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1 **Exercise and Ghrelin – A Narrative Overview of Research**

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17

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25

26 **Abstract**

27 Since its discovery in 1999, ghrelin has been implicated in a multiplicity of
28 physiological activities. Most notably, ghrelin has an important influence on energy
29 metabolism and after the identification of its potent appetite stimulating effects ghrelin
30 has been termed the '*hunger hormone*.' Exercise is a stimulus which has a significant
31 impact on energy homeostasis and consequently a substantial body of research has
32 investigated the interaction between exercise and ghrelin. This narrative review
33 provides an overview of research relating to the acute and chronic effects of exercise on
34 circulating ghrelin (acylated, unacylated and total). To enhance study comparability, the
35 scope of this review is limited to research undertaken in adult humans and consequently
36 studies involving children and animals are not discussed. Although there is significant
37 ambiguity within much of the early research, our review suggests that acute exercise
38 transiently interferes with the production of acylated ghrelin. Furthermore, the
39 consensus of evidence indicates that exercise training does not influence circulating
40 ghrelin independent of weight loss. Additional research is needed to verify and extend
41 the available literature, particularly by uncovering the mechanisms governing acute
42 exercise-related changes and characterising responses in other populations such as
43 females, older adults, and the obese.

44

45 **Key Words**

46 Ghrelin, Acylated Ghrelin, Exercise, Training, Appetite, Food Intake, Energy Balance

47

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50

51 **Introduction**

52 After an arduous search, in 1999 Kojima and colleagues reported the purification and
53 identification of an endogenous ligand able to bind to the orphan growth hormone
54 secretagogue receptor (GHSR-1a) and stimulate growth hormone (GH) secretion via a
55 novel independent pathway (Kojima, 2008; Kojima et al., 1999). The researchers
56 termed this peptide ‘ghrelin’ as a tribute to its potent *GH-RE*leasing action (Kojima,
57 Hosoda, Matsuo, & Kangawa, 2001). Unbeknown at the time, the importance of ghrelin
58 in metabolism would turn out to be much more wide ranging than initially recognised.

59

60 Ghrelin is a 28 amino acid peptide produced primarily from P/D₁ cells in the stomach
61 fundus, with much lesser amounts being synthesised in the intestine, pancreas and other
62 peripheral organs including the testis, heart, adipose tissue and skin (Gutierrez et al.,
63 2008; Stengel & Tache, 2012). Upon fasting, and/or low circulating levels of glucose
64 and insulin, ghrelin is secreted into the circulation where it is present in two forms,
65 acylated and unacylated (~1:4 ratio) (Stengel, Goebel, Wang, & Taché, 2010). Acylated
66 ghrelin is made explicit by the post-translational addition of a medium chain fatty acid,
67 typically octanoate or decanoate, to its third amino acid residue (serine), a modification
68 catalysed by ghrelin O acyltransferase (GOAT) (Gutierrez et al, 2008; Yang, Brown,
69 Liang, & Grishin, 2008) and which is essential for ghrelin to bind to the GHSR-1a to
70 exert its primary hormonal and metabolic actions (Kojima & Kangawa, 2005) (Figure 1).

71

72 *Insert figure 1 near here*

73

74 The biological activities of ghrelin are multifaceted which is consistent with the
75 widespread distribution of its receptor in the brain e.g. hypothalamus, (Schellekens,

76 Dinan & Cryan, 2010) and peripheral tissues e.g. vagal afferents, adipose tissue, spleen,
77 myocardium, thyroid, adrenal gland (Stengel & Taché, 2012). In addition to its well
78 defined role as a regulator of GH secretion, ghrelin is also understood to harbour
79 complex roles in glucose metabolism (Delhanty & van der Lely, 2011), gastrointestinal
80 (Tack et al., 2006; Levin et al., 2006), reproductive (Muccioli et al., 2011), immune
81 (Taub, 2008) and cardiovascular (Nagaya et al., 2001; Vlasova, Järvinen, & Herzig,
82 2009) function. Unquestionably however, the most notable discovery has been the
83 identification of ghrelin's central role in appetite regulation and energy homeostasis
84 whereby ghrelin remains the only known circulating peptide which stimulates appetite
85 and feeding. Research surrounding this unique characteristic of ghrelin has captured
86 significant attention.

87

88 There is an extensive body of literature demonstrating that ghrelin administration
89 augments food intake and over time leads to gains in body weight/adiposity (Asakawa
90 et al., 2003; Nakazato et al., 2001; Shintani et al., 2001; Wren et al., 2000; Wren et al.,
91 2001a). In humans, the appetite stimulating properties of ghrelin were first identified
92 when hunger was reported as a side effect during an investigation examining the
93 influence of ghrelin administration on GH dynamics (Arvat et al., 2001). Thereafter, in
94 a landmark study, Wren and co-workers (2001b) published findings demonstrating a
95 striking increase in hunger perceptions and *ad libitum* energy intake in response to
96 intravenous ghrelin infusion. These results have subsequently been confirmed by other
97 investigators in both lean and obese individuals (Druce et al, 2005; 2006). The diurnal
98 circulating profile of ghrelin is also consistent with the notion that ghrelin influences
99 appetite and feeding with circulating levels peaking before meal times and falling
100 thereafter in proportion to the amount of ingested energy (Callaghan et al., 2004;

101 Cummings et al., 2001). Ghrelin has subsequently been labelled the ‘hunger hormone’
102 (Higgins, Gueorguiev, & Korbonits, 2007).

103

104 The impact of ghrelin on energy metabolism extends beyond appetite regulation.
105 Specifically, ghrelin promotes weight gain and adiposity by reducing energy
106 expenditure (Pfluger et al., 2008) and fat oxidation (increases the respiratory exchange
107 ratio) (Wortley et al., 2004), whilst promoting fat storage and the motivation to seek out
108 energy dense food (Shimbara et al., 2004). In humans, circulating concentrations of
109 ghrelin are inversely associated with body mass index and multiple measures of
110 adiposity (Shiia et al., 2002). Ghrelin levels are reduced in obese individuals
111 (Cummings et al., 2002; Tschöp et al., 2001; Vendl et al., 2004) which may at least
112 partly be mediated by impaired insulin sensitivity/hyperinsulinemia (McLaughlin,
113 Abbasi, Lamendola, Frayo, & Cumming, 2004). Augmented ghrelin therefore does not
114 appear to be a mechanism which perpetuates obesity. Conversely, an attenuated
115 postprandial suppression of ghrelin has been reported in obese individuals requiring a
116 higher energy ingestion before a post-meal suppression is observed (English, Ghatei,
117 Malik, Bloom, & Wilding, 2002; Le Roux et al., 2005). This may contribute to impaired
118 satiety signalling in obesity and the propagation of positive energy balance.

119

120 In addition to its role in mediating the homeostatic control of energy balance, recent
121 research has identified a role of ghrelin in the hedonic component of eating behaviour
122 with studies showing that ghrelin increases the preference for foods with high
123 palatability or high fat content (Egecioglu et al., 2010; Perello et al., 2009). This effect
124 appears to be mediated by the activation of key brain regions associated with pleasure

125 and reward (amygdala, orbitofrontal cortex, anterior insula and striatum) (Malik,
126 McGlone, Bedrossian, & Dagher, 2008).

127

128 With the recognition of the apparent centrality of ghrelin in the control of appetite and
129 energy metabolism it was not long before interest developed concerning the impact of
130 exercise on ghrelin. Exercise influences diverse aspects of energy homeostasis and
131 metabolism including appetite, energy expenditure, substrate utilisation or partitioning,
132 body weight and composition. A decade ago the first studies investigating the impact of
133 exercise on ghrelin appeared in the literature (Dall et al., 2002; Kallio et al., 2001) and
134 since this time there has been an explosion of research within the area. This review aims
135 to provide a narrative overview of studies that have examined both the acute and
136 chronic impact of exercise on circulating levels of ghrelin in adult humans. At this point
137 it is important to emphasise that ghrelin, in the general sense, is composed of two
138 peptide variants, namely acylated and unacylated (Kojima et al., 1999; Yang et al.,
139 2008). References to ‘ghrelin’ typically refer to total ghrelin i.e. measurements based on
140 assays which detect both circulating forms. This distinction is critical given that the
141 physiological actions of acylated and unacylated ghrelin vary considerably. Acylated
142 ghrelin binds and signals through the GHSR-1a to induce GH secretion and to stimulate
143 appetite and feeding. Unacylated ghrelin cannot bind to this receptor, and although it
144 was initially thought of as inactive, it is now known to possess diverse metabolic effects
145 (e.g. effects on insulin sensitivity, glucose and lipid metabolism), some of which may
146 modulate the effect of acylated ghrelin (Delhanty, Neggers, van der Lely, 2012). Of
147 particular note, unacylated ghrelin may even antagonise the orexigenic effect of
148 acylated ghrelin (Asakawa et al., 2005). Due to these inherent functional differences in
149 ghrelin variants it is critical to make this distinction and consequently in this review we

150 will segregate our discussion accordingly. In our text we will use the term ‘ghrelin’ to
151 refer to total ghrelin. Conversely, we will specifically allude to acylated and unacylated
152 ghrelin when talking about the individual ghrelin moieties. The intention of this review
153 is not to provide a systematic or exhaustive account of studies in this area; rather we
154 aim to identify and evaluate the most relevant studies with the objective of clarifying
155 the development and status of research in this burgeoning area and to identify future
156 important avenues of investigation.

157

158 **Acute exercise**

159 *Ghrelin (total)*

160 Initial interest regarding the acute effect of exercise on ghrelin emanated from a
161 hypothesised role of ghrelin as a mediator of exercise-induced changes in GH.
162 Circulating levels of GH rise markedly in response to moderate-high intensity exercise
163 (Godfrey, Madgwick, & Whyte, 2003) and after the discovery of ghrelin’s potent GH
164 releasing action it was thought that ghrelin may orchestrate the exercise-related GH
165 response. However, several early investigations did not observe any changes in
166 circulating levels of ghrelin in response to moderate-high intensity bouts of running
167 (Kraemer et al., 2004a; Schmidt, Maier, Schaller, 2004) or cycling (Dall et al., 2002;
168 Kallio et al., 2001). This was despite notable increases in circulating levels of GH.
169 These findings therefore demonstrate that changes in circulating levels of ghrelin do not
170 mediate GH responses to exercise.

171

172 In subsequent years there was a second wave of interest about the interaction between
173 exercise and ghrelin which was triggered by the identification of ghrelin as a critical
174 regulator of appetite and energy homeostasis (Druce et al., 2005; Wren et al., 2001b).

175 After the cementation of this discovery researchers were keen to investigate how
176 exercise modulates this important appetite regulatory peptide. Questions arose as to
177 whether ghrelin may in part mediate acute appetite changes with exercise e.g. '*exercise*
178 *induced anorexia*' (King et al., 1994), or whether circulating levels of ghrelin would
179 change in response to deviations in energy balance. Unfortunately several initial studies
180 examining the short-term influence of exercise on ghrelin were unable to establish a
181 consensus (Christ et al., 2006; Dall et al., 2002; Erdmann, Tahbaz, Lippl, & Wagenpfeil,
182 2007; Kallio et al., 2001; Kraemer et al., 2004a; Schmidt et al., 2004). However, these
183 early studies were highly diverse in terms of the study designs which make it difficult to
184 compare outcomes. Furthermore, many of these studies harboured significant
185 methodological limitations relating to standardisation of pre-experimental diet and
186 sample collection/assay procedure. Additionally, most of these studies did not
187 implement a non-exercise control group making it impossible to determine whether
188 outcomes were solely related to exercise.

189

190 In 2007 two studies with robust methodologies investigated acute changes in circulating
191 levels of ghrelin during and for up to 1 h after moderate-high intensity exercise. Burns,
192 Broom, Miyashita, Mundy, & Stensel (2007) examined ghrelin responses to 60 min of
193 moderate-high intensity running (74% $\dot{V}O_2$ max) in 18 young, healthy, men and women.
194 Despite hunger being suppressed during and for up to 1 h after exercise, ghrelin levels
195 were unchanged throughout. Similarly, Martins, Morgan, Bloom & Robertson (2007)
196 assessed circulating ghrelin responses to 60 min of moderate intensity cycling (65% of
197 maximum heart rate) in 12 healthy men and women and observed no impact of exercise
198 on ghrelin. It is possible in this study however that consumption of a small meal one

199 hour before exercise may have lowered ghrelin concentrations and masked any effect of
200 exercise.

201

202 The effects of rowing on circulating ghrelin has been the subject of intense investigation
203 by one particular European research group working with elite athletes (Jürämie et al,
204 2007a; Jürämie, Jürämie, Purge, 2007b; Jürämie et al., 2009). In their first publication
205 the researchers examined the ghrelin response to 30 min of sculling at ~ 79% of
206 maximum oxygen consumption. Immediately after exercise circulating ghrelin levels
207 were ~7% higher although this was not quite statistically significant. In a subsequent
208 study these researchers reported a significant increase in ghrelin (24%) immediately
209 after exercise in response to a maximal rowing ergometer test (average duration ~20
210 min, intensity 81% $\dot{V}O_2$ max). This effect was transient however as no differences were
211 apparent 30 min after the end of exercise. Each of these two studies lacked control
212 groups however making it impossible to determine whether changes in ghrelin were
213 solely related to exercise. To address this, in a third investigation these researchers
214 assessed ghrelin responses to a 2 h rowing training session (~67% heart rate max) with
215 participants also completing a non-exercise control trial. The authors reported that
216 exercise significantly increased (15%) ghrelin when measured 30 min after exercise, but
217 not immediately after. The findings from these investigations contradict those of Burns
218 et al (2007) and Martins et al (2007) and the reason for this is not clear. It is possible
219 that these discrepancies are due to factors related to the differing modes of exercise,
220 however it is perhaps more likely that differences in dietary control, sample
221 collection/processing and assay procedure are implicated (Chandarana et al., 2009).

222

223 A handful of studies implementing both aerobic (Malkova, McLaughlin, Manthou,
224 Wallace, & Nimmo, 2008; Toshinai et al, 2007; Vestergaard et al, 2007) and resistance
225 exercise (Ballard et al., 2009; Ghanbari-Niaki, 2006; Kraemer et al., 2004b) have
226 reported decreases in circulating ghrelin in response to single bouts of exercise. Notably,
227 Toshinai et al (2007) examined ghrelin responses to 40 min of graded intensity cycling
228 (four, 10 min stages progressing from light to high intensity) in five healthy males.
229 Plasma ghrelin was suppressed in an intensity dependent fashion. Furthermore, changes
230 in ghrelin were associated with changes in plasma adrenaline ($r = -0.533$) and
231 noradrenaline ($r = -0.603$), an outcome which the authors suggested may indicate a
232 causal mechanism, namely, a sympathetically mediated reduction in gastric blood flow
233 causing decreased delivery of ghrelin into the circulation. An inhibitory effect of GH
234 has also been posited as a mechanism responsible for suppressed ghrelin levels in
235 response to exercise. Specifically, Vestergaard et al (2007) examined the independent
236 and additive effects of GH therapy and acute exercise on post-exercise ghrelin responses.
237 Exercise and GH therapy additively suppressed post-exercise ghrelin concentrations in
238 the circulation with the exercise response being inversely associated with changes in
239 GH ($r = -0.35$).

240

241 *Acylated ghrelin*

242 The appetite stimulating function of ghrelin is now understood to be chiefly determined
243 by acylated ghrelin, via signalling through the GHSR-1a (Kojima et al., 1999). Within
244 appetite related research, emphasis has subsequently shifted to acylated ghrelin, and the
245 relatively recent development of assays specific for acylated and unacylated ghrelin has
246 enabled this change in focus (Hosoda et al, 2004). Accordingly, recent research has
247 unveiled notable differences in the responses of the individual ghrelin moieties to

248 various stimuli including nutrition and energy balance (Liu et al., 2008). Thus, it was
249 not long before researchers became interested in the specific interaction between
250 acylated ghrelin and exercise.

251

252 Broom, Stensel, Bishop, Burns, & Miyashita (2007) were the first to publish data
253 regarding the acute effects of exercise on circulating acylated ghrelin. In their
254 investigation nine healthy males completed an exercise trial and a control trial in a
255 randomised crossover fashion. After an overnight fast, participants completed 60 min of
256 treadmill running at 72% of $\dot{V}O_2$ max and then rested for eight hours. Plasma acylated
257 ghrelin was significantly lower during exercise and immediately after. Moreover,
258 subjective ratings of hunger were significantly reduced over the first three hours of the
259 exercise trial and this was positively associated with suppressed acylated ghrelin ($r =$
260 0.699). These data suggest that acylated ghrelin is transiently suppressed during
261 moderate-high intensity running and this may at least in part contribute to an acute
262 appetite suppression that occurs in response to moderate-high intensity exercise.

263

264 The finding that acylated ghrelin is transiently suppressed by acute exercise, i.e. during
265 and for a limited period after, has been reproduced several times by our research group
266 (Broom, Batterham, King, & Stensel, 2009; King, Miyashita, Wasse, & Stensel, 2010a;
267 King et al., 2011a; Wasse, Sunderland, King, Batterham, & Stensel, 2012). This effect
268 appears to be independent of exercise mode as we have observed this outcome almost
269 identically in response to running, cycling, swimming, sprint interval training, and
270 resistance exercise (Broom et al., 2009; Deighton, Barry, Connon, & Stensel, 2012;
271 King, Wasse, & Stensel, 2011b; Wasse, Sunderland, King, Miyashita, & Stensel, 2013).
272 Exercise intensity stands out as an important determinant of this acute response as low

273 intensity exercise such as walking or cycling (45-50% of $\dot{V}O_2$ max) does not affect
274 circulating acylated ghrelin (King et al, 2010b; Ueda et al, 2009). Broom & Stensel
275 (2006) specifically examined this issue and demonstrated that whilst treadmill running
276 at 75% of $\dot{V}O_2$ max markedly suppressed acylated ghrelin, running at 50% of $\dot{V}O_2$ max
277 had no effect. This mediating influence of intensity may point to possible regulatory
278 mechanisms governing this response, with intensity dependent reductions in splanchnic
279 blood flow and/or augmented sympathetic output at higher exercise intensities
280 potentially interfering with ghrelin production or acylation (Burns et al., 2007; Toshinai
281 et al., 2007). Circulating levels of insulin and glucose are key mediators of prandial
282 ghrelin responses however neither likely affect exercise responses given that circulating
283 insulin concentrations are suppressed during exercise (intensity dependent) (Galbo,
284 Christensen & Holst, 1977) whilst glucose levels remain stable or decrease with
285 prolonged exercise without exogenous carbohydrate (Wagenmakers et al., 1991).

286

287 One of the limitations of many studies which have examined gut hormone responses to
288 exercise is the brevity of observation which is typically limited to sampling before,
289 during and immediately after exercise. As ghrelin, and indeed several other appetite
290 hormones, are regulators of the overall meal response, to capture the more meaningful
291 effect of exercise on ghrelin it is necessary to assess extended responses to exercise and
292 feeding. To this end we examined ghrelin responses to 90 min of moderate-high
293 intensity running with frequent assessment of plasma acylated ghrelin during and for an
294 8.5 h period after exercise, and once on the following morning (King et al., 2010a).
295 Given the intricate relationship between ghrelin and energy balance we hypothesised
296 that ghrelin would be suppressed during exercise, but would increase in the hours
297 thereafter as a compensatory mechanism to promote the restoration of energy balance.

298 Paradoxically, in this study, although we witnessed a transient suppression during and
299 immediately after exercise, circulating concentrations of acylated ghrelin remained no
300 different to control at any point throughout the remainder of the trials. Notably, acylated
301 ghrelin values on the morning after exercise (24 h sample) were almost identical
302 between the exercise and control trials. This was despite participants expending
303 approximately 5324 kJ during exercise. These findings indicate that acylated ghrelin is
304 not sensitive to acute energy deficits induced by exercise. Such a lack of response is in
305 line with the consensus that acute exercise does not immediately augment appetite
306 perceptions (apart from the transient suppression) or energy intake (Blundell, Stubbs,
307 Hughes, Whybrow, & King, 2003; Martins et al., 2008), specifically on the day of
308 exercise. This is in stark contrast to energy deficits induced through acute food
309 restriction whereby rapid and marked compensatory appetite, energy intake and
310 circulating acylated ghrelin responses occur (Hubert, King, & Blundell, 1998; King et
311 al., 2011a). Specifically, we directly compared circulating acylated ghrelin responses to
312 identical acute energy deficits (4280 kJ) induced by exercise verses food restriction and
313 observed a striking compensatory response following consumption of reduced energy
314 meals (King et al., 2011a). Conversely, no such response was observed in response to
315 90 min of running performed at the very beginning of a 9 h trial (Figure 2). It would
316 therefore appear that acutely, acylated ghrelin is sensitive to nutrient/energy ingestion
317 but not to transient perturbations in energy balance that occur with single bouts of
318 exercise.

319

320

Insert figure 2 near here

321

322 There is evidence that females may be less likely to experience favourable changes in
323 body weight and/or composition in response to exercise training compared with males
324 and it is possible that this is due to divergent hormonal responses to exercise (Hagobian
325 & Braun, 2010). Recent investigations have examined whether part of this discrepant
326 response is related to effects on appetite regulatory hormones such as acylated ghrelin,
327 however a recent study has shown that acute acylated ghrelin responses to moderate-
328 high intensity exercise do not differ between sexes (Hagobian et al., 2013). It is possible
329 that larger energy deficits associated with consecutive days of exercise training are
330 necessary before any sex differences emerge.

331

332 Although transient reductions in circulating acylated ghrelin have been consistently
333 observed in response to moderate-high intensity bouts of exercise, the physiological
334 relevance of this response is not clear. Suppressed levels of acylated ghrelin have been
335 found to correlate with suppressed hunger ratings (Broom et al., 2007) suggesting a role
336 of acylated ghrelin in mediating appetite responses to exercise. Whether acute changes
337 in acylated ghrelin after exercise impact up on energy intake is questionable however,
338 given the brevity of responses which typically revert to control values within 30 min
339 post-exercise (King et al., 2010a; Wasse et al., 2012, Wasse et al., 2013). Furthermore,
340 after exercise, circulating pre-meal concentrations of acylated ghrelin do not correlate
341 with subsequent *ad libitum* energy intake (King et al., 2010a; Deighton et al., 2012).
342 Relative energy intake (energy intake corrected for the energy cost of exercise) is an
343 important concept within energy balance research and our group recently examined the
344 relation between exercise, acylated ghrelin and relative energy intake (Deighton et al,
345 2012). In this study we observed no association between acylated ghrelin and relative
346 energy intake. Taken collectively, these data suggest that transient changes in acylated

347 ghrelin with exercise are not tightly linked to changes in absolute or relative energy
348 intake. It is likely that within the short-term other behavioural, psychological or habitual
349 factors have a stronger impact on energy intake/food choices.

350

351 The mechanism(s) responsible for producing transient perturbations in circulating
352 acylated ghrelin with exercise are not clear but must be related to either interference in
353 the production of acylated ghrelin and/or its secretion into the circulation e.g. via effects
354 on GOAT activity within the golgi apparatus, or augmentation of de-acylation by
355 circulating proteases/esterases (De Vries et al., 2004). Findings demonstrating amplified
356 acylated ghrelin suppression when exercising in the heat as compared with a thermo-
357 neutral climate (Shorten, Wallman, & Guelfi, 2009) may implicate attenuated blood
358 flow to the splanchnic regions and/or exertion related stress responses as key mediating
359 mechanisms. Further research is needed to clarify this issue.

360

361 *Unacylated ghrelin*

362 The influence of acute exercise on circulating unacylated ghrelin has been determined
363 recently. Using a sample of young, healthy males, Shiiya et al (2011) collected blood
364 samples before, frequently during, and 90 min after one hour of moderate intensity
365 cycling (50% $\dot{V}O_2$ max). Acylated and unacylated ghrelin were assessed using enzyme-
366 linked immunosorbant assays specific for each peptide variant. Baseline levels of
367 unacylated ghrelin were ~6 fold higher than acylated ghrelin. During exercise,
368 circulating acylated ghrelin was suppressed by approximately 55% however levels of
369 unacylated ghrelin did not change at any point. These data support those relating to the
370 acylated ghrelin literature and suggest that exercise somehow interferes with the
371 acylation of ghrelin, rather than affecting unacylated ghrelin. The authors of this study

372 suggest that gastric mucosal ischaemia and/or increased sympathetic nerve activity may
373 mediate these effects on ghrelin acylation.

374

375 **Exercise Training**

376 *Ghrelin (total)*

377 Several studies have investigated the impact of exercise training (predominantly aerobic)
378 on circulating levels of ghrelin, acylated ghrelin and unacylated ghrelin. Interpreting
379 these outcomes is challenging given stark differences between studies in terms of the
380 designs implemented, the participant groups examined and the methods utilised.

381

382 Ravussin, Tschöp, Morales, Bouchard, & Heiman (2001) were the first to report
383 findings regarding the impact of exercise training on circulating ghrelin. These
384 researchers reported that a 93 day cycling intervention (2 bouts of cycling per day to
385 expend 4184 kJ/day) with associated weight loss (6%) led to a 26% increase in fasting
386 plasma ghrelin concentration within a sample of healthy, young, men. Conversely,
387 chronic overfeeding (351,456 kJ) over 100 days, sufficient to raise body mass by 13%
388 led to a significant decrease (18%) in fasting ghrelin. These findings indicate that
389 ghrelin is highly responsive to changes in energy balance/body weight and this finding
390 has been corroborated by others (Garcia et al., 2006). Conversely, one study reported
391 that fasting and meal related circulating ghrelin levels remained unchanged despite 5%
392 weight loss induced by food restriction and exercise in a group of morbidly obese men
393 and women (Morpurgo et al., 2003). This information may suggest that a threshold
394 exists before changes in ghrelin are seen in response to weight loss interventions which
395 is likely mediated by factors such as sex, baseline weight status and insulinaemia.

396

397 It is thought that one of the primary functions of ghrelin is to regulate food intake on a
398 meal to meal basis. Consequently, to understand how interventions impact on ghrelin it
399 is essential to examine ghrelin responses before and after meals rather than merely
400 assessing fasting levels. To address this, Leidy, Dougherty, Frye, Duke, & Williams
401 (2007) performed 24 h blood sampling in a small group of normal weight women,
402 before and after a 12 week combined exercise and dietary intervention. In this study
403 participants performed moderate-intensity aerobic exercise five times per week for
404 approximately 45 min/session. Dietary intake was also decreased by a quarter. The
405 intervention reduced body weight by ~4% and this was associated with significantly
406 higher circulating ghrelin (area under the curve) across the day (20%). More specifically,
407 compared with baseline, heightened circulating ghrelin peaks were evident at key time
408 points throughout the day and these changes were associated with reduced feelings of
409 fullness (Figure 3). This study clearly demonstrates that exercise interventions with
410 ensuing weight loss augment the ghrelin diurnal profile.

411

412 *Insert figure 3 near here*

413

414 A limitation of the data from the studies previously identified is that we cannot identify
415 whether changes in ghrelin occurred in response to exercise *per se* or to the associated
416 weight loss. Leidy et al (2004) studied the impact of 12 weeks of exercise training on
417 fasting levels of ghrelin in a group of healthy, normal weight women. Participants
418 completed moderate intensity aerobic exercise five times each week for a duration to
419 expend 2092 kJ/session. Diet was controlled immaculately with all participant meals
420 being provided by the research team. This study showed that exercise, without
421 significant weight loss (<1.5 kg) had no impact on fasting plasma ghrelin concentrations.

422 Conversely, ghrelin levels increased two-fold in those who experienced significant
423 weight loss (> 1.5 kg). These findings are supported by those of Foster-Schubert et al
424 (2005) who also observed augmented plasma levels of ghrelin only in participants who
425 experienced weight loss. Specifically, these researchers studied a large group of post-
426 menopausal women over 12 months. Half of the group exercised, performing moderate
427 intensity aerobic exercise five times each week, whilst the other participants were
428 randomised to control. Over the course of the intervention the exercise group lost
429 weight (1.4 kg by 12 months) and this led to an increase in fasting plasma ghrelin
430 concentrations (~5%). Importantly, more detailed analysis of the exercise group
431 revealed that changes in ghrelin only occurred in those who lost body weight.
432 Specifically, fasting ghrelin levels increased in a step-wise fashion, with greater changes
433 being seen in those who lost a large amount of weight (> 3 kg, 18% increase) compared
434 with those who lost a moderate amount (0.5-3 kg, 7% increase). Overall, the change in
435 ghrelin was inversely associated with change in body weight ($r = -0.607$). These
436 findings have also been corroborated by others who reported that fasting levels of
437 ghrelin did not change in response to 12 weeks of supervised moderate-intensity aerobic
438 exercise training (five times per week) in a group of healthy, normal weight women
439 who did not lose weight (Scheid, De Souza, Leidy, & Williams, 2011). Conversely,
440 fasting ghrelin levels increased significantly (~25%) in an exercise group who lost
441 weight (3.2 kg).

442

443 The mechanisms by which changes in energy balance/body mass impact on circulating
444 ghrelin are not fully understood although the adiposity signals leptin and insulin appear
445 to be important. Leptin is produced within adipocytes and circulating levels correlate
446 directly with adipose tissue mass (Maffei et al., 1995). An inverse reciprocal

447 relationship exists between leptin and ghrelin with studies having unveiled a direct
448 inhibitory effect of leptin on the production of ghrelin (Kamegai et al., 2004). Changes
449 in circulating concentrations of ghrelin in response to deviations in body mass e.g. with
450 weight loss or gain, may therefore occur secondary to alterations in leptin. Insulin may
451 also mediate some of the effects of adiposity on ghrelin (Williams and Cummings,
452 2005). Specifically, it has been shown that insulin resistance and hyperinsulinemia are
453 inversely associated with circulating levels of ghrelin (McLaughlin et al., 2004) and this
454 may represent one mechanism by which insulin is implicated in the homeostatic
455 regulation of energy balance.

456

457 *Exercise training & acylated ghrelin*

458 The influence of exercise training on acylated ghrelin has been investigated recently.
459 Hagobian et al (2009) examined acylated ghrelin responses to meal challenges before
460 and after four consecutive days of exercise. Participants were previously sedentary,
461 overweight or obese men and women and each completed two, four day trials in a cross-
462 over fashion. In both trials participants performed daily aerobic exercise to expend
463 ~30% of daily energy expenditure. In one trial participants replaced the energy
464 expended during exercise by increasing their energy intake, whilst in the other condition
465 no dietary changes were made, resulting in an energy deficit. Interestingly, these
466 researchers observed augmented circulating levels of acylated ghrelin after the
467 intervention in females independent of the condition. This outcome suggests that
468 exercise may independently trigger a compensatory acylated ghrelin response in females.
469 In males, neither intervention had an influence on acylated ghrelin and it is possible that
470 this divergent response may indicate the presence of a tighter homeostatic control

471 system in females than males (Hagobian & Braun, 2010). Further research is necessary
472 to confirm this.

473

474 Martins, Kulseng, King, Holst, & Blundell (2010) also reported findings regarding the
475 acylated ghrelin response to exercise training. In this study overweight/obese men and
476 women completed supervised moderate-intensity aerobic exercise training five times
477 per week for 12 weeks. Acylated ghrelin responses to standardised meal challenges
478 were examined before and after the intervention. In accordance with previous reports
479 describing suppressed fasting and meal related changes in ghrelin in obese individuals
480 (Cummings et al., 2002; English et al., 2002; Tschöp et al., 2001), circulating acylated
481 ghrelin levels were low and unresponsive to meals before the intervention. After the
482 intervention fasting levels of acylated ghrelin were increased and this was associated
483 with greater meal related suppression. This change may indicate a beneficial response
484 i.e. enhanced sensitivity to nutrient intake may represent improved appetite control.
485 Notably, this response is consistent with previous data suggesting that exercise training
486 in this population has a dual effect on appetite by increasing fasting hunger and
487 enhancing satiety (King et al, 2009).

488

489 In the study of Martins et al (2010) participants maintained their usual diet and
490 consequently lost weight during the course of the study. It is therefore impossible to
491 determine whether these changes in acylated ghrelin dynamics were due to exercise
492 itself or to weight loss. In contrast to these results, Guelfi, Donges, & Duffield (2012)
493 recently reported that acylated ghrelin fasting levels and meal related profiles do not
494 change in response to exercise training. These researchers studied a cohort of
495 overweight or obese men who were allocated to control, aerobic or resistance training

496 (three times per week) for 12 weeks. The aerobic training group lost ~ 2 kg whilst
497 weight did not change amongst the resistance training group. Nonetheless, circulating
498 levels of acylated ghrelin did not change in either group. Differential outcomes between
499 this investigation and that of Martins et al (2010) may be due to differences in study
500 participants i.e. whether both men and women were included, meal challenges imposed
501 (the latter study used an oral glucose challenge as a stimulus), training frequency (five
502 vs. three times per week) and associated weight loss. Further research is therefore
503 needed to isolate the influence of exercise training on circulating acylated ghrelin,
504 however we may speculate that as for ghrelin, changes will possibly only occur
505 secondary to perturbations in body weight.

506

507 *Exercise training & unacylated ghrelin*

508 The effect of exercise training on circulating unacylated ghrelin concentrations was
509 investigated in a prospective study during which 552 young Finnish men completed six
510 months of military training (Cederberg et al., 2011). In this investigation the authors
511 reported a significant increase in circulating unacylated ghrelin which was weakly
512 inversely associated with changes in body weight, waist circumference and fat mass.
513 Although this study provides a useful starting point for future work investigating the
514 interaction between exercise training and unacylated ghrelin, unfortunately the lack of
515 control over training volume, dietary intake and body weight change make it impossible
516 to derive any concrete inferences from the study.

517

518 **Conclusions and future directions**

519 The first studies to investigate the interaction between exercise and ghrelin were
520 published approximately a decade ago. Outcomes reported from several early

521 experiments produced a confused picture with reports of acute increases, decreases and
522 no change in circulating ghrelin. More recently, with the development of more sensitive
523 assay methodologies, investigators have specifically focused on the individual
524 responses of acylated and unacylated ghrelin to exercise. A large body of data suggests
525 that circulating levels of acylated ghrelin are transiently suppressed in response to acute
526 exercise when performed at moderate intensities or higher. This effect is independent of
527 exercise mode and lasts for approximately 30 min after exercise. After this period no
528 further changes in acylated ghrelin occur on the day when exercise is performed i.e.
529 there is no increase or compensation in acylated ghrelin. Limited data available
530 indicates that circulating levels of unacylated ghrelin do not change with acute exercise.
531 Collectively, it appears that transient changes in ghrelin in response to acute exercise are
532 related to interference with the production of the acylated form of ghrelin. Further
533 research is needed (with more consistent methods i.e. control of participants' pre-trial
534 diet, sample collection/processing procedures, assay protocols) to confirm the impact of
535 exercise on the individual ghrelin variants. Additional work is also needed to define the
536 mechanisms responsible for changes in acylated ghrelin with acute exercise.

537

538 Data regarding the influence of exercise training on ghrelin is more consistent and
539 clearly illustrates that exercise training *per se* has no impact on circulating levels of
540 ghrelin. Instead, changes in ghrelin that are seen over the course of exercise
541 interventions take place secondary to weight loss. This response likely represents a
542 physiological mechanism seeking to defend energy homeostasis. The impact of exercise
543 training on acylated and unacylated ghrelin has received less attention with insufficient
544 data available to derive any meaningful conclusions regarding unacylated ghrelin. The
545 limited findings regarding the effects on acylated ghrelin are mixed, but may suggest

546 that exercise training with associated weight loss improves the acylated ghrelin satiety
547 response to meals in overweight and obese individuals. Moreover, one study suggests
548 that exercise training may exert an independent compensatory effect on acylated ghrelin
549 in females. Additional research is needed however to conclusively determine the extent
550 of sex differences in ghrelin regulation and to determine the independent influence of
551 exercise training (various modes) on the dual circulating ghrelin forms.

552

553 Finally, although this review has focused solely on ghrelin, it is important to note that
554 there are several additional hormones that are involved in the acute and chronic
555 regulation of appetite and energy balance e.g. Peptide YY, glucagon-like-peptide 1,
556 cholekystokinin and leptin. Future research must take a holistic approach and take into
557 account the wider impact of interventions on this hormonal system. **Additionally,**
558 **although this review has focused on ghrelin, it is also important to highlight that food**
559 **intake/energy balance is not solely governed by homeostatic forces but is also**
560 **influenced significantly by non-homeostatic factors which may be physiological (Evero,**
561 **Hackett, Clark, Phelan & Hagobian, 2012, Westerterp-Plantenga, Verwegen, Ijedema,**
562 **Wijckmans & Saris, 1997) cognitive/behavioural (Blundell & Gillett, 2001), social (de**
563 **Castro, 1990) or environmental (Hill, Wyatt, Reed & Peters, 2003). These influences**
564 **have the potential to override homeostatic regulators (Berthoud, 2004; Borer, 2010) and**
565 **must therefore always be considered in the context of food intake regulation.**

566

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934 **Figure Legends**

935 Figure 1: Post-translational processing yielding acylated ghrelin via addition of medium
936 chain fatty acids to serine-3. Adapted from Kojima et al (1999).

937 Figure 2: Acute acylated ghrelin responses to identical energy deficits (4280 kJ)
938 induced by exercise and food restriction. NB: exercise performed 0-1.5 h. ^adifferent
939 from Control $P < 0.05$; ^bdifferent from exercise $P < 0.05$. Values are mean \pm SEM (n =
940 12). Data from King et al (2011a).

941 Figure 3: Circulating concentrations of total ghrelin before and after a 12 week diet and
942 exercise intervention producing a sustained negative energy balance and reduction in
943 body weight. * $P < 0.05$. Values are mean \pm SEM (n = 8). Data from Leidy et al, (2007).

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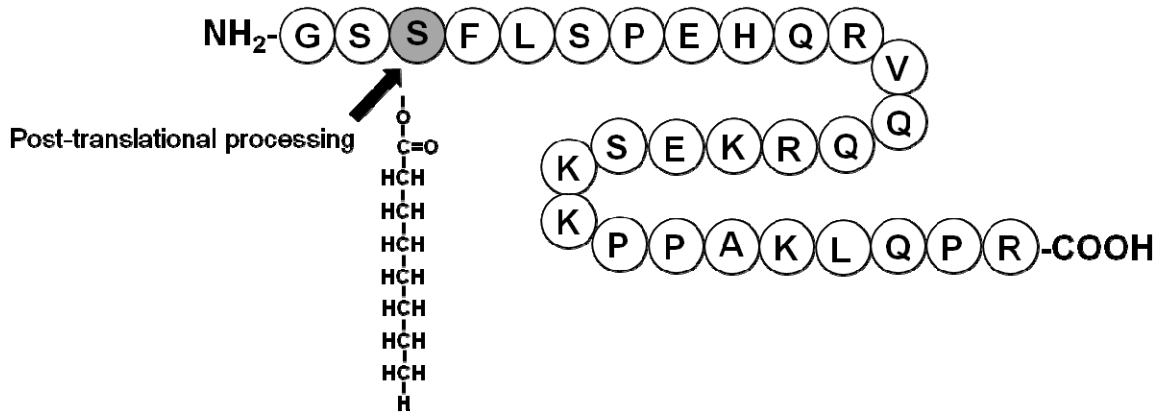
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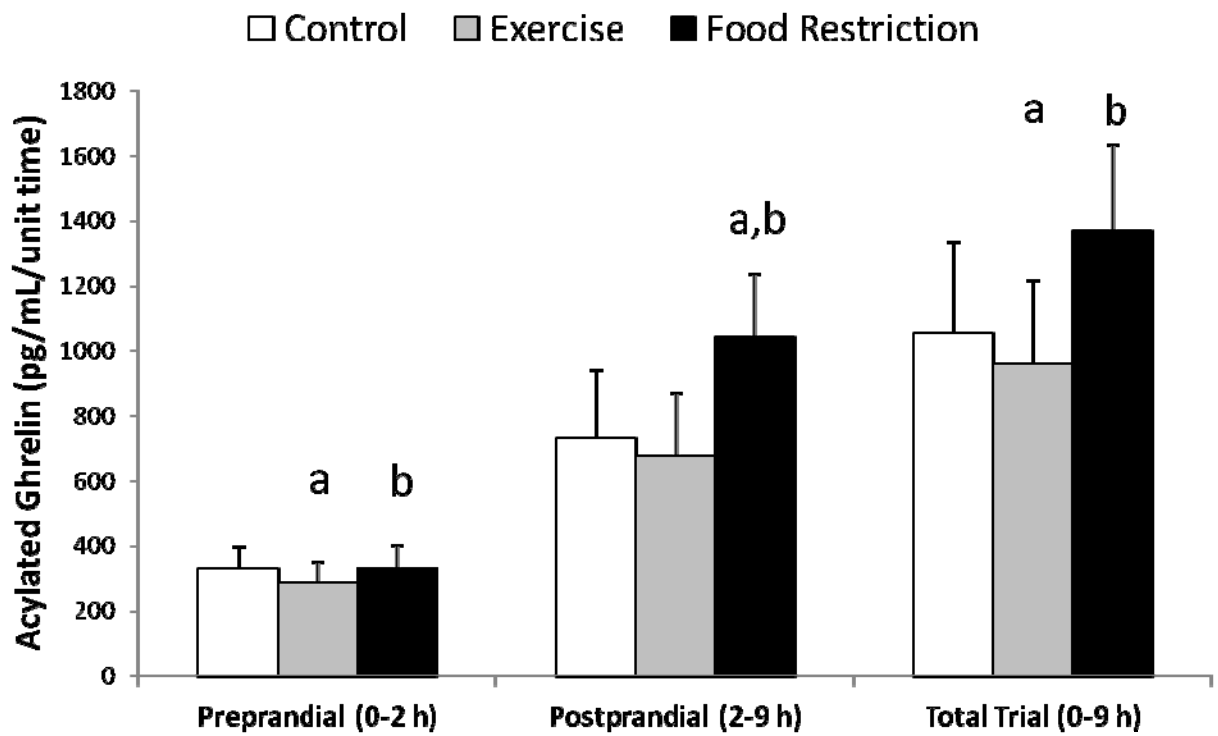
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953 **Figure 1**



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955 **Figure 2**



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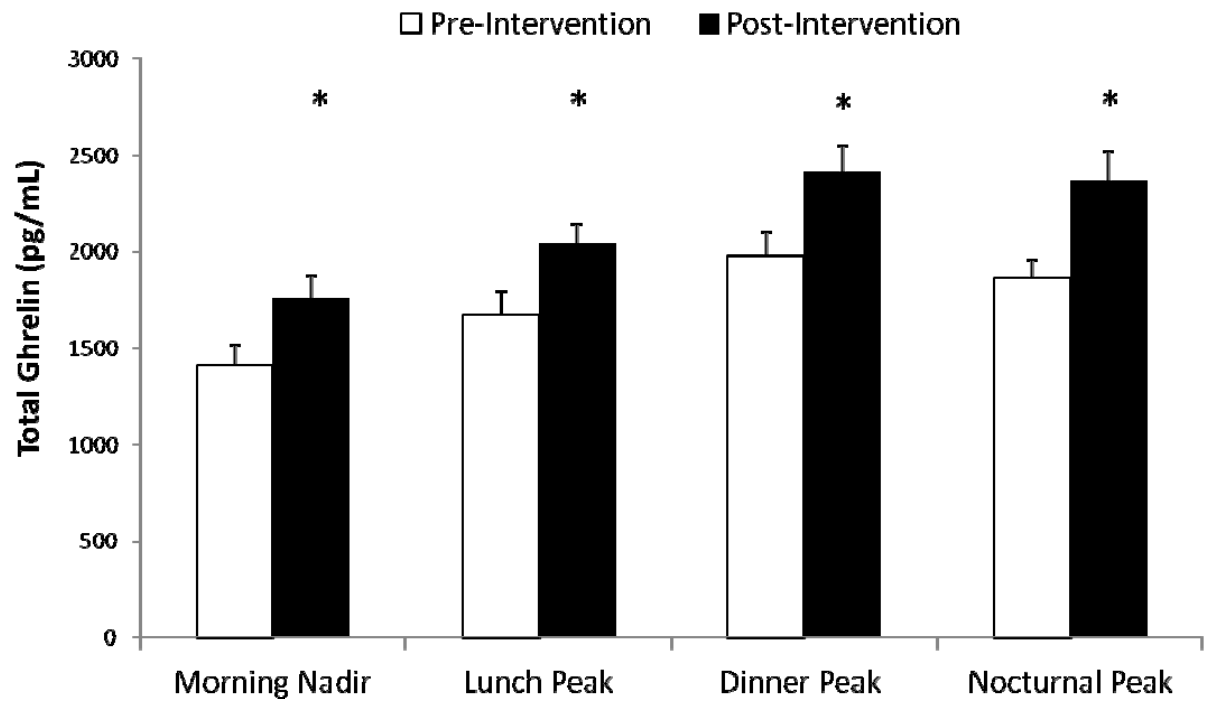
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961 **Figure 3**

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