EFFECT OF EVENING VS. MORNING EXERCISE ON THE APPETITE HORMONES

Penny Akre

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Honors Thesis

EFFECT OF EVENING VS. MORNING EXERCISE ON THE APPETITE HORMONES

by

Penny Akre

Submitted to Brigham Young University in partial fulfillment of graduation requirements for University Honors

Nutritional Science Department
Brigham Young University
April 2024

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Honors Coordinator: Jeff Tessem
ABSTRACT

EFFECT OF EVENING VS. MORNING EXERCISE ON THE APPETITE HORMONES

David W. Coltrin
Nutritional Science Department
Bachelor of Life Science

This paper explores the potential impact of exercise timing on appetite hormones and metabolic outcomes, aiming to enhance understanding of the complex interplay between exercise, appetite regulation, and weight management. A randomized trial involving premenopausal women investigated the effects of morning versus evening exercise sessions over an eight-week intervention period. Participants underwent comprehensive screening and a supervised exercise regimen, with pre-and post-training laboratory sessions assessing body composition and appetite hormone levels. Statistical analyses revealed no significant differences in appetite hormone levels between morning and evening exercise groups, but notable correlations were observed between appetite hormones and body composition measures. Despite limitations such as the exclusive inclusion of female participants, the study's strengths, including a supervised exercise protocol and randomized design, contribute to its scientific credibility. Overall, this research underscores the complexity of metabolic responses to exercise timing and highlights the need for further exploration to optimize weight management strategies.
ACKNOWLEDGMENTS

I am immensely grateful to Professor LeCheminant for his patient guidance and unwavering support throughout this thesis. His assistance was invaluable during times of uncertainty and correction. I also extend my thanks to Professor Clement for his dedicated assistance, despite linguistic barriers. His willingness to review my work outside his field reflects his commitment to academic excellence. Their support has been instrumental in shaping this project, and for that, I am deeply thankful.
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Introduction

Obesity is a significant health issue in the United States (1,2). It is imperative to understand the complex interplay among food, exercise, and weight control to advance individual and public health. Furthermore, it's more important than ever to find efficient weight-management techniques to combat the increasing obesity rates (3,2). Energy balance and weight management research has the potential to transform and enhance the lives of millions of people worldwide by informing best strategies to positively impact health.

Multiple strategies are currently available to assist with weight management and to prevent obesity. The foundation of most weight management programs is behavior, diet, and physical activity improvements (4). In addition, recent advances in bariatric surgery and pharmacotherapy have been shown to significantly enhance weight management outcomes, particularly for those with extreme levels of obesity (5,6,7). Further, there are many nuanced dietary patterns and physical activity strategies that may be beneficial for some individuals, especially for those that require only modest weight loss.

Over the last 10 years, intermittent fasting has emerged as a potential method to control energy intake for some people. A type of intermittent fasting, time-restricted feeding, is used to limit food consumption to certain times of the day(8, 9, 10). Time-restricted feeding has been examined for weight loss, body composition, energy levels, glucose levels, and blood pressure, among other variables (11,12, 13). The concept of time-restricted feeding is partially based on the idea of circadian rhythms and biological clocks, recognizing the importance of aligning eating patterns with the body's internal timekeeping mechanisms. This
approach acknowledges that our bodies have evolved to metabolize nutrients more efficiently during certain times of the day, potentially influencing metabolic health (14).

Recently, some researchers have expanded the concept of eating based on time of day to physical activity and exercise by time of day. In other words, the question has been posed, do some health outcomes, including weight management, improve if a person exercises in the morning vs. the evening (15,16,17). The optimal timing of physical activity remains a subject of considerable debate. Many individuals advocate for morning exercise, citing potential benefits such as enhanced metabolism, increased energy levels throughout the day, and improved adherence to exercise routines (18). Proponents of morning exercise argue that it jumpstarts metabolism, leading to more significant calorie burn throughout the day and potentially aiding in weight management (19). Additionally, the psychological boost from completing a workout early in the day may positively impact mood and motivation, setting a productive tone for the remainder of the day (19).

Some studies suggest that morning exercise uniquely affects appetite regulation, influencing food choices and daily intake patterns (15). However, contrasting opinions exist, with some individuals preferring evening workouts for increased muscle strength and flexibility later in the day, reduced risk of injury due to warmer muscle temperatures, and the ability to unwind and de-stress after a long day (18). Researchers from Nottingham and Loughborough University conducted a study on exercise timing's impact on post-exercise factors. Contrary to expectations, participants consumed more calories after evening exercise,
suggesting timing affects post-exercise energy intake. Exercise performance remained consistent, with no major differences noted between the morning and evening sessions. The study observed daily variations in carbohydrate oxidation and energy expenditure, indicating potential benefits of morning exercise, but these differences did not persist during training, and no significant impact on long-term appetite or body composition changes was found (16). This indicates that timing of exercise may not significantly impact subjects’ long-term appetite and body composition changes.

Other research suggests that exercise can influence energy intake and weight management through regulation of appetite-related hormones, such as leptin or ghrelin (17, 20, 21, 22). This is intriguing for weight management as exercise is posited to have at least the dual effect of increasing energy expenditure, as well as influencing appetite hormones in a way that decreases energy intake. Previous research has demonstrated that appetite hormone responses to exercise can vary depending on exercise intensity, duration, and individual characteristics (23,24). However, there are a few studies that have examined whether there is a nuanced effect of time of day of exercise specifically for appetite-related hormones.

For this paper, I have a unique opportunity to explore the effects of a morning or evening exercise intervention on the effect of multiple appetite hormones, including leptin, amylin, C-peptide, GIP, GLP-1 insulin, PP, and PYY. We hypothesized that there will be no significant difference in these hormones between the morning and evening exercise groups (25). Below, I provide background information on each appetite-related hormone and how physical
activity influences the hormone. Subsequently, I will describe the methods and results of this study. To our knowledge, no study has yet directly compared the effect of time of day of exercise on these hormones.

Background

**Leptin.** Leptin, a hormone primarily secreted by adipose tissue, plays a pivotal role in energy balance and appetite regulation. Acting on the hypothalamus, it suppresses appetite and enhances energy expenditure, contributing to weight maintenance and metabolic control (26, 27). It acts on the hypothalamus in the brain to suppress appetite and increase energy expenditure, helping to maintain body weight and regulate metabolism.

Although there are differences in the research on how exercise affects leptin levels, the link between the two is acknowledged (28,29,30,31). Aerobic activity, four times a week for sixty minutes, was shown to lower leptin levels during a 16-week intervention in obese female youngsters, suggesting a possible function for exercise in the regulation of hunger hormones (32). An analogous investigation conducted on adult participants in a 12-month exercise and diet regimen demonstrated an inverse relationship between baseline adiponectin:leptin ratio and different markers of adiposity and cardiometabolic health, underscoring the intricate relationship between exercise, leptin, and metabolic health. The inconsistent findings across several studies emphasize the need for a more nuanced comprehension of how exercise influences leptin levels, especially in relation to hunger suppression (28, 32). Exercise and leptin levels are related in a complicated way, and the time of day you exercise matters.
Although leptin levels may rise with exercise, this may not always be the case. The release and efficacy of leptin hormones can be impacted by metabolic changes and circadian rhythms, which are also included in the temporal dimension.

Amylin. Amylin, a pivotal hormone in blood sugar control and satiety, is integral to regulating metabolic processes by inhibiting glucagon synthesis, delaying stomach emptying, and promoting feelings of fullness (17, 25, 33). Acute, intensive exercise has been observed to elevate amylin levels, even in well-trained individuals, comparable to insulin. Interestingly, these heightened levels persist during the recovery period following training, presenting a dynamic response that includes a decrease in amylin during the post-prandial state. This emphasizes the vital function that amylin plays in controlling blood sugar levels and promoting satiety, as well as its importance in the complex network of physiological processes that control hunger and metabolic health (25, 26).

Analyzing the research on amylin levels and exercise offers important insights into the dynamic way that this hormone reacts to physical activity (34,35). A particular study with eight young, healthy males found that following a 90-minute treadmill workout at 60% of their VO2 max, amylin levels significantly decreased, in contrast to the typical trend of rising amylin levels during acute exercise. Surprisingly, this decrease in amylin continued after the workout, suggesting that the effects of exercise on amylin are long-lasting and continue into the recovery phase. Exercise's long-lasting effects on amylin levels advance our knowledge of the complex interactions involving movement, metabolic
hormones, and hunger control. Furthermore, it emphasizes the possible impact of exercise scheduling on hormone-mediated appetite regulation. This underlines the need to investigate the potential effects of exercise timing—morning or nighttime—on subtle physiological reactions and underscores the function of amylin in energy balance (35). Deciphering the temporal subtleties in the relationship between exercise and amylin provides important information on how to best use physical activity for controlling appetite and maintaining overall metabolic health.

**C-peptide.** C-peptide is produced when insulin is cleaved and is essential for controlling blood glucose levels (36). Exercise affects insulin sensitivity, glucose absorption, and metabolic activity because training muscles have higher energy demands, which causes variations in C-peptide levels. The fact that regular exercise improves insulin sensitivity and glucose metabolism underscores the complex link between exercise and C-peptide. Regular exercise leads to an improvement in insulin sensitivity, which has important management implications for illnesses including insulin resistance and type 2 diabetes. Regular physical activity increases the effectiveness of insulin's continued use in promoting cells’ absorption of glucose, highlighting the significance of exercise in the long run for managing blood sugar levels.

Short-term increases in insulin secretion during acute exercise sessions have been reported to cause a temporary elevation in C-peptide levels. But the important thing is to stick to a regular exercise schedule, which is crucial for long-term glucose control. Regular physical activity helps to enhance insulin sensitivity
and reduce reliance on C-peptide over time according to research, including studies on young children and longitudinal analyses (36). Exercise's temporal component, particularly when it comes to timing, may have an additional influence on C-peptide levels and long-term metabolic health. Understanding the nuanced relationship between exercise, C-peptide, and age-related trends becomes crucial, as promoting physical activity emerges not only as a cornerstone in enhancing metabolic health but also as a key strategy in reducing the risk of both type 1 and type 2 diabetes by alleviating the burden on insulin-producing beta cells (36).

**Insulin.** Insulin, a hormone produced by the pancreas, is central to glucose metabolism and energy regulation. When carbohydrates are ingested, insulin facilitates glucose uptake into cells for energy production or storage (37). Additionally, it suppresses glucose release from the liver and promotes the storage of excess glucose as glycogen. Insulin's role in regulating fat metabolism involves promoting the uptake of fatty acids into adipose tissue for storage. This hormone is intricately connected to appetite regulation, working in conjunction with other hormones like ghrelin, known as the "hunger hormone" (37). Ghrelin levels rise before meals, stimulating appetite and reducing energy expenditure. Dysregulation of ghrelin has implications for obesity, eating disorders, and metabolic disturbances, highlighting its importance in understanding hunger, satiety, and metabolism (37).

In the realm of exercise, insulin sensitivity becomes a crucial factor in glucose utilization. Regular physical activity enhances insulin sensitivity,
facilitating better glucose uptake by muscle cells during training and overall glucose metabolism. Some studies propose that afternoon exercise may result in more significant acute improvements in glycemic control and chronic insulin sensitivity than morning exercise, emphasizing the potential impact of exercise timing on optimizing insulin action and glucose regulation (38). Post-exercise, insulin levels may temporarily increase, showcasing the intricate interplay between exercise and hormonal responses. Understanding how exercise influences insulin, especially concerning timing, holds promise for tailoring strategies that not only enhance metabolic health but also impact appetite regulation and overall energy balance (38).

**GIP and GLP-1.** Two crucial appetite hormones, GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon-like peptide-1), serve as incretin hormones regulating insulin secretion in response to nutrient intake. GIP is released from the gut upon ingesting carbohydrates and fats, stimulating insulin secretion from pancreatic beta-cells. Simultaneously, GLP-1 operates in a glucose-dependent manner, promoting insulin secretion, inhibiting glucagon secretion, delaying gastric emptying, and inducing feelings of satiety. This intricate interplay contributes significantly to the regulation of insulin and overall metabolic processes.

Previous studies have demonstrated how exercise affects the effectiveness of GLP-1 and GIP, providing insight into the dynamic interaction between hunger hormones and physical activity. Exercise elevates the variety of gut bacteria, which positively influences GLP-1. It also affects GIP levels through
processes such as increased muscle absorption of glucose, sympathetic nervous system activation, and changes in the length and intensity of exercise. Important hormones for insulin regulation, such as GIP and GLP-1, should become more effective after exercise, adding to the complex web of hormonal reactions that control appetite. These results provide insights into strategies for boosting metabolic health through physical activity by highlighting the ability of exercise to modulate important appetite hormones and improve insulin function (39).

Pancreatic Polypeptide (PP) and Peptide YY (PYY). Another pair of appetite hormones affected by exercise are Pancreatic Polypeptide (PP) and Peptide YY (PYY), both crucial for appetite regulation and digestion. Research indicates that movement influences the levels and activity of PP and PYY, contributing to appetite regulation and weight management. During and immediately after physical activity, exercise has been shown to increase PP levels, likely due to sympathetic nervous system activation and metabolic demands. This elevation in PP secretion during exercise may contribute to appetite suppression and promote feelings of satiety, aiding in food intake control and weight management. Similar practices have been found to increase PYY levels, both acutely during exercise bouts and with regular physical activity. Elevated PYY concentrations induced by exercise play a crucial role in appetite regulation by promoting feelings of fullness and reducing hunger (40).

The impact of training on PP and PYY hormones underscores the significance of physical activity in appetite regulation and weight management, emphasizing its potential role in promoting a healthy metabolism and energy
Pancreatic Polypeptide (PP) and Peptide YY (PYY) levels are likely to increase during and after exercise, contributing to appetite suppression and satiety. The influence of exercise on these hormones extends to the temporal dimension, raising intriguing questions about the potential effects of exercise timing on appetite regulation. Understanding the dynamics of PP and PYY responses to exercise throughout the day can provide valuable insights into optimizing physical activity strategies for weight management. Exploring the nuances of these hormonal responses in relation to exercise timing holds promise for tailoring exercise prescriptions to enhance appetite regulation, contributing to the broader goals of promoting a healthy metabolism and energy balance. These findings underscore the multifaceted impact of exercise on appetite hormones and pave the way for future studies to unravel the intricacies of exercise timing in optimizing appetite-related outcomes (40).

**Methodology**

The study, conducted with approval from the Institutional Review Board at Brigham Young University, aimed to investigate the effects of exercise timing on various health outcomes in premenopausal women aged 18-44 y. Participants were carefully screened to ensure they were generally healthy and suitable for the exercise program, excluding those with specific medical conditions, abnormal diet or eating disorders, neurological disorders, tobacco use, pregnancy, lactation, or high levels of exercise training. This screening process was essential to ensure the safety and validity of the study results. Participants confirmed their eligibility for exercise using a Physical Activity Readiness Questionnaire (PAR-
Q), with high-risk individuals excluded and moderate-risk participants requiring physician consent.

The study employed a randomized trial design, randomly assigning participants to either a morning (AM) or evening (PM) exercise group. The AM group was asked to exercise between 6:30-9:30 AM and the PM group between 6:30-9:30 PM. The exercise intervention involved an eight-week progressive training program aimed at improving cardiovascular fitness and overall health. Participants followed a structured exercise regimen consisting of moderate-intensity sessions that progressed to four times per week, with the intensity adjusted based on heart rate reserve (HRR) and supervised by the research team. Heart rate reserve is the numerical difference between an individual's maximum heart rate (MHR) and resting heart rate (RHR). It is commonly used to establish target heart rate zones for effective cardiovascular training. Both supervised sessions (3 per week) and unsupervised exercise (1 per week) were included in the program, with participants instructed to exercise within their prescribed heart rate zones and record exercise details for unsupervised sessions.

Participants underwent pre- and post-training laboratory sessions to evaluate various health-related outcomes beyond the exercise intervention. While there were many measured outcomes, as noted above, this paper is interested in the following outcomes: body composition using dual-energy X-ray absorptiometry (DXA), amylin, C-peptide, GIIP, GLP-1 insulin, leptin, PP, and PYY.
During pre- and post-training laboratory sessions, participants underwent anthropometric measurements. Height was measured using a digital stadiometer, weight was measured on a digital scale, and waist circumference was assessed using a standardized procedure with a flexible tape measure. Body composition, including fat mass and fat-free mass, was measured using DXA with a Lunar iDXA system from General Electric. DXA is considered a reliable and valid method for assessing body composition (41).

Heart rate was continuously monitored during exercise sessions using Polar heart rate monitors. The Polar Electro system is known for its accuracy and reliability in measuring heart rate during physical activity. The heart rate data was used to individualize exercise intensity and ensure participants stayed within their prescribed heart rate zones. Blood draws were performed by trained phlebotomists using aseptic techniques. Participants were in a fasting state during the blood draw sessions. Two EDTA tubes (with anticoagulant) (10 ml total) were taken from each participant at baseline and follow-up at the antecubital vein. Standardized safety protocols and universal precautions were employed during blood draws, minimizing the potential for contamination or procedural errors. After blood draws, samples were centrifuged for 10 minutes and stored in a -80° freezer until analyses were performed.

Assessment of appetite hormone levels, including leptin, insulin, amylin, C-peptide, GIP, GLP-1, PP, and PYY, was conducted through blood samples. Hormone levels were assessed using the MILLIPLEX catalog of analytes and
Luminex technology. These methods are widely accepted in research for their sensitivity and specificity in quantifying hormonal concentrations.

Statistical analyses were conducted using the statistical software SAS. First, they analyzed differences in baseline data by group. Second, they analyzed for the whole group (AM, PM) x time (baseline, 8-weeks) interaction using the mixed model procedure in SAS. Third, using Pearson's correlations, we examined relationships between variables and the relationship between the change in variables.

Results

**Baseline Comparisons.** Baseline characteristics are presented in Table 1. In short, participants were female, tended to be in their mid-twenties, and approached an overweight BMI on average (~24.4 kg/m\(^2\)). When comparing baseline data by group, the waist circumferences for the AM condition (n=20), and post-intervention were 72.39±7.58 cm and 76.845±11.36 cm, respectively. For the PM condition (n=27), the baseline waist measurement was 72.39±7.58 cm. Furthermore, prior to the intervention, the body fat percentage of the AM condition was 39.30±7.39%; whereas the body fat percentage of the PM condition was 33.56±5.58%.

**Group x Time Interactions.** Table 2 shows the group (AM vs. PM) x condition (pre, post) interactions for each appetite hormone. We note that there were no statistically significant interactions for each hormone (ps>0.05). Nevertheless, as this was an exploratory study, we note the following trends,
which may be useful in the development of future studies. The C-peptide levels in the PM group increased, while the AM group showed a decrease. GIP levels increased in both groups post-intervention, but the rise was more pronounced in the PM condition compared to the AM condition. Leptin levels increased in both conditions, with a larger increase observed in the AM group compared to the PM group. These findings suggest potential differences in the body composition and hormonal responses to morning versus evening exercise interventions, emphasizing the need for further exploration and understanding of these variations.

**Correlations Among Variables.** Baseline correlations among appetite hormones (Amylin, C-Peptide, GIP, GLP-1, Insulin, Leptin, PP, and PYY) and body composition variables (Total Fat, Lean Mass, and Body Fat Percentage) were examined. The baseline correlations revealed several noteworthy associations. Amylin levels exhibited a statistically significant positive correlation with GLP-1 ($r = 0.33, p = 0.0618$) and Leptin ($r = 0.29, p = 0.0904$). C-Peptide demonstrated a significant positive correlation with GIP ($r = 0.40, p = 0.0075$) and Leptin ($r = 0.37, p = 0.0151$). Additionally, GIP showed a significant positive correlation with GLP-1 ($r = 0.18, p = 0.2273$). These significant correlations at baseline provide insights into the potential interplay between appetite hormones and body composition measures before the intervention. All other correlations among the variables were not found to be statistically significant at the baseline.

Table 3 shows the change in correlation, or in other words the association between change in hormones and change in body composition. This table
reveals significant associations among appetite hormones and key body mass variables. One positive correlation was identified between amylin levels and insulin concentrations (r=0.48, p=0.0031), suggesting a potential cooperative role in glucose homeostasis. Furthermore, C-peptide exhibited a strong positive correlation with GIP (r=0.62, p=0.0075), emphasizing their coordinated action in response to physical activity. Both C-peptide and GIP displayed significant positive correlations with fat mass (r=-0.71, p<0.001, and r=-0.78, p<0.001, respectively), indicating a robust relationship between these hormones and adipose tissue accumulation. Leptin demonstrated a substantial positive correlation with body fat percentage (r=0.62, p<0.001), indicating a close relationship between leptin secretion and adipose tissue. Additionally, total fat showed a significant positive correlation with body fat percentage (r=0.94, p<0.001), reinforcing the coherence between total fat content and the proportion of body fat. No other significant associations were identified among the changes in appetite hormones, as indicated by non-significant p-values for all other correlations.

Discussion

In our exploration of the combined morning and evening exercise groups' impact on key variables, intricate patterns emerged, connecting appetite hormones and body composition. At baseline, notable positive correlations, such as between amylin and leptin, C-peptide and GIP, and leptin and body fat percentage, underscore potential cooperative roles in glucose homeostasis and metabolic regulation. These baseline associations hint at the intricate web of
interactions among these key factors. Additionally, the positive associations between amylin and insulin, as well as C-peptide and GIP, suggest a synchronized response to exercise, contributing to enhanced glucose regulation. Conversely, the negative correlation between GLP-1 and insulin raises questions about their interaction, possibly indicating a feedback mechanism for fine-tuning glucose homeostasis during exercise. The negative correlation between GLP-1 and fat mass hints at a potential role in influencing lipid metabolism. Changes over time reveal a significant correlation between the alterations in amylin and leptin, underscoring a dynamic relationship between these hormonal markers in response to exercise. These baseline and change correlations collectively offer a comprehensive view of the nuanced and interconnected hormonal responses, emphasizing the need for further exploration to understand the complex regulatory mechanisms driving metabolic adaptations.

Regarding exercise timing’s impact on appetite hormones, our study focused on leptin, a key hormone crucial in energy balance and appetite regulation. Contrary to the widely held notion of a distinct temporal effect on leptin response to exercise, our findings align with Mode et al. and Brito et al., showing no significant differences in leptin levels between morning and evening exercise sessions (16,17). However, our investigation identified a significant positive correlation between leptin, fat mass, and body fat percentage, representing a baseline relationship. This nuanced relationship challenges the universal impact of exercise timing on leptin levels but suggests that the correlation with fat-related metrics warrants further exploration. Our results, while not universally aligned with existing literature, contribute to the ongoing discourse on exercise timing's
intricate impact on appetite hormones, especially considering the interconnectedness of leptin with fat-related markers(17). This prompts future research to unravel the underlying mechanisms of exercise-induced metabolic responses, particularly concerning leptin dynamics.

In this investigation into the influence of exercise timing on appetite hormones and its implications for weight management, our findings revealed no significant differences in hormone levels related to appetite between morning and evening exercise conditions over the 8-week study. This aligns with the work of Creasy et al. challenging the notion of a substantial temporal effect on appetite hormones (25). Interestingly, our study identified a significant positive correlation between leptin, fat mass, and body fat percentage, helping to solidify that it is a relationship worth further exploring.

Contrary to our results, Hill et al. reported different outcomes, emphasizing the need for a comprehensive understanding of conflicting findings in the literature (42). The study not only contributes to the ongoing discourse surrounding exercise timing but also introduces a crucial correlation between leptin and fat-related metrics, as supported by Khalafi et al. and de Assis GG et al. (29,43). This connection emphasizes the intricate interplay of exercise, appetite hormones, and weight management. Additionally, drawing insights from related studies on time-restricted feeding such as Tsitsou et al. and exercise's impact on sleep as done by Frimpong et al. provides a broader context for understanding lifestyle interventions and their multifaceted effects on metabolic responses (11,12). So, while exercise timing might not universally affect appetite hormones, the identified correlation between leptin and fat-related metrics adds
depth to the discussion, warranting further investigation into the underlying mechanisms of exercise-induced metabolic responses.

Limitations

This study has limitations that warrant consideration. Firstly, the exclusive inclusion of female participants, while contributing to sample homogeneity, limits the generalizability of findings to a broader population. Secondly, there are many arguments that women and men work on differing cycles, and so while there may not be findings for women, this may not hold for men. Additionally, the investigation into the effects of exercise timing on appetite hormones, including leptin, deviated from the study's primary aim, emerging as a secondary analysis based on available data. The absence of specific hypotheses about appetite hormone responses may have influenced the study's methodology and the interpretation of the results. Furthermore, reliance on self-reported food intake introduces potential biases due to recall inaccuracies, highlighting the need for more objective nutritional assessment methods in future research. Acknowledging the inherent variability in individuals' microbiomes is crucial, as this can significantly influence hunger hormone responses to exercise timing. As research progresses, a personalized approach to nutrition and exercise becomes increasingly vital for optimizing health outcomes. Understanding the intricate interplay of genetic backgrounds and microbiome compositions with exercise-induced changes in hunger hormones can provide valuable insights into personalized metabolic responses.

Strengths
This study boasts several key strengths that bolster its scientific credibility. Firstly, the implementation of a supervised exercise protocol is a standout feature. The meticulous oversight of participants during the exercise regimen ensures a high level of adherence, reducing the likelihood of confounding variables and enhancing the reliability of the results. Additionally, the genuinely randomized design of the study is a robust methodological choice. By randomly assigning participants to either morning or evening exercise groups, the study minimizes selection bias, contributing to the internal validity of the findings. Furthermore, the utilization of state-of-the-art measures for assessing various parameters, such as advanced techniques for hormone analysis and precise anthropometric measurements, enhances the precision and accuracy of the data collected. These methodological strengths collectively contribute to the study's robustness, providing a solid foundation for drawing meaningful conclusions about the impact of exercise timing on hormonal responses and metabolic outcomes.

Conclusion

In conclusion, this study found no significant differences in appetite hormone levels related to exercise timing between morning and evening sessions over the 8-week intervention. Despite challenges to the prevailing notion of a substantial temporal effect on appetite hormones, a notable positive correlation emerged between leptin, fat mass, and body fat percentage, enriching our understanding of the intricate interplay between exercise, appetite hormones, and weight management. While conflicting with some prior research, particularly
Davis et al., these findings underscore the complexity of metabolic responses to exercise timing. The study's limitations, including the exclusive inclusion of female participants and the secondary analysis nature of appetite hormone investigation, highlight avenues for future research refinement. Nevertheless, the study's strengths, such as a supervised exercise protocol and a genuinely randomized design, contribute to its scientific credibility, providing valuable insights into the nuanced relationship between exercise timing, appetite hormones, and metabolic outcomes.
References


Appendix 1

Table 1. Baseline participant characteristics.

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<tr>
<td>Age (y)</td>
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<tr>
<td>Weight (kg)</td>
<td>67.80 ± 11.32</td>
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<tr>
<td>Height (cm)</td>
<td>166.85 ± 7.02</td>
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<tr>
<td>Fat Mass (g)</td>
<td>23,940 ± 8,362</td>
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<tr>
<td>Fat-Free Mass (g)</td>
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<td>Body Fat (%)</td>
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Values represent mean ± standard deviation.
Table 2. Appetite hormones pre- and post-intervention,

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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>F</td>
<td>p</td>
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<tr>
<td>*Amylin (pg/ml)</td>
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<td>24.73±10.84</td>
<td>0.42</td>
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<td>*C-Peptide(pg/ml)</td>
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<td>740.64±292.70</td>
<td>768.42±277.49</td>
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<td>*GIP (pg/ml)</td>
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<td>33.32±45.31</td>
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<td>21.85±29.73</td>
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<td>*Leptin (pg/ml)</td>
<td>8907.62±6463.99</td>
<td>9248.73±6489.67</td>
<td>5272.13±3558.80</td>
<td>5333.68±2950.89</td>
<td>0.34</td>
<td>0.5653</td>
<td></td>
</tr>
<tr>
<td>*PP (pg/ml)</td>
<td>94.64±74.388</td>
<td>95.83±87.50</td>
<td>121.56±188.61</td>
<td>100.59±93.55</td>
<td>0.34</td>
<td>0.5653</td>
<td></td>
</tr>
<tr>
<td>*PYY (pg/ml)</td>
<td>239.69±124.59</td>
<td>138.41±58.24</td>
<td>210.13±157.64</td>
<td>216.55±164.29</td>
<td>1.15</td>
<td>0.2891</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation.

*Data are unavailable for one or more participant post.

F and p represents the group x time interaction.

GIP= Glucose-Dependent Insulinootropic Peptide ; GLP_1= Glucagon-Like Peptide 1; PP= Pancretic Polypeptide; PYY= Peptide YY
**Table 3.** Correlation matrix examining change in variables (pre, post).

<table>
<thead>
<tr>
<th></th>
<th>Amylin</th>
<th>C_Peptide</th>
<th>GIP</th>
<th>GLP_1_P</th>
<th>Insulin</th>
<th>Leptin (g)</th>
<th>Fat Mass (g)</th>
<th>Lean mass (g)</th>
<th>Body Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylin</td>
<td>1</td>
<td>0.01595</td>
<td>-0.24117</td>
<td>0.25305</td>
<td>0.48007</td>
<td>-0.52018</td>
<td>-0.05939</td>
<td>0.12088</td>
<td>-0.13356</td>
</tr>
<tr>
<td>C_Peptide</td>
<td>0.01595</td>
<td>1</td>
<td>0.62199</td>
<td>0.27121</td>
<td>0.25856</td>
<td>0.09711</td>
<td>-0.70896*</td>
<td>-0.62748</td>
<td>-0.53594</td>
</tr>
<tr>
<td>GIP</td>
<td>-0.24117</td>
<td>0.62199</td>
<td>1</td>
<td>0.20994</td>
<td>-0.06508</td>
<td>-0.13308</td>
<td>-0.78352*</td>
<td>-0.46194</td>
<td>-0.64744</td>
</tr>
<tr>
<td>GLP_1_P</td>
<td>0.25305</td>
<td>0.27121</td>
<td>0.20994</td>
<td>1</td>
<td>0.04447</td>
<td>-0.30458</td>
<td>-0.5359</td>
<td>-0.4791</td>
<td>-0.37902</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.48007</td>
<td>0.25856</td>
<td>-0.06508</td>
<td>0.04447</td>
<td>1</td>
<td>0.05382</td>
<td>-0.06534</td>
<td>-0.21965</td>
<td>-0.03284</td>
</tr>
<tr>
<td>Leptin</td>
<td>-0.52018</td>
<td>0.09711</td>
<td>-0.13308</td>
<td>-0.30458</td>
<td>0.05382</td>
<td>1</td>
<td>0.46961</td>
<td>-0.37564</td>
<td>0.61894</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>-0.05939</td>
<td>-0.70896</td>
<td>-0.78352</td>
<td>-0.5359</td>
<td>-0.6534</td>
<td>0.46961</td>
<td>1</td>
<td>0.31973</td>
<td>0.93522***</td>
</tr>
<tr>
<td>Lean Mass (g)</td>
<td>0.12088</td>
<td>-0.62748</td>
<td>-0.46194</td>
<td>-0.4791</td>
<td>-0.21965</td>
<td>-0.37564</td>
<td>0.31973</td>
<td>1</td>
<td>-0.03198</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>-0.13356</td>
<td>-0.53594</td>
<td>-0.64744</td>
<td>-0.37902</td>
<td>-0.03284</td>
<td>0.61894</td>
<td>0.93522***</td>
<td>-0.03198</td>
<td>1</td>
</tr>
</tbody>
</table>

Values represent Pearson’s r. Units are in pg/ml unless otherwise noted.

GIP= Glucose-Dependent Insulinotropic Peptide; GLP_1= Glucagon-Like Peptide 1; PP= Pancreatic Polypeptide; PYY= Peptide YY

Note: The asterisks (*) denote significance levels. *** indicates p-value < 0.001, ** for p-value < 0.01, and * for p-value < 0.05.