Exercise and ghrelin. A narrative overview of research

Exercise and Ghrelin – A Narrative Overview of Research

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Abstract

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

Key Words

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

Introduction

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

Ghrelin is a 28 amino acid peptide produced primarily from P/D_1 cells in the stomach fundus, with much lesser amounts being synthesised in the intestine, pancreas and other peripheral organs including the testis, heart, adipose tissue and skin (Gutierrez et al., 2008; Stengel & Tache, 2012). Upon fasting, and/or low circulating levels of glucose and insulin, ghrelin is secreted into the circulation where it is present in two forms, acylated and unacylated (~1:4 ratio) (Stengel, Goebel, Wang, & Taché, 2010). Acylated ghrelin is made explicit by the post-translational addition of a medium chain fatty acid, typically octanoate or decanoate, to its third amino acid residue (serine), a modification catalysed by ghrelin O acyltransferase (GOAT) (Gutierrez et al, 2008; Yang, Brown, Liang, & Grishin, 2008) and which is essential for ghrelin to bind to the GHSR-1a to exert its primary hormonal and metabolic actions (Kojima & Kangawa, 2005) (Figure 1).

Insert figure 1 near here

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

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

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

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

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

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

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

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

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

Acute exercise

Ghrelin (total)

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

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

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

In 2007 two studies with robust methodologies investigated acute changes in circulating levels of ghrelin during and for up to 1 h after moderate-high intensity exercise. Burns, Broom, Miyashita, Mundy, & Stensel (2007) examined ghrelin responses to 60 min of moderate-high intensity running (74% $\dot{V}O_2$max) in 18 young, healthy, men and women. Despite hunger being suppressed during and for up to 1 h after exercise, ghrelin levels were unchanged throughout. Similarly, Martins, Morgan, Bloom & Robertson (2007) assessed circulating ghrelin responses to 60 min of moderate intensity cycling (65% of maximum heart rate) in 12 healthy men and women and observed no impact of exercise 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

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

Acylated ghrelin

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

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

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

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

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

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

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

Unacylated ghrelin

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

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

Exercise Training

Ghrelin (total)

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

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

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

Insert figure 3 near here

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

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

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

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

Exercise training & acylated ghrelin

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

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

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

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

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

Exercise training & unacylated ghrelin

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

Conclusions and future directions

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

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

Data regarding the influence of exercise training on ghrelin is more consistent and clearly illustrates that exercise training *per se* has no impact on circulating levels of ghrelin. Instead, changes in ghrelin that are seen over the course of exercise interventions take place secondary to weight loss. This response likely represents a physiological mechanism seeking to defend energy homeostasis. The impact of exercise training on acylated and unacylated ghrelin has received less attention with insufficient data available to derive any meaningful conclusions regarding unacylated ghrelin. The 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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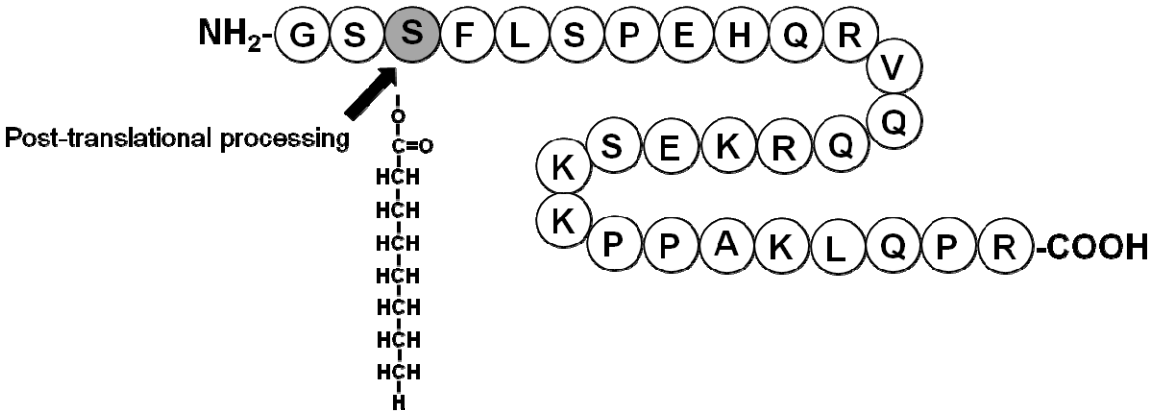
Figure Legends

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

Figure 2: Acute acylated ghrelin responses to identical energy deficits (4280 kJ) induced by exercise and food restriction. NB: exercise performed 0-1.5 h. [a]different from Control $P < 0.05$; [b]different from exercise $P < 0.05$. Values are mean ± SEM (n = 12). Data from King et al (2011a).

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

953 **Figure 1**



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955 **Figure 2**

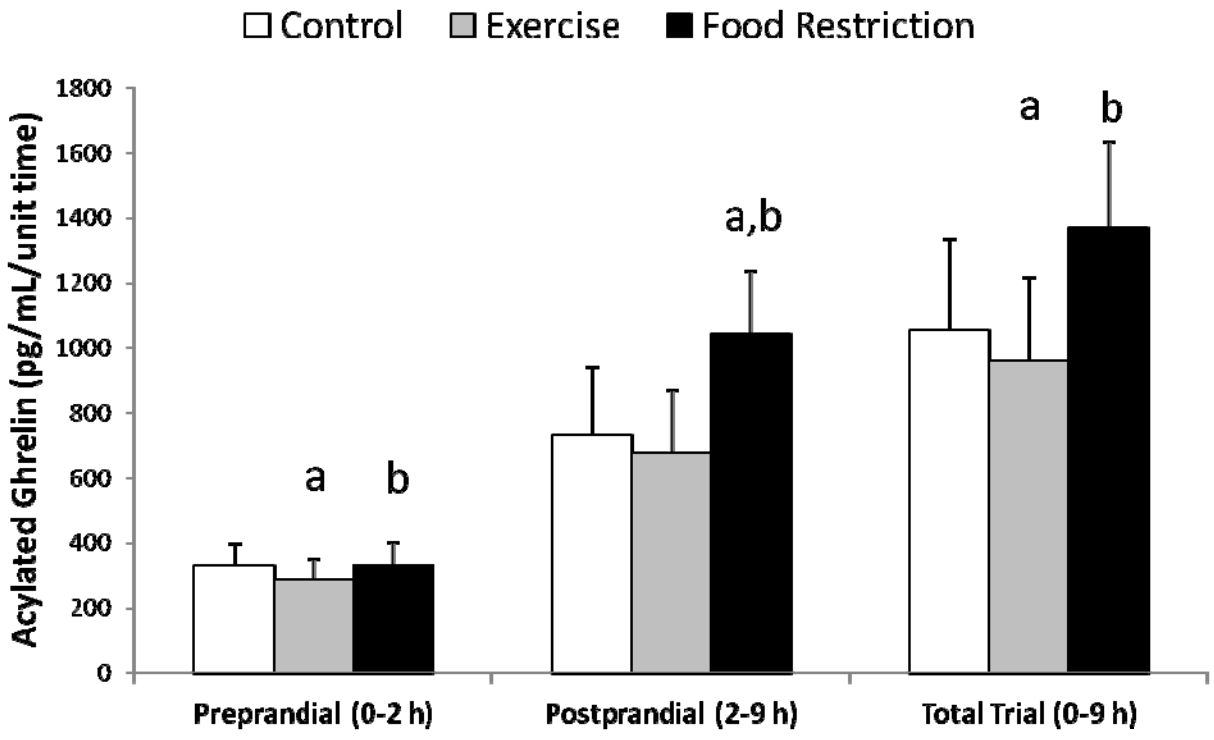

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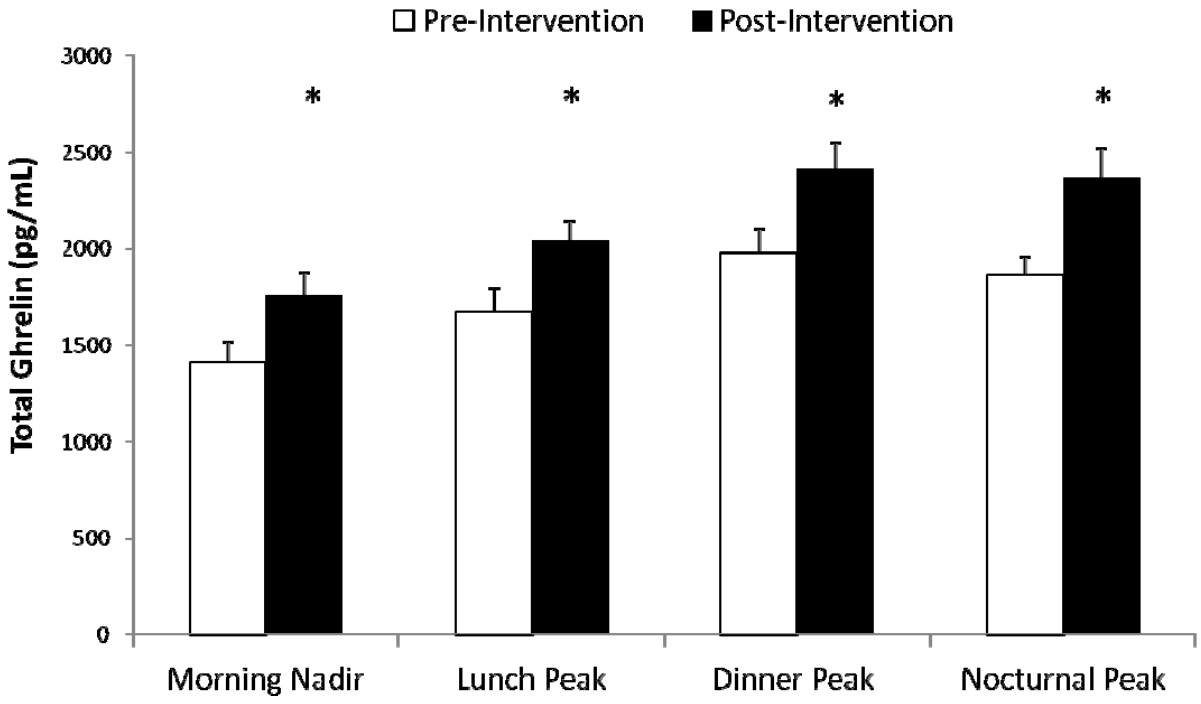
Figure 3


Pre-Intervention
Post-Intervention
Total Ghrelin (pg/mL)
3000
2500
2000
1500
1000
500
0
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*
*
Morning Nadir
Lunch Peak
Dinner Peak
Nocturnal Peak