

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

KING, J.A., DEIGHTON, K, BROOM, David <<http://orcid.org/0000-0002-0305-937X>>, WASSE, L.K., DOUGLAS, J.A., BURNS, S.F., CORDERY, P.A., PETHERICK, E.S., BATTERHAM, R.L., GOLTZ, F.R., THACKRAY, A.E., YATES, T and STENSEL, D.J.

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Published version

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

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30 **Abstract length : 275 words**

31 **Manuscript length: 6558 words**

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35 **ABSTRACT**

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

57

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

59

60 INTRODUCTION

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

76

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

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

87

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

101

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

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

114

115 **METHODS**

116 **Research studies and participants**

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

131

132 **Exercise protocol characteristics**

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

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

143

144 **Anthropometry and standardisation**

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

155

156 **Hunger analyses**

157 The primary analyses of interest in this study relating to hunger were: 1) individual variation
158 in fasting hunger (n = 192); 2) the immediate (during exercise, n = 178) and prolonged (up to
159 8 h post-exercise, n = 118) effects of exercise on perceived hunger. In each of the studies

160 included within these analyses participants reported their perceived hunger at intervals of 30
161 min using pen and paper based 100 mm visual analogue scales (14). The impact of exercise
162 on hunger was assessed by comparing mean hunger ratings calculated during and after
163 exercise with paired values calculated on each participant's control trial. In the post-exercise
164 hunger analysis mean hunger scores were calculated from data available until the end of trials
165 or until the occurrence of a buffet meal (when standardised appetite scores were no longer
166 comparable). The reproducibility of fasting perceived hunger was determined from baseline
167 hunger ratings at the start of paired exercise and control trials. Individual variation in hunger
168 responses during and after exercise were calculated by subtracting mean hunger ratings
169 calculated during control trials from mean hunger ratings observed during the same periods
170 within exercise trials. For all post-exercise analyses, hunger ratings obtained within the first
171 30 min after exercise was excluded to eliminate any latent impact of the exercise bout.

172

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

178

179 **Energy intake analyses**

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

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

196

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

208

209

210 **Acylated ghrelin analyses**

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

235 and intra-subject variation for circulating acylated ghrelin was ± 46 pg/mL and 17.2%,
236 respectively. Over a longer period of 2.5 h the corresponding values were ± 38 pg/mL/h and
237 14.4%.

238

239 **Statistical analyses**

240 Data was analysed using the Statistical Package for the Social Sciences (SPSS) software
241 version 22.0 (IBM SPSS, Inc., Chicago, IL). Area under the curve (AUC) was calculated for
242 plasma acylated ghrelin using the trapezoidal method. Repeated measures analysis of
243 covariance (ANCOVA) were used to assess differences in hunger (fasting and mean values),
244 energy intake and circulating acylated ghrelin (fasting and AUC) between paired control and
245 exercise trials. Study was included as a covariate for all analyses whilst additional covariates
246 were added if they correlated significantly with dependent variables. In effect, age and fat
247 mass were included as additional covariates in the fasting hunger analyses whilst fat mass
248 was included as a covariate in the post-exercise hunger analyses. Variation in fasting hunger
249 ratings and circulating acylated ghrelin concentrations were expressed as the co-efficient of
250 intra-subject variation ($CV_{\text{intra}} = \text{SDd} / (m\sqrt{2})$) and co-efficient of repeatability ($CR = 2 \times \text{SD}$)
251 as described by Horner et al (21). The Person product-moment correlation co-efficient was
252 used to examine relationships between key variables with the correlations interpreted as small
253 (0.1), medium (0.3), and large (0.5) (8). Within the correlation analyses exact participant
254 numbers are stated in parenthesis when this deviates from the number included within the
255 main outcome analysis. Effect sizes were calculated to determine the magnitude of statistical
256 effects using Cohen's d which adopts the following values to represent small (0.2), medium
257 (0.5) and large (0.8) effects (8). All data are presented as mean \pm standard deviation.
258 Statistical significance was identified if $P < 0.05$.

259

260 **RESULTS**

261 **Hunger responses**

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

273

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

285 was suppressed to an extent greater than the boundaries of normal variation whilst 3% (n = 5)
286 demonstrated an increase. The remaining 60% (n = 108) lay within this boundary. Further
287 scrutiny of these data revealed a weak inverse relationship between percent carbohydrate
288 oxidation during exercise and mean hunger (n = 152; r = -0.177; P = 0.030). There were no
289 relationships between mean hunger during exercise and fat oxidation (n = 152; r = 0.079; P =
290 0.332), exercise intensity (n = 162; r = -0.100; P = 0.204), energy expenditure (n = 162; r = -
291 0.105; P = 0.182) or $\dot{V}O_2$ peak (n = 164; r = -0.088; P = 0.260).

292

293

Insert figure 1 here

294

295 Hunger responses after exercise were analysed using data collected up until the end of trials,
296 or until the provision of an ad libitum meal (range 3-8 h post-exercise). There was no
297 significant difference in mean hunger ratings after exercise between the paired exercise
298 (44±17 mm) and control trials (44±18 mm) (P=0.142; d = 0.01). Figure 1b shows the
299 aggregate of each participant's post-exercise mean hunger responses which varied widely
300 (-52 to +30 mm). Fifty percent (n = 59) of participants reported lower mean post-exercise
301 hunger whilst 47% (n = 56) demonstrated higher mean post-exercise hunger (3% reported no
302 difference between trials). Importantly, when normal variation is considered, 90% (n = 106)
303 of participants' responses lay within the boundaries of normal variation with 4% (n = 5)
304 demonstrating higher mean hunger after exercise and 6% (n = 7) reporting lower. Within
305 these studies, we detected a small significant correlation between post-exercise hunger and
306 fat oxidation during exercise (n = 106; r = -0.247; P = 0.011). No relationships were found
307 between mean post-exercise hunger and carbohydrate oxidation (n = 106; r = -0.011; P =
308 0.911), age (n = 118; r = -0.062; P = 0.504), BMI (n = 118; r = -0.055; P = 0.552), weight (n
309 = 118; r = 0.032; P = 0.730), fat-free mass (n = 107; r = -0.081; P = 0.404), fat mass (n =

310 107; $r = 0.082$; $P = 0.402$), energy expenditure ($n = 116$; $r = 0.162$; $P = 0.082$) or exercise
311 intensity ($n = 116$; $r = 0.108$; $P = 0.250$).

312

313 **Energy intake responses**

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

328

329

330 **Insert figure 2 here**

331

332 In this cohort there was no relationship between post-exercise energy intake and prior energy
333 expenditure ($r = 0.054$; $P = 0.720$), exercise intensity ($r = 0.029$; $P = 0.850$), carbohydrate (r
334 $= 0.113$; $P = 0.454$) or fat oxidation ($r = -0.049$; $P = 0.746$) ($n = 46$). Hunger ratings

335 immediately before the first post-exercise meals were lower after exercise, likely reflecting a
336 delayed appetite suppressive effect (exercise 59 ± 28 mm; control 64 ± 23 mm; $P = 0.006$; d
337 $= 0.36$). Despite this, pre-meal hunger did not correlate with subsequent energy intake at the
338 first post-exercise meal in the control ($r = 0.158$; $P = 0.229$) or exercise trials ($r = -0.019$; $P =$
339 0.886) ($n = 60$).

340

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

358

359

360 **Acylated ghrelin responses**

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

375

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

385 period ($\pm 17.2\%$, obtained from our new data) it can be seen that 27% ($n = 32$) of participants
386 demonstrate responses which fall within this normal range, with 66% ($n = 78$) and 7% ($n = 8$)
387 showing a suppression and increase beyond of this range, respectively. No significant
388 correlations were found between acylated ghrelin concentrations during exercise and exercise
389 intensity ($r = -0.111$; $P = 0.251$) or carbohydrate oxidation ($r = 0.122$; $P = 0.223$). Fat
390 oxidation during exercise was positively associated with acylated ghrelin concentrations ($r =$
391 0.286 ; $P = 0.004$).

392

393

Insert figure 3 here

394

395 The prolonged effects of exercise on circulating acylated ghrelin concentrations were
396 assessed by comparing paired post-exercise acylated ghrelin AUC values across nine studies
397 ($n = 89$, see the table in Supplementary Digital Content 8). Plasma acylated ghrelin
398 concentrations were measured between 3-8 h after exercise. As a group, the post-exercise
399 acylated ghrelin AUC was 16% lower after exercise (108 ± 101 pg/mL/hour) compared to
400 control (128 ± 120 pg/mL/hour) ($P = 0.024$; $d = 0.61$). Individually, Figure 3b shows that
401 74% ($n = 66$) of participants demonstrated reduced levels of acylated ghrelin whilst 26% ($n =$
402 23) showed an increase after exercise. Notably, again, when comparing these responses with
403 the natural acylated ghrelin sampling variation seen across an extended period ($\pm 14.4\%$),
404 42% ($n = 37$) of participants' responses were within the boundaries defined by this normal
405 variation whilst 10% ($n = 9$) and 48% ($n = 43$) of participants' responses were above and
406 below this range, respectively.

407

408

409

410 **DISCUSSION**

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

421

422 The hunger outcomes reported here are consistent with previous findings published within
423 and external to our laboratory which have shown that single bouts of moderate- to high-
424 intensity aerobic exercise transiently suppress hunger but have little impact in the hours
425 afterwards (22,23,25,26,29,30,37). Specifically, in our pool of 178 individuals, group-level
426 analyses showed that mean hunger perceptions are suppressed by approximately one-third
427 during exercise which represents a medium- to large-sized statistical effect. Interestingly,
428 there was marked variation in hunger responses which ranged from an extensive suppression
429 to hunger stimulation. Importantly though, even when we accounted for the natural day-to-
430 day variation in hunger assessment that occurs when using visual analogue scales, we saw
431 that just over one-third of the study sample reported suppressed hunger below this boundary
432 of variation whilst only a handful of individuals reported increased hunger above this level.
433 The remainder of participants' responses lay within the boundaries of normal variation and

434 therefore it is uncertain whether or not these responses represent true effects or random
435 variation.

436

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

451

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

459 definitive variation in post-exercise hunger responses, with only 10% of individuals
460 demonstrating changes in post-exercise hunger outside of the normal variation boundaries. In
461 future studies it would be interesting to see whether these responses are consistent across
462 additional trials for this sub-set of individuals as opposed to representing random events.

463

464 Given the large number of fasting hunger ratings ($n = 192$) obtained at the beginning of the
465 paired control and exercise trials, we examined the variation between repeated assessments.
466 We identified a rather large variation in fasting hunger ($38\%, \pm 44$ mm) which is consistent
467 with results from previous studies. Specifically, in a sample of 12 active males, Gonzalez et
468 al (16) reported a 21% co-efficient of variation whilst in a similar population others have
469 calculated higher estimates (24-30%) (32). Furthermore, Horner et al (21) reported a higher
470 estimate in a sample of overweight and obese males (35%). Collectively, these data identify
471 the expected variation in fasting hunger ratings across repeated assessments in young, healthy
472 males and these data have implications for sample size calculations within experimental
473 research trials. Such high co-efficients of variation also support the measurement of hunger
474 perceptions at multiple time-points in response to an intervention rather than single fasted
475 values.

476

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

484 In our analyses we also examined the impact of acute exercise on ad libitum energy intake at
485 buffet meals consumed within 60 min after exercise as well as at meals consumed over
486 several hours post-exercise. Consistent with previous data collected outside of our laboratory
487 (25, 26, 28, 33), our pooled analysis showed that at group-level, energy intake was unaffected
488 at meals consumed within the first post-exercise hour. This outcome was apparent, despite
489 hunger ratings being significantly lower (8%) immediately before ad libitum meals following
490 exercise. Indeed, we actually found that 85% of participants' net energy intake responses
491 (aggregate of control and exercise values) lay within the boundaries of normal day-to-day
492 variation, as determined by our own repeatability experiment which was conducted with a
493 similar population and buffet meal. This is an important finding because it demonstrates that
494 there is actually very little true variation in ad libitum energy intake beyond the summated
495 boundaries of biological variation and technical measurement error. Previously, researchers
496 have attempted to categorise individual participants as 'compensators' or 'non-compensators'
497 with regards to the effect of exercise on energy intake based upon aggregated energy intake
498 responses after paired acute exercise and control trials (13,20). In these previous studies, it
499 can be seen however, that the net impact of exercise on energy intake is actually less than the
500 natural variation in energy intake from an ad libitum meal which has been defined as ± 1406 -
501 1477 kJ (9-12%) with ad libitum homogenous meals (17,21) and ± 1937 kJ (18.9%) with ad
502 libitum buffet meals (latter reported in this paper). Moreover, a recent study has elegantly
503 demonstrated that energy intake responses after exercise show a marked degree of
504 inconsistency; collectively meaning that individuals cannot reliably be classified as
505 'compensators' or 'non-compensators' based upon their energy intake responses to acute
506 exercise (38). Consequently, it is likely that in our analyses, the 15% of participants who
507 reported exercise-induced alterations in energy intake beyond normal variation boundaries
508 | may not exhibit this same response if trials were repeated.

509 In our energy intake analysis it is worth noting that the identified variability estimates for our
510 ad libitum buffet meals were considerably higher (± 1937 kJ, 18.9%) than previously
511 reported when homogenous meals are provided (17,21). This is most likely because a small
512 change in food selection with a buffet meal on one occasion can produce large differences in
513 energy intake across paired eating assessments. The implication of this is that for studies
514 simply concerned with intervention effects on ad libitum energy intake, rather than food
515 selection, a homogenous meal will reduce the variance in energy intake measurement and
516 increase statistical power.

517

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

525

526 In this manuscript we report the test-retest variability in circulating fasting acylated ghrelin
527 concentrations which has been calculated from a large sample of healthy males. We saw no
528 significant difference in fasting acylated ghrelin concentrations between paired trials. This
529 outcome supports the findings of Chandarana et al. (7) who also observed no differences in
530 fasting or postprandial plasma acylated ghrelin concentrations, with or without dietary
531 standardisation. Despite this, in our analyses, we identified a rather large variance in fasting
532 plasma concentrations (~19%) even with prior (24 h) dietary and physical activity
533 standardisation. This variance is composed of the technical error associated with the assay

534 measurement (typically 6-8% in our laboratory) and biological variation in ghrelin secretion
535 and clearance. For the participants in these analyses, dietary standardisation relied on
536 individuals accurately maintaining and subsequently following food diaries and it is possible
537 that biological error could be reduced if diet is standardised for a longer period, or if
538 participants are provided with all of their foods during the standardisation phase. Future
539 research should examine these methodological factors as it has direct relevance for appetite
540 and gut hormone assessment in experimental appetite-regulation research.

541

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

559 In the present analysis we identified a statistically significant reduction in circulating acylated
560 ghrelin over the course of several hours post-exercise. This finding is interesting given that
561 on an individual study basis a prolonged reduction in circulating acylated ghrelin in the hours
562 after exercise has not been identified consistently. The substantially larger study sample used
563 in this pooled analysis was therefore necessary to identify this small statistical effect.
564 Interestingly, our data shows that this persistent effect of exercise can be seen robustly in
565 almost half of participants who exhibited suppressed ghrelin levels after exercise that were
566 beyond the calculated range associated with normal variation. Research is now needed to
567 identify the mechanisms producing this effect and to understand its physiological/metabolic
568 significance.

569

570 The analyses in this paper have provided a novel insight regarding the interaction between
571 exercise, hunger, ad libitum energy intake and circulating acylated ghrelin. These analyses
572 have been made possible by the integration of over 10 years of experimental appetite research
573 in our laboratory using study protocols with a high degree of similarity. Our findings do
574 however have some limitations which should be recognised. The first important consideration
575 is the generalisability of our data. Because all of our participants were young, healthy men,
576 we do not know whether our findings would generalise to other populations such as women,
577 children, those who are inactive or obese. A second limitation of our data is that our
578 homogenous sample may have inhibited the ability to identify associations between key
579 variables reported in this paper. Thirdly, it is feasible that the energy intake response to
580 exercise may differ between a laboratory controlled environment and an ecologically valid
581 social setting. However, the aim of this study was to understand the physiological effects of
582 exercise on appetite and energy intake responses in a tightly controlled laboratory
583 environment to control against other confounding factors. Finally, it should be recognised

584 that the studies included in the present investigation involved acute exercise protocols that
585 commenced either in the fasted state (n = 13) or after a breakfast snack (n = 4). Although our
586 group have shown previously that appetite and energy intake responses to acute exercise do
587 not differ depending on feeding status (11), there is the possibility that this factor could have
588 interacted differently across the various studies in our pooled analyses.

589

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

599

600 **ACKNOWLEDGEMENTS**

601 This research was supported by the National Institute for Health Research (NIHR) Diet,
602 Lifestyle and Physical Activity Biomedical Research Unit based at University Hospitals of
603 Leicester and Loughborough University. The views expressed are those of the authors and
604 not necessarily those of the NHS, the NIHR or the Department of Health.

605

606 **CONFLICT OF INTEREST**

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

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754 **FIGURE LEGENDS**

755 **Figure 1:** mean hunger ratings (exercise minus control) obtained during (a, n = 178) and after
756 exercise (b, n = 118). Values above zero indicate increased hunger during or after exercise;

757 values below zero indicate reduced hunger. Horizontal lines represent zones of natural
758 variation across 1 h (1a: ± 30 mm) and 2.5 h (1b: ± 20 mm).

759

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

765

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

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779 **SUPPLEMENTAL DIGITAL CONTENT**

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

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

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

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

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

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

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

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