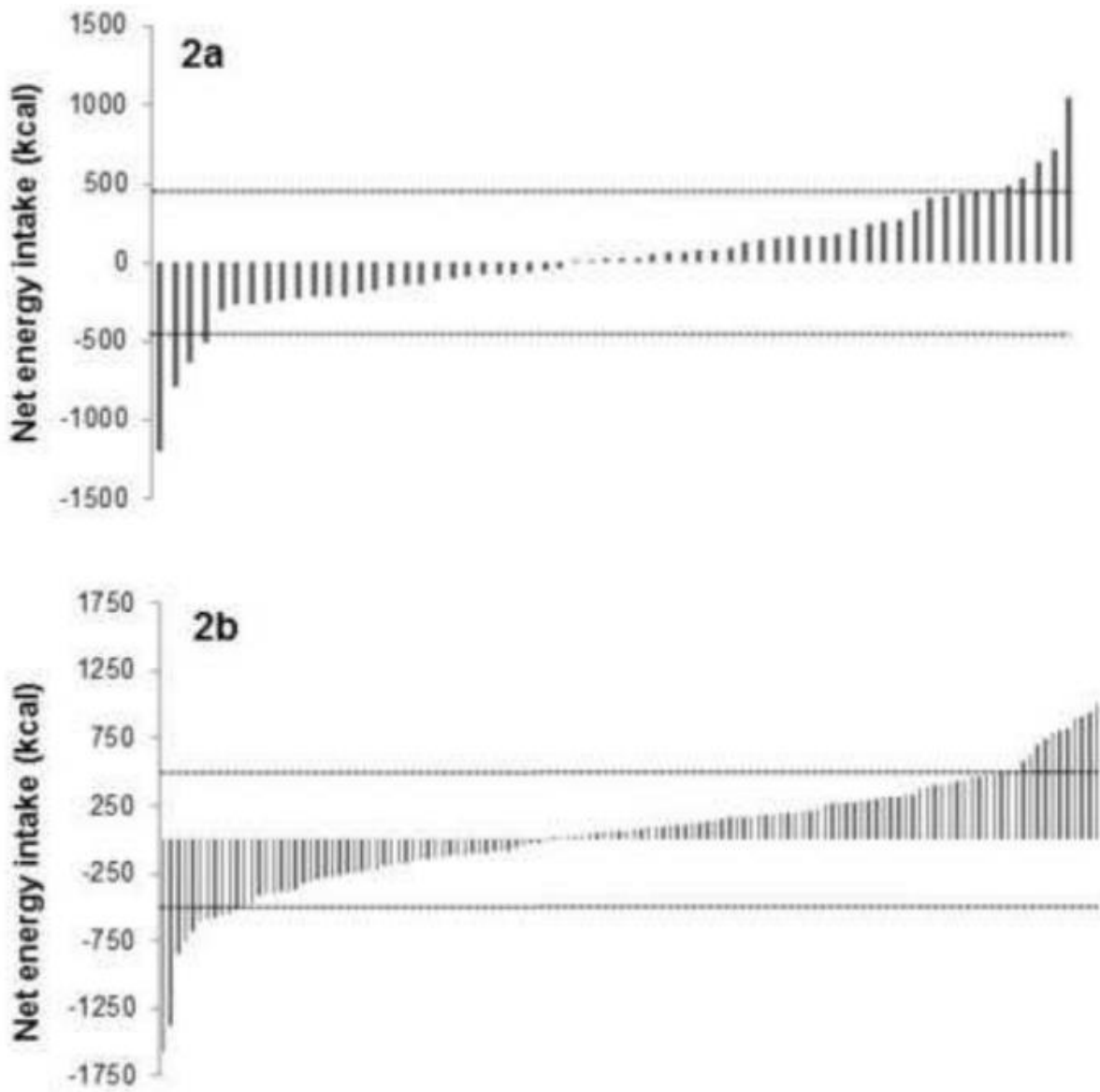


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799 **Figure 2**

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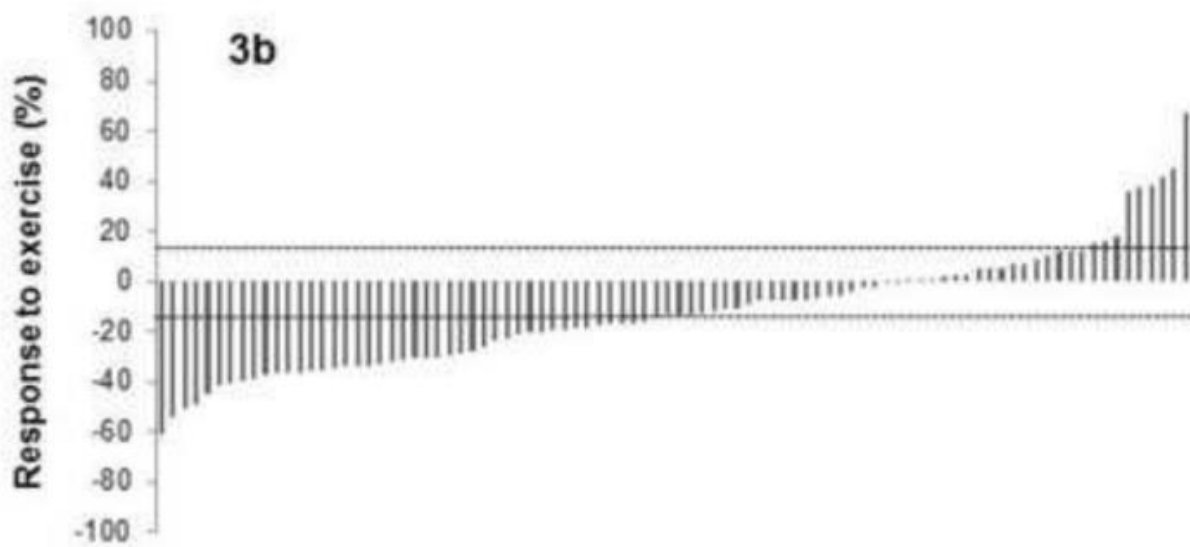
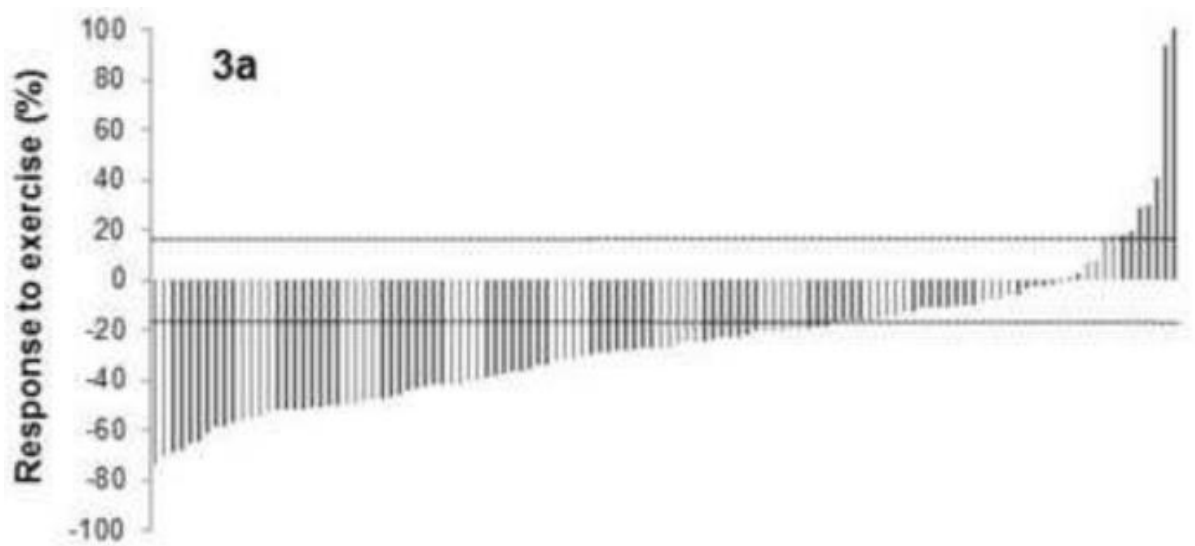
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804 **Figure 3**

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