THE ACUTE EFFECT OF EXERCISE ON ENERGY INTAKE IN OVERWEIGHT WOMEN

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Introduction: The role of exercise on short-term appetite regulation is not known. Furthermore mechanisms mediating this relationship need to be established. **Purpose:** The purpose of this study was to examine how a single bout of exercise influenced energy intake, subjective feelings of hunger, GLP-1 and acylated ghrelin concentrations compared to an exercise condition. **Methods:** A total of 19 overweight/obese women (BMI: 32.5 ± 4.3 kg/m^2; age 28.5 ± 8.3 years) underwent two experimental testing sessions (exercise and rest) which were separated by at least 2 days. For the exercise session, subjects walked on a treadmill at a moderate intensity (70-75% of age-predicted maximal heart rate) until an energy expenditure of 3.0 kcals/kg of body weight was achieved. During the resting condition, subjects rested quietly for a similar length of time. Blood was drawn prior to exercise/rest, immediately post-exercise/rest, 30-minutes post, 60-minutes post, and 120-minutes post-exercise/rest and was analyzed for acylated ghrelin and GLP-1 concentrations. Subjective feelings of hunger were measured using a Likert scale prior to each blood draw. From 1-2 hours post-exercise subjects were provided ad-libitum access to a buffet-style meal and energy intake was calculated based upon food intake during this period. **Results:** There was no difference in energy intake between conditions (exercise: 551.5 ± 245.1 vs. rest: 548.7 ± 286.9 kcals). However, relative energy intake, taking into account the energy cost of exercise, was significantly lower in the exercise condition (197.8 ± 256.5 kcals) compared to the resting condition (504.3 ± 290.1 kcals; $p<0.001$). Exercise did not significantly alter acylated ghrelin, GLP-1, or subjective feelings of hunger from pre-testing to post-testing, nor were differences observed between conditions across the entire experimental testing session ($p>0.05$). **Conclusion:** Exercise does not appear to acutely influence energy intake in an overweight/obese population, thus making it a valuable component for managing body weight. Future studies should explore potential physiological or psychological mechanisms to explain why energy intake is not increased following a bout of moderate-intensity exercise in this population.

TABLE OF CONTENTS

1.0 INTRODUCTION..1

1.1 PHYSICAL ACTIVITY 2

1.2 CLINICAL RATIONALE...3

1.2.1 Acute Effects of Exercise on Energy Intake 4

1.2.2 Glucagon-like Peptide 1 5

1.2.3 Ghrelin...6

1.3 THEORETICAL RATIONALE 7

1.4 SPECIFIC AIMS 10

1.5 HYPOTHESES 11

1.6 SIGNIFICANCE...12

2.0 REVIEW OF LITERATURE 13

2.1 INTRODUCTION 13

2.2 ENERGY BALANCE..13

2.3 ACUTE EFFECT OF EXERCISE ON ENERGY INTAKE IN LEAN SUBJECTS...15

2.4 EXERCISE CONSIDERATIONS...17

2.4.1 Exercise Intensity 17

2.4.2 Total Energy Expenditure..18

2.5 ENERGY INTAKE CONSIDERATIONS....19

2.5.1 Assessment of Energy Intake....19

2.5.2 Prior Feeding Status....20

2.5.3 Macronutrient Composition of Foods Provided....21

2.6 SUBJECT CHARACTERISTICS....23

2.6.1 Training Status of Subjects....23

2.6.2 Body Weight of Subjects....24

2.7 ACUTE EFFECT OF EXERCISE ON ENERGY INTAKE IN OVERWEIGHT SUBJECTS....25

2.8 ACUTE EFFECT OF EXERCISE ON SUBJECTIVE FEELINGS OF HUNGER....29

2.9 APPETITE REGULATION....30

2.10 APPETITE REGULATING HORMONES....31

2.10.1 Ghrelin....32

2.10.2 Glucagon-Like Peptide 1....34

2.11 POTENTIAL FACTORS THAT MAY AFFECT ENERGY INTAKE....36

2.11.1 Dietary Restraint....37

2.11.2 Mood....37

2.11.3 Sleep....38

2.12 CONCLUSION....39

3.0 METHODOLOGY....40

3.1 SUBJECTS....40

3.2 RECRUITMENT AND SCREENING PROCEDURES....41

3.3 EXPERIMENTAL DESIGN 42
3.3.1 Assessment Visit 43
3.3.2 Testing Sessions 43
3.4 ASSESSMENT COMPONENTS 48
3.4.1 Height..48
3.4.2 Body Weight 48
3.4.3 Resting Energy Expenditure 48
3.4.4 Body Composition 50
3.4.5 Graded Exercise Test 50
3.5 PRIMARY OUTCOME MEASURES 51
3.5.1 Blood Analysis 51
3.5.2 Mood/Hunger Questionnaire 52
3.5.3 Measurement of Energy Intake 53
3.6 STATISTICAL ANALYSIS 54
3.7 POWER ANALYSIS 55
4.0 RESULTS 57
4.1 SUBJECTS..57
4.2 DURATION AND ENERGY EXPENDITURE OF EXPERIMENTAL SESSIONS 61
4.3 ANALYSIS OF DATA BY SPECIFIC AIM...63
4.3.1 Specific Aim 1: Comparison of Ad-Libitum Energy Intake Following the Resting and Exercise Experimental Sessions 63

4.3.2 Specific Aim 2: Comparison of Acylated Ghrelin Concentrations Following Exercise and Rest....68

4.3.3 Specific Aim 3: Comparison of GLP-1 Concentrations Following Exercise and Resting Conditions....72

4.3.4 Specific Aim 4: The Effect of Exercise on Subjective Feelings of Hunger 74

4.4 EXPLORATORY ANALYSES....79

4.4.1 Mood....79

4.4.1.1 Comparison of Mood Constructs Between Experimental Testing Sessions 80

4.4.1.2 Influence of Positive Affect on Energy Intake....83

4.4.2 Correlational Analyses....85

5.0 DISCUSSION....87

5.1 INTRODUCTION....87

5.2 SUMMARY OF MAJOR FINDINGS....88

5.2.1 The Influence of Exercise on Energy Intake....88

5.2.2 The effect of Exercise on Acylated Ghrelin Concentrations....95

5.2.3 The Effect of Exercise on GLP-1....99

5.2.4 The Effect of Exercise on Subjective Feelings of Hunger....101

5.2.5 Energy Expenditure of the Exercise Bout....104

5.2.6 Psychological Parameters....105

5.3 LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH....108

5.4 CONCLUSIONS 111
APPENDIX A 113
APPENDIX B 126
APPENDIX C 129
APPENDIX D 131
APPENDIX E 133
APPENDIX F 135
APPENDIX G 137
APPENDIX H 141
APPENDIX I 142
APPENDIX J 145
BIBLIOGRAPHY 148

LIST OF TABLES

Table 1 - Descriptive statistics 60

Table 2: Testing and resting sessions..62

Table 3: Correlational matrix for the difference in energy intake between testing conditions and descriptive variables...65

Table 4: Ad-libitum energy intake 1-2 hours post-testing and fluid intake throughout the testing period 66

Table 5: Changes in appetite-regulating hormone concentrations throughout the testing days 71

Table 6: Change in hunger ratings throughout the exercise and resting testing days 78

Table 7: Changes in mood across the exercise and resting conditions...........................81

LIST OF FIGURES

Figure 1: Theoretical pathways by which exercise may influence body weight.................8

Figure 2: Potential ways in which exercise can influence energy balance.......................10

Figure 3: An example of how exercise can influence energy intake 10

Figure 4: Experimental design 47

Figure 5: Study enrollment and randomization...58

Figure 6: Difference in energy intake between experimental sessions* for each individual subject 64

Figure 7: Energy intake expressed relative to body weight for each BMI category.........67

Figure 8: Change in acylated ghrelin concentrations over time for testing condition 70

Figure 9: Change in total GLP-1 concentration over time for testing conditions.............74

Figure 10: Changes in subjective feelings of hunger throughout the course of testing...77

Figure 11: Changes in positive affect over time 83

Figure 12: Difference in energy intake between conditions for those with improved and worsened positive affect 85

1.0 INTRODUCTION

Obesity is currently a major health concern for millions of Americans. Using body mass index (BMI) to classify individuals, 65% of adults in the United States are overweight (BMI$\geq$25.0 kg/m^2) and over 30% are obese (BMI$\geq$30 kg/m^2) [1]. Excess body weight is associated with many adverse health consequences including an increased risk of mortality [2, 3], cardiovascular disease [4-6], diabetes [7-9], and certain forms of cancer [10,11]. Despite the serious health risks associated with being overweight, the incidence of obesity is rapidly rising [1]. These alarming statistics support the need to further examine the factors that may impact the regulation of body weight.

Body weight is controlled by two variables: energy intake (EI) and energy expenditure (EE). When energy intake is equal to energy expenditure a person is weight stable, or in a state of energy balance. However, any disruptions to this equilibrium can result in either an increase or decrease in body weight. When energy intake is greater than energy expenditure, a positive energy balance ensues and weight gain occurs. Conversely, when energy expenditure is greater than energy intake, a state of negative energy balance is achieved, resulting in a reduction in body weight. Current recommendations for weight loss include reducing caloric intake and increasing physical activity, which influence energy intake and energy expenditure respectively [12].

However, research has not thoroughly examined the interaction between these two behaviors and how this may impact body weight regulation.

1.1 PHYSICAL ACTIVITY

The role of physical activity in the prevention and treatment of obesity is still somewhat controversial. In regards to initial weight loss, exercise is thought to produce modest reductions in body weight. In a literature review conducted by Wing et al. [13], it was estimated that exercise alone produced an average weight loss of 1-2 kg compared to control conditions. Conversely, a diet only lifestyle modification program has been shown to produce a 9 kg weight loss in a group of overweight subjects at the end of 6 months [14]. The Expert Panel on the Identification, Evaluation and Treatment of Overweight and Obesity in Adults concluded that when 30-60 minutes of exercise, 3 times per week, was used in conjunction with calorie restriction, the addition of exercise resulted in an extra 1.9 kg weight loss compared to calorie restriction alone [15]. Thus, exercise appears to only have a modest impact on weight loss, compared to dietary alterations.

In terms of weight maintenance, the role of exercise appears to be more prominent. Physical activity has been shown to be one of the most important predictors of long-term weight maintenance [16]. Previous prospective [17] and retrospective [18] studies indicate that high levels of physical activity are needed to maintain significant weight losses over time. Individuals in the National Weight Control Registry (NWCR) who have succeeded in losing at least 30 pounds and keeping that weight off for at least one year, have reported expending over 2500 kcals/week in physical activity [18]. However, the question remains as to why physical activity is so important for weight maintenance.

This positive effect of physical activity in the regulation of body weight may be due to the fact that physical activity increases energy expenditure, which impacts energy balance. However, some research also suggests that physical activity may assist in weight control by influencing energy intake or altering metabolic pathways that affect hunger/satiety [19]. Recent work by Hubert et al. [19] demonstrated that exercise training leads to an improved ability to regulate energy intake in response to either a high or low-energy preload meal. Other studies have indicated that trained individuals may have an improved awareness of energy needs [20], and this response may be governed by a change in hormonal output [21] or differences in substrate utilization during and following exercise [22, 23]. Whether an acute bout of physical activity also regulates energy intake in sedentary and overweight individuals is not yet known.

1.2 CLINICAL RATIONALE

Previous work in both human and animal models has demonstrated that a negative energy balance induced through calorie restriction results in a compensatory increase in food intake to restore energy homeostasis [24, 25]. However, this concept does not necessarily apply when a negative energy balance of a similar magnitude is achieved through exercise [26]. In general, there appears to be a loose coupling between energy intake and energy expenditure implying that a compensatory increase in energy intake (equal to the energy expenditure of exercise) is rarely observed shortly following a single bout of exercise. These findings indicate that exercise uniquely alters some mechanism of energy homeostasis preventing this compensatory response in energy intake to occur post-exercise [24]. A better understanding of the role of exercise in weight control can be established by understanding how an acute bout of exercise influences subsequent food intake in an overweight, sedentary population and identifying the mechanisms that mediate the relationship between exercise and energy intake.

1.2.1 Acute Effects of Exercise on Energy Intake

Studies examining the acute effects of exercise on energy intake have produced mixed findings. Some studies have reported a slight increase in caloric intake following exercise compared to a resting condition [27-30], others have found that exercise served as an appetite suppressant [31], while the majority of studies reported that energy intake was unaltered shortly following a single bout of exercise [22, 31-34]. However, in some studies where no difference in energy intake was detected following exercise compared to a control condition, it is important to note that subjective feelings of hunger were lower in the exercise condition compared to the resting condition [22, 32, 34]. Overall, these inconclusive findings may be the result of methodological differences between studies which include the exercise intensity and duration utilized, the time at which food intake was monitored following exercise, levels of dietary restraint, and differences in body weight between the subjects.

The majority of the studies conducted in this area of research have focused on lean individuals [22, 28, 29, 32, 34-36]. However, it has been suggested that energy intake immediately following exercise may vary between obese and non-obese subjects [31, 37, 38]. Since a better understanding of the effect of exercise on energy intake is most critical for the overweight and/or obese population, more studies should be conducted to determine whether exercise contributes towards weight loss and weight maintenance efforts or whether it positively assists in the regulation of energy intake, thus aiding in weight control. Additionally, the majority of the studies that have examined food intake immediately following a bout of exercise have not explored possible mechanisms governing the relationship between physical activity and food intake. A better understanding of how certain appetite-regulating hormones are altered during exercise may lead to an increased knowledge of this relationship between exercise and energy intake.

1.2.2 Glucagon-like Peptide 1

Glucagon-like peptide 1 (GLP-1) is involved in the short-term regulation of appetite and has been shown to decrease food intake and increase satiety in both lean [39] and obese [40] subjects. However, very little research has been conducted to examine the acute effect of a single bout of exercise on GLP-1 concentrations. One study found that in response to an acute bout of moderate intensity exercise, GLP-1 was significantly elevated above resting conditions both during exercise and for one hour following the cessation of exercise in normal weight subjects [30]. Conversely, another study conducted in athletes found that running at a high-intensity had no effect on postprandial GLP-1 concentrations compared to a resting condition [41]. Currently, only one study has explored how GLP-1 is affected by a bout of exercise in an overweight and/or obese population. In a group of obese males and females, Adam et al. [42] found that GLP-1 was not increased immediately following a low-intensity bout of exercise and that this response was different than what was seen in a group of normal weight subjects. Thus, future studies are warranted to examine how GLP-1 is altered following exercise in overweight/obese individuals.

1.2.3 Ghrelin

Ghrelin is a hormone involved in the short-term regulation of appetite and is classified as an orexigenic hormone, meaning that it has an appetite stimulating effect. Ghrelin has been shown to increase energy intake when administered both peripherally and centrally [43]. In response to a single bout of exercise, the majority of the studies have reported ghrelin levels to be unaltered [30, 44, 45]. However, these studies only examined total ghrelin concentrations. Ghrelin exists in two forms: nonacylated and acylated, and only acylated ghrelin is involved in appetite regulation [46]. Only two studies have examined how acylated ghrelin concentrations respond to an acute bout of exercise. Broom et al. [47] found levels of acylated ghrelin to be reduced for nine hours following exercise (running 72% VO_{2max} for 60 minutes) in young, physically active males while Marzullo et al. [48] examined the effect of an incremental maximal cycle ergometer test on acylated ghrelin concentrations in 8 obese and 8 normal weight subjects. In this study, both groups of subjects had a decrease in peak acylated ghrelin concentrations but this response was lower in the obese subjects.

Due to the lack of research in this area, there is clearly a need for future studies to examine how GLP-1 and acylated ghrelin respond to an acute bout of exercise in an overweight/obese population. Additionally, it is important to determine if changes in the concentration of these two hormones influence hunger and energy intake.

1.3 THEORETICAL RATIONALE

Despite the significant role that exercise plays in weight maintenance, the mechanism through which exercise exerts its effect on body weight is currently unknown. Figure 1 illustrates three potential theoretical pathways through which exercise may influence body weight. One theory is that the energy expended during exercise simply creates an energy deficit, resulting in a state of negative energy balance. Another possibility is that an acute bout of exercise may regulate appetite through alterations in hormone concentrations, which may directly influence body weight. Lastly, the theoretical pathway that is being examined in this study is highlighted. In theory, exercise may have the ability to alter various metabolic parameters including some appetite regulating hormones that would then influence feelings of hunger, and alter energy intake. Thus, particular attention will be paid to the acute effect of physical activity on energy intake.

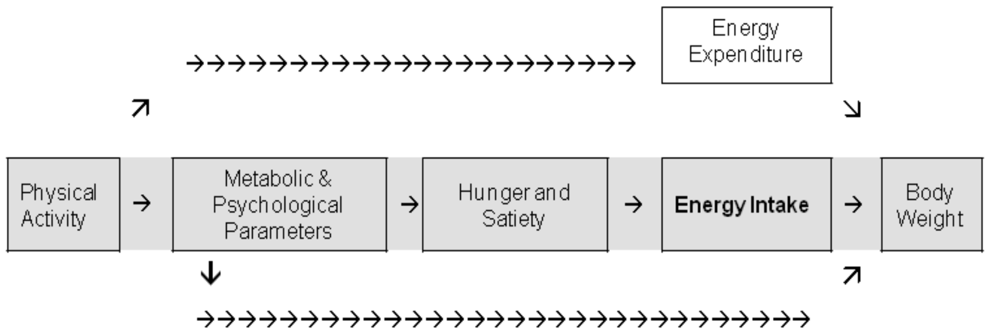

Figure 1: Theoretical pathways by which exercise may influence body weight

Theoretically, when compared to a resting condition, exercise could impact energy intake in one of three ways (see Figure 2). First, exercise could result in an increase in hunger and subsequent increase in energy intake compared to a resting condition. However, the degree of compensation would determine whether a state of negative, positive, or neutral energy balance was achieved. When the energy expended during exercise is partially compensated for, a state of negative energy balance would occur. For example, if an individual expends 300 calories in a session of exercise and only 100 calories while resting for the same period of time, the person would have created an energy deficit of 200 calories through exercise. However, suppose immediately following exercise, an individual then consumes 100 calories more than what he/she consumed following the resting condition. This would leave the subject in a negative energy deficit of 100 calories (see Figure 3). However, when the energy expended during exercise is fully compensated for, or overcompensated for, states of energy balance and positive energy balance would occur respectively. In the example previously described, if an individual consumes 200 calories more following exercise compared to a resting condition, this would cause a full compensation, leaving him/her in a state of energy balance. If he/she were to consume 300 calories more than a resting condition, this would be classified as overcompensation and result a positive energy balance equivalent to 100 calories.

The second manner through which exercise could possibly influence energy intake would be through a suppression of hunger and reduction in energy intake compared to a resting condition. This decrease in energy intake would result in a state of negative energy balance, above and beyond that which was produced by exercise alone. In the example used in Figure 3, if the individuals were to consume 200 calories fewer following exercise compared to what he/she consumed following the rest period, it could be said that exercise suppressed his/her hunger, thus resulting in an energy deficit of 400 calories.

Lastly, it is possible that exercise has no influence on energy intake or hunger, making energy intake following exercise comparable to a resting condition. In this case, although energy intake would not be reduced, a state of negative energy balance would still prevail and be equal to the energy expended during exercise. In the case of the previous example, a negative energy balance of 200 calories would result.

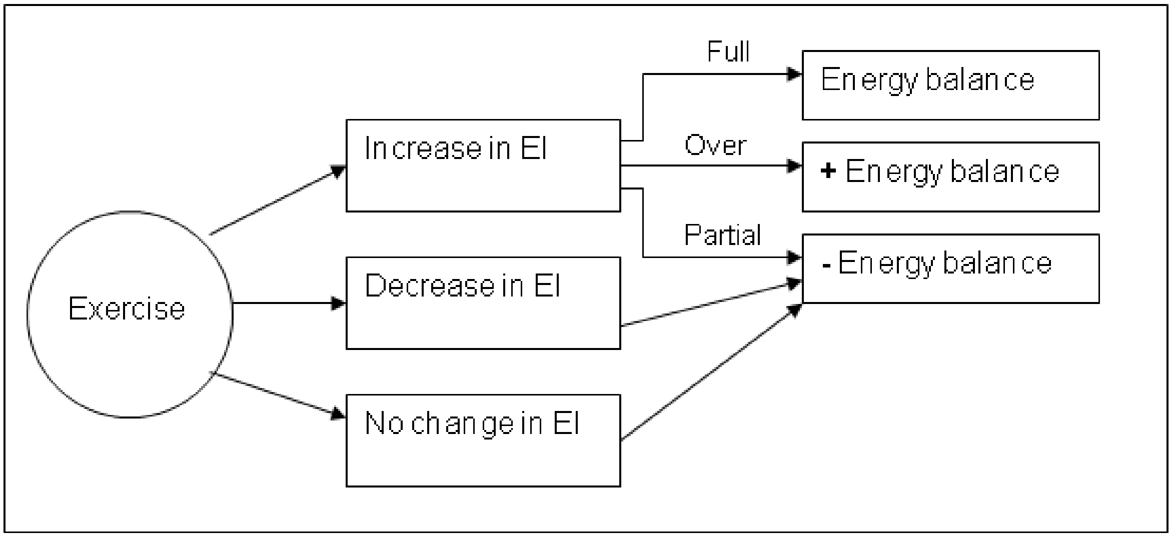

Figure 2: Potential ways in which exercise can influence energy balance

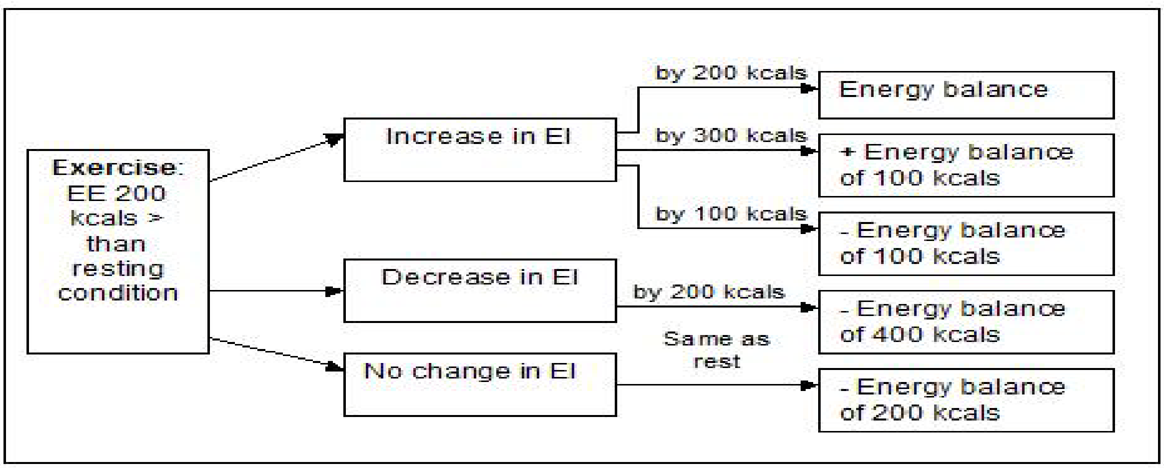

Figure 3: An example of how exercise can influence energy intake

1.4 SPECIFIC AIMS

The specific aims of this study include:

1) To examine whether an acute bout of moderate intensity exercise alters energy intake during one to two hours post-exercise compared to a resting condition.
2) To examine whether an acute bout of moderate intensity exercise alters acylated ghrelin concentrations immediately following exercise and up to one-hour post-exercise compared to a resting condition.
3) To examine whether an acute bout of moderate intensity exercise alters GLP-1 concentrations immediately following exercise and up to one-hour post-exercise compared to a resting condition.
4) To examine whether an acute bout of moderate intensity exercise alters subjective feelings of hunger immediately following exercise and up to one-hour post-exercise compared to a resting condition.

1.5 HYPOTHESES

1) An acute bout of moderate intensity exercise will not alter absolute energy intake compared to a resting condition. However, relative energy intake (accounting for the energy expended during exercise) will be lower following exercise compared to a resting condition.
2) Acylated ghrelin concentrations will be reduced at each time point up until one-hour post-exercise compared to a resting condition.
3) Glucagon-like peptide-1 concentrations will be increased at each time point up until one-hour following exercise compared to a resting condition.

4) There will be a reduction in subjective feelings of hunger immediately following exercise and up to one-hour post-exercise compared to a resting condition.

1.6 SIGNIFICANCE

Overweight and obesity are significant public health problems in the United States. Current recommendations for weight loss include reducing caloric intake and increasing physical activity. It is assumed that the primary contribution of physical activity to body weight regulation occurs from an increase in energy expenditure resulting from physical activity. However, there is some evidence that physical activity may play a role in the regulation of energy intake, yet this area of inquiry has not been thoroughly examined. This study proposed to address the gaps in the literature regarding whether physical activity acutely impacts energy intake in overweight, sedentary adults. We hypothesized that physical activity would result in an acute relative reduction in energy intake, ultimately favoring weight maintenance or weight loss. However, it is possible that the energy deficit resulting from an acute bout of physical activity could trigger a compensatory mechanism resulting in an increase in energy intake, which would resist weight loss and possibly increase body weight. These questions have not been thoroughly examined. Moreover, if an effect of exercise were detected, this would justify the need for additional studies to explore the mechanisms through which physical activity may contribute to the regulation of energy intake and ultimately body weight.

2.0 REVIEW OF LITERATURE

2.1 INTRODUCTION

Obesity is a serious health concern for millions of Americans, primarily due to the link between excess body weight and a number of severe medical conditions and chronic diseases. High levels of body fat have been shown to be involved in the pathogenesis of hypertension, insulin resistance, hyperlipidemia, and various forms of cancer [49]. Additionally, excess body weight is considered to be a primary risk factor for cardiovascular disease [50]. The Framingham Heart Study revealed that the risk of death, over a 26-year period, increased by 1% for every pound increase in body weight for subjects aged 30-42 and by 2% for subjects between the ages of 50 and 62 [3]. Thus, in order to improve the health of the American public, appropriate and effective strategies for weight loss and weight control need to be implemented.

2.2 ENERGY BALANCE

Over the past 25 years, higher calorie consumption and lower levels of physical activity both appear to contribute to the increased prevalence of obesity in today's society [49].

Thus, the current weight loss recommendations include increasing physical activity levels and decreasing caloric intake. These recommendations are based on the theory of energy balance and imply that whenever a significant energy deficit is created through one or both of these avenues, weight loss will occur. In theory, exercise assists in weight control by creating an energy deficit through an increase in energy expenditure. However, this does not account for the potential relationship that may exist between exercise and appetite regulation. Thus, the relationship between the two components influencing energy balance (exercise and food intake) should be fully investigated and understood.

There are two ways to induce a state of negative energy balance. An individual can either reduce energy intake through food restriction or increase energy expenditure through exercise. As long as the acute energy deficit induced through both methods is of a similar magnitude, theoretically both techniques should exhibit a comparable long-term energy deficit. However, this is highly dependent upon the extent to which these two methods alter appetite and one’s compensatory food intake response. For example, skipping a meal (food restriction) or exercising could positively or negatively influence long-term weight control. If a single bout of exercise or food restriction results in an increase in hunger following this time period, these methods would be futile towards one’s weight control efforts. Conversely, if these two situations give rise to suppressing appetite and attenuating food intake, they could be considered effective strategies for regulating body weight. However, a study by Hubert et al. [19] suggested that an acute energy deficit induced through exercise may result in a dissimilar food intake response compared to an energy deficit achieved through meal omission or a reduction in meal size. For this reason, it is imperative to examine how exercise acutely impacts energy intake and determine its effects on short-term appetite control. A better knowledge of this acute relationship between exercise and food intake will have practical implications for the prevention and treatment of obesity.

2.3 ACUTE EFFECT OF EXERCISE ON ENERGY INTAKE IN LEAN SUBJECTS

The majority of studies examining the acute effect of exercise on energy intake in lean subjects have been conducted using moderately-to-highly active individuals [19, 22, 27, 29, 32, 35, 51, 52] and only two studies have used untrained subjects [30, 53]. Despite the common misconception that exercise causes an immediate increase in appetite, the majority of studies have observed no impact on absolute energy intake minutes to hours following an exercise bout [19, 22, 32, 35, 51-53]. Although one study found a decrease in energy intake following a vigorous intensity bout of exercise compared to a moderate intensity bout, no comparison was made between the exercise bouts and a resting condition [31]. Conversely, a few studies found energy intake to be higher following a bout of exercise [27-30]. Martins et al. [30] found absolute energy intake to be increased following a 60-minute, intermittent bout of moderate-intensity exercise (913 ± 363 kcals) compared to a resting condition (762 ± 252 kcals) in 12 untrained males and females. Similarly, using a between-subjects design, Verger et al. [29] found energy intake to be significantly higher following a two hour bout of exercise (2109 ± 127 kcals) compared to a resting condition (1672 ± 111 kcals) in lean young men (BMI: 21.3 ± 1.5 kg/m^2). One possible explanation for the increase in energy intake found following exercise in these two studies could be that the EE of the exercise bout was higher than many of the previous studies (approximately 500-800 kcals). However, Pomerleau et al. [27] found similar results following a high-intensity bout of exercise (70% $VO_{2\ peak}$) despite a lower EE (approximately 350 kilocalories).

A potential limitation in this area of research is that data analyses in many of these studies fail to take into account the energy cost of exercise, which could lead to a misinterpretation of results. For instance, in the two studies previously described that found a significant increase in energy intake following exercise [29, 30], one could conclude that exercise may negatively impact weight loss progress and thus should not be prescribed. However, if the high-energy cost of exercise in the study conducted by Martins et al. [30] is taken into account, the relative energy intake (REI) is significantly lower in the exercise condition (421 $\pm$ 92 kcals) compared to the resting condition (565 $\pm$ 226 kcals). Factoring in REI, a more appropriate conclusion would be that despite an increase in energy intake following a bout of exercise, the magnitude of compensation is less than the exercise EE, inducing a state of negative energy balance and providing a beneficial effect for weight control.

In studies that reported no change in absolute energy intake following an exercise condition compared to a resting condition [19, 22, 32, 35, 51-53], the final conclusion about the acute effect of a bout of exercise on food intake was changed when REI was calculated. Despite no difference in energy intake, the majority of the studies found REI to be lower in the exercise condition compared to a resting condition [19, 22, 32, 35, 51-53]. Of those studies reviewed, King et al. [32] were the only investigators to find no difference in REI between resting and exercise conditions.

However, there was a trend for both the low-intensity (REI = 1290 $\pm$ 344 kcals) and high-intensity (1199 $\pm$ 434 kcals) exercise conditions to have a lower REI compared to the resting condition (1485 $\pm$ 312 kcals) in a group of lean males (BMI: 24.2 $\pm$ 1.5 kg/m^2). Thus, in physically active, lean subjects, it appears that a single exercise session results in an acute state of negative energy deficit, which has beneficial applications for weight loss.

2.4 EXERCISE CONSIDERATIONS

2.4.1 Exercise Intensity

There is some evidence indicating that the intensity of a bout of exercise may impact feelings of hunger and energy intake. It has been suggested that high-intensity exercise may lead to a suppression in hunger, often referred to as exercise-induced anorexia. King et al. [32] examined this phenomena in healthy, lean men and found that hunger was suppressed during and immediately following exercise. The suppression of hunger was greatest during a longer duration (50 minutes), high-intensity (75% VO_{2max}) bout of exercise. The authors also noted that this decreased appetite led to a delay in the onset of eating, despite having no effect on total energy intake.

Other studies examing the effect of exercise intensity on subsequent food intake found conflicting findings [22, 27, 32, 51]. For example, using a crossover design, Imbeault et al. [51] compared the effect of low-intensity (35% VO_{2max}) and high-intensity (75% VO_{2max}), equicaloric bouts of exercise to a resting condition in a group of lean males. Absolute food intake, consumed buffet-style 15 minutes post-exercise, was found to be similar between all conditions, indicating that energy intake following exercise was not dependent upon the intensity of the exercise bout. However, there was a trend for energy intake to be lower following the high-intensity bout of exercise compared to the low-intensity bout. Conversely, Pomerleau et al. [27] observed a trend for absolute energy intake to be higher following high-intensity exercise (70% $VO_{2\ peak}$) compared to a lower-intensity (40% $VO_{2\ peak}$) exercise bout. These equivocal findings indicate the need for future research to examine whether the intensity of exercise impacts subsequent food intake.

In the current study, the intensity of exercise during the testing session was classified as moderate (70-75% HRmax). This intensity was chosen because it is consistent with the American College of Sports Medicine's exercise recommendations for improved health [50]. Additionally, this intensity is appropriate for a sedentary, overweight population and is often what is recommended for individuals in a weight loss program [54].

2.4.2 Total Energy Expenditure

Another factor that may influence food intake is the total energy expenditure of an exercise bout. Studies reviewed have utilized exercise sessions ranging anywhere from 3 minutes [55] to 2 hours [28]. Furthermore, the energy cost of exercise varied quite significantly between these studies. In some cases, the duration of exercise was altered while energy expenditure was held constant for all subjects [19, 28-30, 35, 52]. Other studies used a constant duration and allowed EE to fluctuate [27, 32, 51]. Both methods have their advantages and disadvantages. However, the only study that controlled for the body weight of subjects was conducted by Thompson and colleagues [22]. In that study, exercise was terminated once a subject reached an EE of 4.1kcals/kg of body weight, allowing both duration and EE to fluctuate between subjects.

Since subjects in the current study were in different BMI categories, a protocol similar to Thompson et al. [22] was utilized and both duration and EE of the exercise testing session varied between subjects. The duration of the exercise bout was dependent upon the treadmill grade and body weight of the subject. Total energy expenditure of the session was expressed in two ways: absolute EE and relative to body weight (kcals/kg/body weight). This was done to ensure that the energy deficit created through exercise was similar across all body weight classifications. The exercise session was terminated once an individual reached an energy expenditure equivalent to 3.0 kcal/kg body weight using the ACSM prediction equation for walking. It was estimated that the average energy expenditure and exercise duration would be approximately 300 kilocalories and 35 minutes.

2.5 ENERGY INTAKE CONSIDERATIONS

2.5.1 Assessment of Energy Intake

The assessment of energy intake following an exercise or resting condition can be measured in one of two ways. Subjects can either be aware of, or blinded to the fact that energy intake will be assessed. However, a limitation of previous research is that many studies have not reported the degree of a subject's awareness about the study purpose [19, 27, 29, 32, 35, 51, 52], thus making it difficult to determine the extent to which cognitive factors influence energy intake when a subject's level of awareness is not revealed to the reader.

Similar to Martins et al. [20], the current study attempted to blind subjects to the monitoring of food intake following both resting and exercise conditions, not informing subjects until the end of the study that food consumption was being measured. This design is unique in that it is the first study conducted in an overweight/obese population that has sought to examine the differences between energy intake following resting and exercise conditions while ensuring that subjects are unaware energy intake will be measured. Following the completion of both testing sessions, subjects were asked if they were aware that food consumption during the test meal was being monitored. This questionnaire was included following the cessation of all testing in order to determine if subjects were blinded to the purpose of the study.

2.5.2 Prior Feeding Status

Another issue that may influence feeding and hunger levels post-exercise are levels of satiety prior to the testing period. Hubert et al. [19] reported post-exercise energy intake to be higher following a low-energy (~64 kcals) breakfast compared to a high-energy breakfast (~500 kcals), thus indicating that the feeding state of an individual prior to testing may impact energy intake post-exercise. Study protocols have differed on this issue with some studies utilizing subjects in a fasted state [22, 53], while other studies provided subjects with a breakfast prior to the testing session [19, 27, 30-32, 35, 51].

Additionally, several studies made no effort to standardize energy intake between subjects prior to the testing session [28, 29, 37, 38, 52]. For example, Verger et al. [28] suggested that subjects consume their "normal" breakfast on the day of testing but did not report that the calorie content of this meal was measured. Even when standardized breakfasts were given, all subjects consumed a similar calorie content, despite differences in energy needs between individuals. Martins et al. [30] fed all subjects a standardized breakfast of 500 kcals one-hour prior to the testing session. However, this did not take into account differences in body weight or metabolism, predisposing some subjects to higher or lower states of energy balance before testing began. The extent to which prior feeding status affected the results of these studies is not known.

Since most individuals are unlikely to exercise in a fasted state, the proposed study provided the subjects with a breakfast 2 hours prior to arriving at the testing facility. To limit some of the problems associated with the provision of a standardized breakfast, subjects were given a liquid meal replacement equivalent to 15% of their measured resting metabolic rate. This helped to ensure that subjects in different weight categories began the testing sessions in a similar state of energy balance. Subjects were asked to consume the liquid meal replacement at home, prior to reporting to the testing center, to reduce the time spent at the facility.

2.5.3 Macronutrient Composition of Foods Provided

Some research has suggested that exercise alters food preferences and food selection. This theory is based on the depletion hypothesis which states that following an exercise bout, subjects are expected to replace the substrate predominately utilized during that bout of exercise [56]. Findings from animal studies suggest that the respiratory quotient (RQ), which indicates the primary substrate oxidized during exercise, will influence the macronutrient composition of the foods chosen [57]. If this were true, higher intensity exercise would result in increased carbohydrate intake post-exercise due to an increased reliance on carbohydrate oxidation at higher exercise intensities. However, the validity of this theory is still uncertain and results in humans have been inconclusive [22, 28, 32, 36, 51].

Based on the depletion hypothesis, the selection of foods available to subjects post-exercise in experimental studies could greatly impact the caloric content of the feeding session. For example, Tremblay et al. [36] found that the magnitude of the energy deficit created by a bout of exercise was dependent upon the macronutrient composition of the foods offered to an individual post-exercise. This study found energy intake to be reduced when a low-fat diet was provided compared to either a mixed diet, or high-fat diet. However, the observation period of this study was 48 hours post-exercise. Another study found those individuals who had the greatest reduction in RQ, or an increased reliance on fat oxidation during exercise, also reported lower energy intakes following a bout of exercise, predisposing them to a state of greater negative energy balance compared to those with a higher RQ [23]. Thus, the relationship between substrate oxidation during exercise and post-exercise energy intake and macronutrient composition needs to be explored further.

Overall, previous research suggests that RQ during exercise and the macronutrient composition of the food available to the subject following exercise could influence food intake in a post-exercise meal. The current study monitored RQ continuously to help determine if a relationship exists between substrate utilization during exercise and post-exercise energy intake. Additionally, to mimic conditions of a free-living environment, a mixed diet, offering a wide range of foods, was provided to the subjects following a one-hour rest period post-exercise. These foods were available to the subjects from hours 1-2 post-exercise or rest.

2.6 SUBJECT CHARACTERISTICS

2.6.1 Training Status of Subjects

As previously illustrated, the majority of studies that have examined the acute effect of exercise on energy intake in lean subjects (BMI < 25 kg/m^2) have been conducted using subjects who exercised regularly and were considered to be at least moderately active. Considering that overweight and obese individuals have a tendency to be more sedentary compared to their normal weight peers, it is important to discuss the differences in appetite regulation that may exist between regular exercisers and the sedentary population.

Overall, research indicates that there is a loose coupling between energy intake and energy expenditure. However, there is some evidence to suggest that the relationship between these two variables may be dependent upon habitual physical activity levels. It appears that trained individuals may have a better ability to regulate their energy needs in comparison to those who are untrained [20, 58]. For example, Long and colleagues [58] found a decrease in energy intake following a high-energy preload (607 kcals) compared to a low-energy preload (246 kcals), in a group of exercisers, but not in non-exercisers. Similarly, Martins et al. [20] demonstrated that following a 6-week training period, previously sedentary individuals were better able to recognize their energy needs and regulate food intake accordingly. These findings indicate that the regulation of food intake following preloads may vary between habitual exercisers and sedentary individuals. However, the effect of training status on food intake post-exercise is not yet known.

To date, no study examining the effect of a single bout of exercise on energy intake has included both trained and untrained subjects, thus making it difficult to draw conclusions about whether both groups of subjects would have a similar or different energy intake response following a bout of exercise. Studies have included subjects who were untrained [30, 31, 37, 38, 53], moderately active [19, 27, 51, 52], and highly trained [22, 32, 35]. However, these studies employed different exercise protocols making it is difficult to determine the extent to which prior training status affected energy intake post-exercise. Future research is warranted and caution should be taken prior to making generalizations about the effect of exercise on energy intake to those in different trained states.

2.6.2 Body Weight of Subjects

It can be argued that BMI can influence post-exercise energy intake, with a few studies reporting a relationship between body weight and food intake [31, 37, 59]. For example, George et al. [37] found overweight subjects to have a higher food intake 30 minutes post-exercise compared to lean subjects. However, differences in metabolism due to body weight were not controlled for in this study. Conversely, Kissileff et al. [31] found no difference in energy intake between overweight and normal weight subjects following either a resting or exercise condition. However, no study has ever stratified overweight subjects into different BMI classifications to examine if differences exist between groups.

The current study examined the acute effect of a moderate-intensity bout of exercise on energy intake in overweight, Class I, and Class II obese subjects. This unique stratification allowed for a broad range of body weights to be examined in the context of this research question. Additionally, the subjects' body weight could be controlled for in the current study both in the pre-exercise meal and also when determining the total energy expenditure of the exercise session. This study was one of the first studies to determine how a moderate bout of exercise influences food intake in an overweight/obese population when subjects are given a broad range of foods to choose from in a post-exercise meal, in order to mimic a free-living environment.

2.7 ACUTE EFFECT OF EXERCISE ON ENERGY INTAKE IN OVERWEIGHT SUBJECTS

The majority of research in this area has examined the acute effects of a single bout of exercise on food intake in lean subjects. However, the quantity of literature reporting on overweight or obese subjects is minimal and thus warrants further investigation. To the author's knowledge, only three studies have been performed using this population and there are some limitations to these studies.

Kissileff et al. [31] sought to examine the effects of moderate or vigorous exercise, on food intake 15-minutes post-treatment in 9 overweight and 9 normal weight sedentary women. Subjects reported to the laboratory on 3 separate occasions and either rested quietly for 40 minutes or cycled for 40 minutes at either 90 (vigorous) or 30 (moderate) watts. On the day of testing, subjects reported to the laboratory having fasted overnight and were given a standardized breakfast of 300 kilocalories and asked to return to the laboratory 2 hours later for testing. Following the testing period, subjects were given a post-exercise meal in the form of a strawberry yogurt shake, and told to eat as much as they wished. The main finding of this study was that energy intake was reduced after vigorous exercise compared to moderate intensity exercise in normal weight but not obese subjects. However, no comparison was made between an exercise and resting condition in either of the subject groups. Additionally, this study examined the differences in energy intake between normal weight and overweight subjects and found there to be no significant differences between the different weight classifications across all 3 conditions.

There are limitations of this study that should be noted. First, relative energy intake, accounting for the energy cost of exercise, was not reported. Consequently, drawing proper conclusions from this data is difficult. Second, subjects in this study were only given a strawberry yogurt shake to consume during their post-test meal. Although the authors only included subjects who stated having a liking to this drink, it is possible that this minimal food choice could have confounded the results. Previous research indicates that following exercise individuals may have an increased craving for selected macronutrients and thus a buffet-style test meal should be offered to subjects to properly mimic free-living conditions [60]. Also, the authors of this study did not report energy intake in calories consumed, rather energy intake was reported in grams of the shake consumed, thus making it hard to compare the results of this study to previous studies. Lastly, the energy expenditure of the exercise sessions was not held constant across exercise conditions. Thus, a larger energy deficit was induced as a result of vigorous-intensity exercise compared to the moderate-intensity session, possibly impacting subsequent food intake.

George and Morganstein [37] examined 12 normal weight and 12 overweight, inactive women who participated in a randomized cross-over design that included a resting and exercise condition. The exercise session consisted of walking on a treadmill for 60 minutes at 60% of maximal heart rate, eliciting an approximate EE of 150-200 kilocalories. Before reporting to the laboratory on testing days, subjects ate a standardized breakfast in their home two and a half hours before their testing session. Energy intake was analyzed thirty minutes following the testing session, when subjects consumed an ad libitum meal in the university cafeteria, unaware that their food intake was being monitored.

The primary finding of this study was that energy intake was greater in the overweight group compared to the normal weight group following both exercise and non-exercise conditions. However, a limitation of this study was that it did not account for differences in body weight or energy needs between the two groups of subjects. Additionally, this study did not conduct statistical analyses to determine whether energy intake following the resting condition was different than energy intake following the exercise condition in the overweight subjects. However, from the data presented, there appears to be no difference between the exercise (energy intake = 576 kcals) and non-exercise (energy intake = 525 kcals) conditions in terms of absolute energy intake. However, REI was not calculated, thus making it difficult to know if a greater energy deficit resulted following the exercise condition.

Westerterp-Plantenga and colleagues [38] examined ten normal weight and ten overweight, untrained males using a crossover design in which subjects underwent 4 resting sessions and 4 exercise sessions. The exercise consisted of 120 minutes on a cycle ergometer at 60% W_{max} and for the resting condition subjects read or studied for two hours. All subjects ate breakfast at home. Ten minutes before and 10 minutes after each test session subjects were offered food in a buffet-style fashion and were told to eat as much as they liked. Energy intake was significantly lower following the cycling session (549 $\pm$ 48 kcals) versus the resting condition (740 $\pm$ 71 kcals). Interestingly, these results maintained even though caloric intake was greater (not significantly) during the feeding period prior to the resting session.

A strength of this study was that each subject underwent 4 resting sessions and 4 exercise sessions. However, a limitation is that food intake was not controlled for prior to the testing sessions, during which subjects were offered food in a buffet-style form. Additionally, similar to many other studies, relative energy intake was not calculated. Failure to calculate REI makes it difficult to draw conclusions about the relative state of energy balance achieved following a bout of exercise and a post-exercise meal. Lastly, the generalization of these findings is limited due to the fact that 120 minutes of exercise may be unrealistic for many overweight and sedentary individuals. Thus, the current study sought to use a more realistic duration of exercise (approximately 40 minutes) to determine how a single bout of exercise acutely impacts food intake.

2.8 ACUTE EFFECT OF EXERCISE ON SUBJECTIVE FEELINGS OF HUNGER

Hunger is defined as strong and compelling desire for food. Logically, an increase in hunger, would lead to a subsequent increase in food intake. However, studies that have examined hunger ratings and food intake following an exercise bout have not necessarily seen these two variables fluctuate accordingly. Contrary to public perception, higher intensity bouts of exercise have been shown to suppress hunger [32, 35, 36, 38, 53]. However, these studies have generally found that this response does not remain following the cessation of exercise and that it does not typically correspond to a reduction in food intake post-exercise [61]. Other studies that have examined this relationship have found no effect of exercise on hunger. However, the fact that hunger may be unaltered as a result of exercise means that exercise can still be beneficial for weight control purposes. As long as hunger is not increased following exercise, the energy deficit induced by exercise remains.

A limitation to this area of research is that many studies have not examined the relationship between hunger and food intake post-exercise, and this has not been thoroughly explored in the overweight/obese population. Thus, the proposed study will examine hunger and energy intake in response to a moderate intensity bout of exercise in sedentary, overweight adults.

2.9 APPETITE REGULATION

The regulation of appetite is multifaceted and complex. Unlike early theories (ie - glucostatic [62] or lipostatic [63] theories) that hypothesized that energy intake was regulated by one single factor, more recent evidence indicates that the mechanism which drives an individual to eat is multifaceted and involves many different body systems [64]. The central nervous system is responsible for receiving and processing hormonal signals produced by the gastrointestinal tract, adipocytes, pancreas, and bloodstream, ultimately producing feelings of hunger or satiety. In particular the brainstem and hypothalamus have been recognized to exert both inhibitory and excitatory signals resulting in a decrease or increase in food intake.

There is some evidence to suggest that the regulation of appetite may depend upon the manner through which an energy deficit is induced. For example, when an energy deficit is created through a reduction in food intake, a compensatory increase in energy intake occurs [25, 65]. However, when an energy deficit is created by a single bout of exercise, there is typically no compensatory increase in energy intake [26, 56]. Hubert et al. [19] compared the energy intake response to an energy deficit created by exercise and diet in normal weight subjects (age: 23.2 ± 2.7 years, BMI: 21.5 ± 1.1 kg/m^2). This study found hunger and energy intake to be significantly higher following a low-energy breakfast of approximately 64 kilocalories (9% protein, 5% fat, 86% carbohydrate) compared to a high-energy breakfast of 500 kilocalories (14% protein, 16% fat, 70% carbohydrate). These findings suggest that a greater caloric deficit may elicit a physiological response that triggers a compensatory increase in hunger and food intake to restore energy homeostasis. However, in this same study, a 317-kilocalorie bout of exercise failed to have any effect on subsequent hunger or energy intake compared to a resting condition. These findings suggest that exercise did not weaken post-ingestive satiety signals following breakfast, leaving the subjects in a state of negative energy balance following an ad libitum lunch. For this reason, it is critical to investigate the physiological mechanisms driving this attenuated need to restore energy homeostasis following exercise, as opposed to what is seen following a period of calorie restriction.

2.10 APPETITE REGULATING HORMONES

Various hormones in the body appear to influence appetite. These appetite-regulating hormones that are involved in the regulation of food intake are often categorized by their effect on feeding behaviors (appetite stimulants or suppressants). The major hormones that are positively correlated with energy intake are ghrelin, cortisol, and neuropeptide-Y. Higher concentrations of these factors lead to increased feelings of hunger, resulting in their classification as orexigenic hormones. The hormones that increase satiety and decrease appetite are cholecystokinin, glucagon-like peptide 1 (GLP-1), peptide-YY, corticotropin-releasing hormone, leptin, and insulin. Higher levels of these hormones lead to a suppression of appetite and energy intake and therefore are termed anorexigenic.

Although the involvement of these hormones in the regulation of appetite is fairly well-known, previous research examining the acute effect of exercise on these hormones is sparse. Only a few studies have examined how acylated ghrelin and GLP-

1 are affected by exercise [30, 41, 42, 47, 66] and even fewer have examined energy intake or hunger ratings following exercise in addition to examining these neuroendocrine parameters [30, 47]. However, there is some evidence to suggest that both acylated ghrelin and GLP-1 may be affected acutely by exercise.

2.10.1 Ghrelin

Ghrelin is an orexigenic hormone that is produced mainly by the oxyntic cells of the stomach [67] as well as the arcuate nucleus of the hypothalamus [68]. It serves as an appetite stimulant and has been shown to increase energy intake when administered both peripherally and centrally [43]. Levels of ghrelin fluctuate according to an individual’s feeding state. During fasting, ghrelin levels rise and peak immediately before a meal to initiate eating and fall following a meal to signal an individual to stop eating [67]. It has also been suggested that ghrelin may stimulate the secretion of growth hormone (GH) from the pituitary [69] and since GH increases as a result of exercise, it has been suggested that ghrelin concentrations might also rise during exercise. However, in a recent review article by Kraemer and Castrancane [70], this hypothesis was rejected and the authors stated that ghrelin does not appear to regulate GH release during exercise.

The effect of an acute exercise bout on plasma ghrelin concentrations has been extensively studied in recent years. The majority of these studies have reported ghrelin levels to be unaltered [30, 44, 45, 71-73]. However, one study did find ghrelin levels to be increased following 3 hours of exercise in endurance trained men [74] while another study reported a decrease in ghrelin concentrations for up to an hour following exercise

[75]. It is important to note that a potential limitation of these studies is that they only examined total ghrelin concentrations. It was recently found that ghrelin exists in two forms: nonacylated and acylated. Although 80-90% of ghrelin is in the nonacylated form [46], research has demonstrated that this form of ghrelin is not involved in appetite regulation. Acylated ghrelin has been found to stimulate food intake in both fed and fasted states [76]. For this reason, this study examined acylated ghrelin concentrations.

To the author's knowledge, only two studies have examined the impact of a single bout of exercise on acylated ghrelin concentrations. Broom et al. [47] examined the impact of a 60-minute bout of running on a treadmill at 75% of maximum oxygen uptake on concentrations of acylated ghrelin in nine college-aged, physically active males. Subjects reported to the lab in a fasted state, performed an exercise session, rested for eight hours, and then consumed a test meal 2 hours post-exercise. The main finding of this study was that acylated ghrelin concentrations were significantly lower 30 minutes into exercise compared to a resting condition. Additionally, there was a trend for acylated ghrelin concentrations to be suppressed, for up to eight hours post-exercise, although this was not significant. Furthermore, a trend towards a decrease in hunger was also seen during and following the exercise session until the feeding period.

The second study that has examined acylated ghrelin concentrations in response to an acute bout of exercise found similar results [48]. Following a maximal cycle ergometer test in both lean and obese subjects, there was a reduction in acylated ghrelin concentrations at the peak of exercise with a greater suppression in the obese subjects compared to the normal weight subjects. However, this study reported sampling blood at 20 and 40 minutes post-exercise, but these results were not reported.

Thus, it is difficult to know if the changes in acylated ghrelin concentrations persisted following exercise.

Due to the limited number of studies examining the effect of an acute bout of exercise on acylated ghrelin concentrations, the current study explored how this hormone responds to a single bout of moderate-intensity exercise in a group of sedentary, overweight women. Under resting conditions, previous research has found that obese individuals tend to have lower total plasma ghrelin levels compared to their lean counterparts [77]. However, research is unclear whether this is also true for acylated ghrelin.

2.10.2 Glucagon-Like Peptide 1

Glucagon-like peptide 1 is a anorexigenic gastrointestinal hormone that is secreted from the L-cells of the ileum and colon when macronutrients (carbohydrate, protein, fat) are present [67]. It has been shown to rapidly decrease food intake and increase satiety in both lean [39, 78] and obese [40, 79] subjects. One suggested mechanism through which GLP-1 inhibits food intake is through an inhibition of gastric emptying [80]. Another possible mechanism is through its effect on the GLP-1 receptors present in the hypothalamus [39]. In addition to its role in controlling appetite, GLP-1 is also an important regulator of both insulin and glucagon production. Studies have shown that GLP-1 enhances insulin secretion and decreases glucagon secretion from the pancreas, and thus may also inhibit food intake through these indirect mechanisms [81].

Very little research has been conducted examining the acute effect of a single bout of exercise on GLP-1 concentrations, with only 5 studies identified that have examined this question. Of these studies, one was performed using adolescents [82], two utilized trained endurance athletes [41, 66], another used healthy, normal weight males and females [30], and the last utilized both normal weight and obese subjects [42]. These subject characteristics are relevant since previous research has found that obese subjects may have an attenuated GLP-1 response following a meal compared to normal weight controls [83]. Furthermore, Adam et al. [42] found that following a 60-minute bout of low-intensity exercise, there was an increase in concentrations of GLP-1 in a group of normal weight subjects, but not obese subjects. This study also demonstrated that the attenuation in GLP-1 release in obese subjects during and following exercise can be reversed with a modest weight loss of 3.5 kg. Due to the physiological differences in lean and obese subjects, it is necessary for the current study to examine the impact of a single bout of exercise on GLP-1 concentrations in a sedentary, overweight population.

In addition to the lack of research examining the response of GLP-1 to exercise in an overweight population, GLP-1 was chosen to be examined in the current study due to a recent finding by Martins et al. [30]. In this study, GLP-1 along with ghrelin, polypeptide YY, pancreatic polypeptide, and insulin were examined in response to an intermittent bout of moderate-intensity exercise in a group of lean subjects. The authors found GLP-1 to be significantly increased during exercise and at the last measurement period 1-hour post-exercise when compared to a non-exercise condition. For the majority of the hormones examined in this study, there was either no effect of exercise or the effect of exercise was short-lived (with the exception of pancreatic polypeptide) thus, providing a rationale for including this hormone in the current study. Furthermore, the study by Martins et al. [30] was unique in that it was the first study to simultaneously measure subjective feelings of hunger and energy intake 1-hour post-exercise. It is necessary to replicate these findings in an overweight population.

2.11 POTENTIAL FACTORS THAT MAY AFFECT ENERGY INTAKE

Thus far, appetite control has only been discussed in the context of physiological responses to energy deficits. However, it is important to mention that there is also a cognitive or behavioral component involved in the regulation of energy intake. For example, palatable foods may increase one's temptation to eat [84]. Additionally, for many individuals, food provides pleasure and is oftentimes used as a reward for a particular behavior. Thus, it is plausible that food could serve as a reward following a bout of exercise. Also, food availability and one's access to certain foods could also influence energy intake. Moreover, exercise could alter one's cognitive state, driving a person to eat or refrain from eating post-exercise due to their conscious awareness of an energy deficit created by an exercise session and not physiological hunger. Hence these psychological influences (i.e. – dietary restraint & mood state) on food intake need to be controlled for when designing studies that examine the physiological mechanisms driving food intake post-exercise.

2.11.1 Dietary Restraint

Dietary restraint refers to an individual's effort to manage weight through consciously controlling food intake [85]. Hence, restrained eaters cognitively control their food intake as opposed to discontinuing eating when satiated. Conversely, unrestrained eaters do not diet and food consumption is not cognitively regulated. This group of individuals is typically driven to eat by physiological sensations of hunger. Generally, studies aiming to assess the impact of exercise on food intake have excluded restrained eaters. However, Lluch et al. [86] found no relationship between levels of dietary restraint and relative energy intake under exercise conditions. Thus, the current study will not exclude subjects based upon dietary restraint scores. However, these values were measured so that this factor could be controlled for when performing the statistical analyses.

2.11.2 Mood

Another factor that must be controlled for when proposing a study that examines the physiological effects of exercise on food intake is mood. Overall, several reviews of literature have stated that exercise has mood-enhancing effects [87, 88] and that this improvement in mood is not just found with chronic exercise but also following an acute exercise bout [89]. However, there is some data opposing this finding, indicating that exercise may adversely impact mood in some individuals [90]. Ekkekakis et al. [91] found that when subjects exercised above their ventilatory threshold, a worsened mood state (assessed by the Feeling Scale) was observed post-exercise. It is theorized that individuals may combat a deterioration in mood by increasing food intake following exercise to improve their mood state [55], since food is generally viewed as pleasurable. Whether a negative mood state indirectly impacts weight control efforts through its influence on food intake is not yet known.

The majority of studies conducted in this area have utilized active, lean individuals; therefore it is difficult to know how exercise impacts mood in a sedentary, overweight population. It is plausible that lean and overweight individuals would experience similar changes in mood in response to a bout of exercise. However, it is also possible that overweight individuals would have a greater deterioration in mood following exercise due to lower ventilatory thresholds, reduced aerobic capacities, negative views of exercise, greater pain with exercise, or feelings of embarrassment while exercising [55]. For these reasons, the current study monitored changes in mood in response to a bout of exercise in a sedentary, overweight population. Thus, any alterations in mood could be considered when examining food intake post-exercise.

2.11.3 Sleep

Cross-sectional data indicates that low-levels of sleep are associated with higher rates of obesity [92, 93]. One possible explanation for these findings could be attributed to the impact that sleep has on hunger and various appetite regulating hormones. Although this area of research is still wide open, there is some evidence to suggest that acute sleep deprivation in both rats [94] and humans [95] leads to an increase in hunger which may be explained by an increase in ghrelin concentrations. In fact, one study performed in humans demonstrated that 2 days of sleep restriction increased ghrelin concentrations by 28% and hunger levels by 24% in a group of 12 healthy, young males. Thus, in the current study, only those individuals reporting a minimum of 6 hours of sleep per night on average were eligible to participate. Additionally, to minimize the acute effect of sleep on hunger and hormone concentrations, subjects were instructed to receive at least 6 hours of sleep each of the 3 nights prior each testing session.

2.12 CONCLUSION

An understanding of the relationship between exercise and energy intake is critical for the prevention and treatment of obesity. However, this relationship has not been thoroughly examined, specifically in the overweight population. Although potential mechanisms that could influence this relationship have been reviewed here, little is known about the effect of a single bout of exercise on appetite regulation in individuals with excess body weight. Additionally, potential mediators and moderators of this relationship need to be explored. It is hypothesized that a bout of exercise will influence appetite-regulating hormone concentrations, which will then influence hunger and satiety levels, and ultimately alter food intake. However, only one study in lean subjects and no studies in overweight subjects have examined changes in these three variables simultaneously. Thus, the current study examined the acute effect of a moderate intensity bout of exercise on hunger, energy intake, and appetite regulating hormone concentrations in sedentary, overweight women.

3.0 METHODOLOGY

3.1 SUBJECTS

A total of 21 pre-menopausal, overweight women were recruited to participate in this study. Subjects were between the ages of 18 and 45 and had a BMI between 25.0 - 39.9 kg/m^2, with an equal number of participants (n=7 per group) classified as overweight (25.0 – 29.9 kg/m^2), Class I obese (30.0 – 34.9 kg/m^2), and Class II obese (35.0 - 39.9 kg/m^2). Furthermore, subjects were sedentary, defined as exercising at a moderate-intensity for less than 30 minutes/week over the past six months. Exclusionary criteria for this study were as follows:

1) History of cancer, heart disease, Type I or Type II diabetes
2) Presence of any medical condition that may alter one's metabolism (i.e., thyroid disease)
3) Presence of any condition that may limit one's ability to exercise (i.e., orthopedic limitations or severe arthritis)
4) Currently a smoker
5) Recent weight loss of ≥ 10 pounds within the previous 6 months

6) Uncontrolled hypertension (currently taking blood pressure medication or having a resting systolic blood pressure of $\geq$ 140 mmHg or a diastolic blood pressure $\geq$ 90 mmHg)
7) Women who were pregnant, planning on becoming pregnant within 2 months, or those previously pregnant within the past 6 months
8) Currently taking any medication that would alter heart rate (i.e., beta blocker) or metabolism (i.e., synthroid)
9) Currently taking psychotropic medication or currently being treated by a doctor or other medical person for a psychological disorder
10) Reporting irregular menstrual cycles (<25 days or >35 days between cycles)
11) Getting an average of <6 hours of sleep/night

3.2 RECRUITMENT AND SCREENING PROCEDURES

All subjects were recruited into this study in one of two ways. First, subjects participating in other research studies at the Physical Activity and Weight Management Research Center were informed of this study at one of their regularly scheduled visits and were asked by a staff member if they were interested in learning more about the current study. Secondly, subjects were recruited through local advertisements and were instructed to call the Physical Activity and Weight Management Research Center for additional study information and to see if they would be eligible to participate. If an individual was still interested in participating in the study after hearing about additional study procedures, potential subjects then underwent a brief telephone screen to ensure initial eligibility. If deemed eligible for the study, research participants were mailed a physician consent document (Appendix B) to have signed prior to their first visit and an information packet with pre-test guidelines (Appendix C).

During the subjects' initial visit, the study was explained in complete detail and research participants were given the opportunity to ask any questions that may have arisen concerning any of the study procedures, prior to signing an informed consent document (Appendix A). At this time, subjects were also asked to complete a Physical Activity Readiness Questionnaire (PAR-Q) [96] to ensure that exercise was not contraindicated. The Institutional Review Board at the University of Pittsburgh approved all study procedures.

3.3 EXPERIMENTAL DESIGN

Subjects reported to the Physical Activity and Weight Management Research Center on three separate occasions: 1) initial assessment visit, 2) exercise testing session, 3) sedentary testing session. This study utilized a randomized cross-over design, thus the order in which the subjects completed the testing sessions was randomly assigned. However, the two testing sessions were always separated by at least 2 days and testing was conducted between days 7 and 21 of a subject's menstrual cycle in order to minimize the effect of hormone concentrations on outcome measures.

3.3.1 Assessment Visit

During the first visit, subjects were asked to report to the center having fasted overnight. Upon arrival, they underwent formal assessments of height, weight, blood pressure, body composition, resting metabolic rate, and physical fitness (Appendix D). Upon the completion of the first visit, subjects were provided with a liquid meal replacement (equivalent in kilocalories to 15% of resting metabolic rate) to take home and consume on the morning of their next testing session. The macronutrient composition of this liquid meal replacement was 47% carbohydrate, 28% fat, and 25% protein. Subjects were also given a list of guidelines to adhere to during the days leading up to their next scheduled testing session. These guidelines were as follows: 1) abstain from any form of exercise for 2 days prior to the testing session, 2) keep a detailed food record for 2 days prior to the testing session, 3) consume the liquid meal replacement on the morning of testing, two hours prior to the scheduled testing time, and 4) abstain from all other food or beverages on the morning of testing, 5) get at least 6 hours of sleep on each of the 3 nights leading up to the testing visit.

3.3.2 Testing Sessions

Subjects reported to the Physical Activity and Weight Management Research Center on the morning of their testing session, having followed all of the guidelines previously explained. Upon arrival, testing procedures were reviewed with the subject, a body weight was taken, and then the subject was equipped with a heart rate monitor. She was then asked to complete the Subjective Exercise Experience Scale (SEES) and the

Positive and Negative Affect Schedule (PANAS) with questions about hunger interspersed throughout. Immediately following the completion of these questionnaires, the subject underwent an initial blood draw, which was immediately followed by the start of the exercise or sedentary condition.

During the exercise testing session, the subject walked on a treadmill at 3.0 mph and a grade that induced a heart rate between 70-75% of age-predicted maximal heart rate. Heart rate was monitored continuously and the grade of the treadmill was adjusted appropriately if the subject's heart rate fell outside the target heart rate range for 2 consecutive minutes. Oxygen consumption (VO_2) was monitored continuously using a facemask and breath-by-breath analysis of VO_2 was averaged every minute. The exercise testing session was terminated once the subject had achieved an energy expenditure of 3.0 kcal/kg of body weight, calculated by the American College of Sports Medicine's (ACSM) metabolic equation for the energy expenditure of walking [50]. The average time that a subject spent walking on the treadmill to elicit this level of energy expenditure was 42 minutes. By expressing energy expenditure relative to body weight, it allowed for a similar relative energy deficit to be created by the bout of exercise in all subjects. The subject's perceived effort during exercise was also assessed every 3 minutes using the Borg's Rating of Perceived Exertion (RPE) scale [97]. Immediately following exercise, the subject completed the SEES questionnaire which was followed by a post-exercise blood draw. Following the post-exercise blood draw, the PANAS questionnaire was completed and the subject was given a water bottle so that fluid intake could be monitored. See Appendix F for data collection form.

The same data collection procedures were followed for the sedentary testing condition (Appendix E). However during this session, the subject rested quietly in a seated, upright position for a predetermined length of time. This period of time was calculated prior to the testing session and was based upon the ACSM's metabolic equations for the energy expenditure of walking using an expected walking grade and speed (determined from the initial GXT), and total energy expenditure. During this time frame, the subject was permitted to watch a video and heart rate and oxygen consumption were monitored. Although the total time of the exercise and sedentary conditions varied slightly due to the estimations involved, this study design allowed for a close approximation of total resting time for the sedentary condition.

Upon completion of the post-exercise/rest blood draw, the subject was instructed to rest quietly for the next two hours and was allowed to watch a video for the first hour and read magazines during the second hour. Additional blood draws occurred at 30, 60, and 120 minutes post-exercise/rest and these blood draws were preceded by the completion of the SEES and PANAS questionnaires. After the first hour of rest, the subject was provided access to a variety of snacks and they were told to help themselves to the snacks provided, unaware that their food intake was actually being monitored. Details regarding the procedures related to assessing energy intake from the snacks offered are provided in the Primary Outcome Assessment section of this paper. Subjects had access to these snacks for a total of one hour (1-2 hours post exercise). A summary of all testing procedures is shown in Figure 4.

Once the subject had completed both the exercise and sedentary testing sessions, they were asked to complete the Three-Factor Eating Questionnaire to measure dietary restraint [98] and to rate the pleasantness of their post-exercise ad libitum test meal (Appendix G). The Three-Factor Eating Questionnaire allowed for the determination of whether individual differences in dietary restraint may have influenced energy intake during the testing sessions. Similarly, asking subjects to rate the pleasantness and palatability of their test meal assisted in the determination of whether their like or dislike of the meal may have impacted the amount of food consumed during each testing session. Additionally, subjects were asked to complete a brief questionnaire to assess their knowledge of the research question. They were asked if they thought that their food intake was being measured, whether they believed that exercise increases or decreases their appetite, how many calories they think they expended in the bout of exercise, and how many calories they believe they consumed during the last testing session. Following the completion of these questionnaires, subjects were debriefed about the researcher's primary aim of this study and were provided with additional information explaining that food intake was actually monitored (Appendix H). Lastly, upon completion of all 3 visits, subjects were paid $300 for their participation in the study.

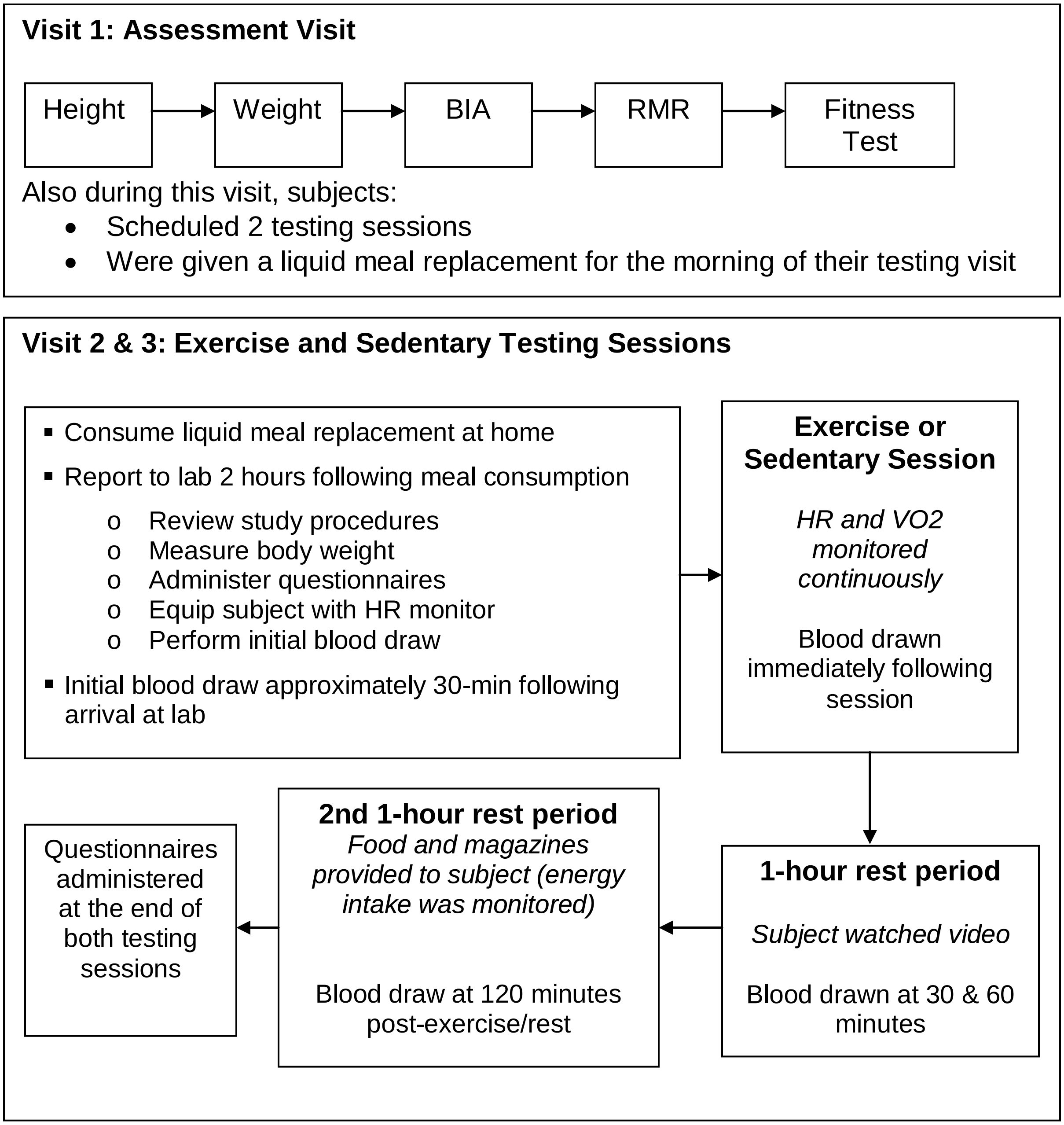

Figure 4: Experimental design

3.4 ASSESSMENT COMPONENTS

3.4.1 Height

Height was measured using a wall-mounted stadiometer. This measurement was only performed during the initial assessment visit and it was used to calculate body mass index (BMI). The subject's height was measured to the nearest 0.1cm.

3.4.2 Body Weight

Body weight was assessed at the initial assessment using a digital scale. Measurements were taken to the nearest quarter of a pound. Furthermore, body weight was also measured on each of the testing visits. Subjects were instructed to take off their shoes and remove items from their pockets prior to being weighed.

3.4.3 Resting Energy Expenditure

Resting energy expenditure (REE) was measured via the dilution technique using a Sensor-Medics 2900 metabolic measuring cart (Yorba Linda, CA) and a plastic canopy. Subjects were instructed to fast for at least 12 hours the night before testing, and to avoid consumption of any over-the-counter medications. Subjects were also instructed to abstain from all vigorous physical activity the day before testing, and to transport themselves to the Physical Activity and Weight Management Research Center in a vehicle on the morning of testing. Patients were questioned verbally regarding their adherence to the aforementioned pre-testing recommendations.

Resting energy expenditure was measured between 7:30-10:30 in the morning. REE measurements were taken at the completion of the subject resting in a supine position in a darkened room for a period of 25 minutes. Following this 25-minute rest period, subjects were placed under the canopy in a supine position for a 20-minute steady state measurement period. Criteria for establishing a stable measure of REE was a steady state consisting of five consecutive data points with a range of no more than 150 kcal/d which approximates the 5% criteria used by Jakicic et al. [99] that significantly correlated ($r = 0.92$, $p < 0.001$) with Foster's techniques finding steady state at a coefficient of variation (standard deviation/mean) of no more than 5% for both VO_2 and CVO_2 [100].

The purpose for measuring resting energy expenditure in this study was two-fold. First, subjects ranged in BMI from 25.0 - 39.9 kg/m^2. Thus, it was assumed that larger subjects would also have larger resting energy expenditures, which could influence food intake based upon higher or lower energy needs of certain individuals. Therefore, when performing the statistical analyses, this variable could be controlled for. Second, prior to each testing session, subjects were given a liquid meal replacement to be consumed on the morning of testing. To standardize this across all body weight classifications, REE was used to ensure that each subject received an equal percentage of her energy needs as opposed to a standard caloric value that would place subjects at different degrees of energy balance.

3.4.4 Body Composition

Body composition was assessed using Bioelectrical Impedance Analysis (BIA). The tetrapolar method was used and 4 small electrodes were placed on the hand, wrist, ankle and foot on the right side of the body. A low-level electrical current was then transmitted between the electrodes to measure impedance, or the opposition to the flow of the current through the body. From this value, fat-mass and fat-free mass were calculated.

Although body composition was not a primary outcome measure of this study, exploratory analyses were performed to determine if differences in fat mass (FM) and fat-free mass (FFM) influenced energy intake and hormone concentrations. Due to the known association between REE and FFM, it was hypothesized that subjects with higher levels FFM would have higher energy needs, and thus it was important for body composition to be controlled for when looking at differences in energy intake between testing conditions.

3.4.5 Graded Exercise Test

Cardiorespiratory fitness was assessed using the Modified Balke protocol in which a subject walked at 3.0 mph on a treadmill while the grade of the treadmill increased by 2.5% every three minutes. A 12-lead electrocardiogram (EKG) was used to assess heart rate throughout and the test was terminated when the subject reached 85% of age-predicted maximal heart rate, determined by the equation: 220 – age.

This graded exercise test (GXT) was included in the methodology of this study for numerous reasons. First, during the exercise testing session subjects exercised at 70-75% of age-predicted maximal heart rate. Thus, this GXT was an appropriate method for determining the incline at which the subject should begin to walk during the exercise testing session to achieve a heart rate in this pre-determined heart rate range. Second, although this was not a diagnostic test, the EKG utilized during the GXT assisted in determining whether it was safe for the subject to undergo the exercise testing session. All EKGs were sent to a cardiologist prior to the beginning any additional testing. Lastly, previous research has indicated that trained individuals have an improved awareness of energy needs [20]. Thus, although all subjects in this study were sedentary, differences in physical fitness could influence one's ability to regulate energy intake and thus initial fitness level could be controlled for in the statistical analyses.

3.5 PRIMARY OUTCOME MEASURES

3.5.1 Blood Analysis

Venous blood was collected in chilled tubes containing EDTA for analysis of GLP-1 and acylated ghrelin at five separate time points for each testing condition. For total GLP-1, the blood sample was stored on ice until it was centrifuged at 1000G for 10 minutes at 4ºC. One milliliter of plasma was then aliquotted into storage tubes and stored at -70ºC until the assay could be run using the ELISA kits from ALPCO (Salem, NH; cat # 48-GP1-HU-E01).

In preparation for the acylated ghrelin assay, 10µl of p-hydroxymercuribenzoic acid (PHMB) was added per ml of blood to prevent the degradation of acylated ghrelin by protease. Samples were then centrifuged at 1000 G's for 10 minutes at 4ºC. The supernatant was transferred and 100µl of 1N HCl was added per ml of plasma collected. The sample was then centrifuged at 3500 rpm for 5 minutes at 4ºC and 1mL was aliquoted into tubes and stored at -70ºC. The assay for acylated ghrelin was run using an ELISA kit from ALPCO (Salem, NH; cat # A05106).

All blood samples were processed at the Heinz Nutrition Laboratory in the Graduate School of Public Health at the University of Pittsburgh. All samples were assayed at the completion of the study and all samples from a single person were performed on the same kit to reduce the risk of intra-individual variability.

3.5.2 Mood/Hunger Questionnaire

In order to blind subjects to the fact that hunger and energy intake were primary outcome measures of the proposed study, questions about hunger were interspersed within the SEES questionnaire and were administered immediately before each blood draw. The questions related to appetite utilized a Likert scale format and a visual analogue scale was also used to assess hunger. The Positive and Negative Affect Schedule (PANAS) was used to assess mood and has been shown to be both valid and reliable [101]. The PANAS questionnaire was included in this study because there is some evidence to suggest that sedentary individuals may experience a deterioration in mood following a bout of exercise [102, 103]. Thus it is possible that changes in mood could influence food intake post-exercise.

3.5.3 Measurement of Energy Intake

Energy intake was assessed based upon an individuals' food consumption during the 1-2 hour time frame following the cessation of exercise or rest. For the first few subjects in this study, a survey was used to get a general feel for the types of foods that the subjects typically eat in the morning/early afternoon. Based upon this information and wanting to provide the subjects with a wide variety of food types, the selection of foods provided was as follows: mixed fruit, yogurt, bagels, cream cheese, butter, donuts, cereal, milk, nutrition bars, coffee, and tea.

During the feeding period, subjects were provided with the above-mentioned selection of foods and were instructed to help themselves to the snacks provided, unaware that their food intake was being monitored. All foods were weighed prior to giving the subject access to them and were weighed again following the subject's departure. The difference in weights between the foods provided and that which was left on the table after the 1-hour feeding period was used to calculate energy intake (Appendix I). All subjects were presented with the same variety of foods and this was held constant across testing sessions.

3.6 STATISTICAL ANALYSIS

Descriptive analyses were performed for subject characteristics (age, height, weight, BMI) as well as physical fitness, physical activity levels, body composition, resting metabolic rate, cognitive restraint, and disinhibition towards food. Additionally, mean ghrelin, GLP-1 concentrations and hunger levels at each of the time points were measured, and substrate utilization during exercise and resting conditions, and mean absolute and relative energy intake for each testing session were calculated.

A 2 x 3 mixed analysis of variance (ANOVA) was used to determine if various measurements of energy expenditure and energy intake were significantly different between the resting and exercise conditions between BMI classifications (overweight, class I obese, class II obese). The main effect of group (BMI categories) was examined to determine if there was a significant difference in any of these variables between body weight classifications averaged across treatment condition. The main effect of condition was examined to determine if there was a significant difference in any of these variables between the resting and exercise conditions averaged across body weight classifications. The interaction effect was analyzed to determine if the pattern of difference in any of these variables among treatment conditions was different between the various body weight classifications. If necessary, post-hoc tests using the Bonferoni adjustment were performed to determine where the difference was found. The assumption of homogeneity of variance was tested using the Brown-Forsythe test and the assumption of normality was tested using the Shapiro-Wilk test.

In order to determine if hunger levels, hormone concentrations, or measures of affect were different between conditions (exercise and rest) over time (baseline, post, 30, 60, 120), multiple 5 x 2 within-subjects ANOVAs were performed. The main effect of condition, the main effect of time, and the time x condition interaction were analyzed and post-hoc analyses were performed when necessary. Additionally, in order to determine if BMI influenced these findings, multiple 5 x 2 x 3 (time x condition x BMI) ANOVAs were performed using hunger, hormone concentrations, and various measures of affect.

Lastly, Pearson correlations were performed between many of the descriptive variables and food intake, hunger, and hormone concentrations in order to determine if any of these variables should be controlled for in the primary analyses. If a variable was found to be significantly correlated with one of these primary outcome measures, the ANOVA would be performed with and without including that particular variable as a covariate. All analyses were conducted using SPSS for Windows (SPSS Inc., Chicago, IL) and the alpha level was set at $p<0.05$.

3.7 POWER ANALYSIS

The primary aim of this study was to examine whether an acute bout of moderate intensity exercise alters energy intake one to two hours post-exercise compared to a resting condition. Therefore, a power analysis was performed to provide an estimate of sample size. Based upon previous research in lean subjects, a moderate-to-large effect size of 0.70 appears to be a reasonable estimate when analyzing differences in relative energy intake. A total of 19 subjects needed to be recruited to detect an effect size of 0.70 when statistical power was set at 0.80 and alpha at 0.05. Due to the possibility that subjects would have incomplete data from not completing both testing sessions, an additional 2 subjects were recruited to ensure adequate statistical power. Thus, a total of 21 subjects were recruited for this study.

4.0 RESULTS

The purpose of this study was to examine whether a single bout of moderate intensity exercise acutely influences food intake, hunger, GLP-1 and acylated ghrelin concentrations following exercise compared to a resting condition. This study utilized a randomized cross-over design and the results from this study are presented in the following sections.

4.1 SUBJECTS

A total of 21 overweight/obese women (mean BMI: 32.5 ± 4.3 kg/m^2) between the ages of 18 and 45 (mean age: 28.5 ± 8.3 years), were recruited for the current study. An equal number of subjects were classified as overweight (BMI: 25-29.9 kg/m^2), Class I obese (BMI: 30-34.9 kg/m^2), and Class II obese (BMI: 35-39.9 kg/m^2). All subjects completed their initial assessment visit; however following this visit, one participant was ineligible due to an abnormal EKG finding and another was unable to complete additional testing due to irregularities with her menstrual cycle. Thus, complete data were collected on a total of 19 subjects (see Figure 5).

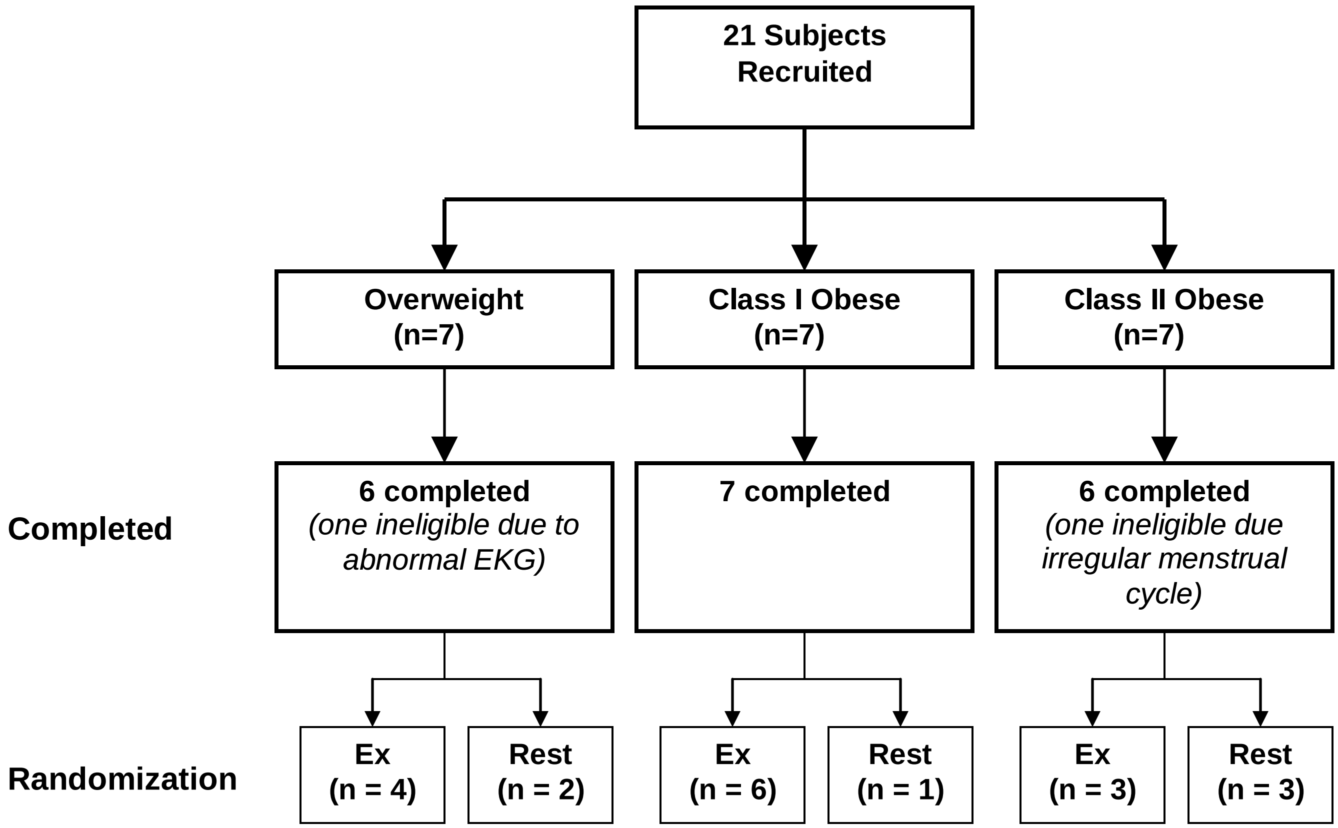

Figure 5: Study enrollment and randomization

Descriptive statistics (mean ± standard deviation) for the total sample, and for each of the previously described BMI classifications, are shown in Table 1. A series of one-way analysis of variance (ANOVA) revealed that there was no significant difference between BMI groups (overweight, Class I obesity, Class II obesity) for age, height, fitness, or physical activity levels. By design there was a significant difference between BMI groups for BMI, body weight, and body composition expressed as percent body fat ($p<0.05$). Resting metabolic rate (kcal/d) was significantly higher in the Class II obese group compared to the overweight group ($p<0.05$); however, there was no significant difference between BMI groups for resting metabolic rate when expressed relative to kg of fat-free mass. There was also a significant difference between the BMI groups for baseline cognitive restraint ($p<0.05$); however, disinhibition and trait hunger did not differ between BMI categories.

Prior to each experimental session (rest or exercise) subjects were instructed to consume a standardized breakfast prior to arriving at the laboratory. The calorie level of this meal was based on a percentage of the measured resting metabolic rate and therefore by design there was a significant difference between the BMI groups ($p<0.05$). All subjects reported consuming this meal as instructed by the investigator. These data are presented in Table 1.

Table 1 - Descriptive statistics

	All groups (n=19)	Overweight (n=6)	Class I Obese (n=7)	Class II Obese (n=6)	p-value**
Age (years)	28.5 ± 8.3	26.8 ± 5.5	33.1 ± 9.7	24.8 ± 7.6	.170
Height (cm)	163.0 ± 4.5	159.9 ± 2.7	165.2 ± 5.5	163.4 ± 3.4	.102
Weight (kg)	191.0 ± 29.7	154.6 ±11.0	198.9 ± 14.4	218.3 ± 15.4	<0.001[A,B]
BMI (kg/m^2)	32.5 ± 4.3	27.4 ± 1.5	33.0 ± 1.5	37.0 ± 2.0	<0.001 [A,B,C]
Fitness (seconds)	646.3 ± 169.8	753.3 ± 162.3	605.7 ± 187.2	586.7 ± 123.1	.175
Physical activity levels (kcal/wk)	586.4 ± 529.9	719.8 ± 295.2	666.9 ± 777.3	359.3 ± 326.3	.465
Body composition (% body fat)	41.7 ± 4.9	36.2 ± 2.8	42.1 ± 2.3	46.7 ± 2.3	<0.001 [A,B,C]
Resting metabolic rate (kcals/day)	1403.6 ± 272.7	1192.0 ± 211.7	1422.4 ± 225.1	1593.5 ± 253.7	.026 [B]
Relative REE (kcals/kg FFM)	28.0 ± 4.9	25.5 ± 4.1	27.3 ± 4.5	30.4 ± 6.1	.376
Cognitive restraint construct	10.28 ± 4.3	7.40 ± 2.9	9.57 ± 4.3	13.50 ± 3.5	.043 [B]
Disinhibition construct	9.89 ± 2.7	10.20 ± 2.3	10.86 ± 3.1	8.50 ± 2.3	.294
Hunger construct	7.39 ± 3.1	6.80 ± 2.7	8.14 ± 3.2	7.00 ± 3.8	.740
Calories in liquid pre-meal*	210.6 ± 41.1	178.3 ± 31.6	213.5 ± 34.0	239.5 ± 37.7	.024 [B]

[A] Overweight is significantly different from Class I obese (p<0.05)
[B] Overweight is significantly different from Class II obese (p<0.05)
[C] Class I obese is significantly different from Class I obese (p<0.05)
* Liquid pre-meal was consumed 2 hours prior to arrival at the lab and approximately 2.5 hours prior to the beginning of testing
**p-value based on one-way ANOVA with post-hoc analysis using a Bonferroni adjustment

4.2 DURATION AND ENERGY EXPENDITURE OF EXPERIMENTAL SESSIONS

The design of this study attempted to equate the duration of the resting and exercise experimental sessions based on the procedures described in the Methods Chapter of this document. Despite these efforts, examination of the data showed that the exercise session was significantly longer in duration than the resting experimental session (42.3 ± 7.7 minutes vs. 35.3 ± 5.1 minutes) ($p<0.001$).

Measured energy expenditure during the experimental exercise session was 353.6 ± 71.9 kcal, which was significantly higher than the measured energy expenditure during the experimental resting session (54.1 ± 13.5 kcal) ($p<0.001$). A 3 x 2 (BMI group X experimental condition) mixed ANOVA revealed that the pattern of difference for the measured energy expenditure differed by BMI group, and energy expenditure was consistently higher in the exercise condition compared to the resting condition across these BMI categories ($p<0.001$; Table 2). The respiratory exchange ratio (RER) during the exercise session was significantly higher than during the resting session ($p<0.05$), but there was no significant difference between BMI groups ($p=0.381$).

Table 2: Testing and resting sessions

	All groups (n=19)	Overweight (n=6)	Class I (n=7)	Class II (n=6)	Group	Condition	Condition x Group
Energy Expenditure (kcals)					<0.001	<0.001	<0.001
Exercise	353.6 ± 71.9	273.6 ± 40.3	373.4 ± 51.9	410.6 ± 40.2			
Rest	44.3 ± 8.9	37.4 ± 6.8	46.9 ± 8.8	48.3 ± 7.4			
Testing Time (min)					0.121	<0.001	0.131
Exercise	42.3 ± 7.7	36.7 ± 5.3	44.1 ± 8.8	45.9 ± 5.8			
Rest	35.3 ± 5.1	32.7 ± 3.9	36.1 ± 6.2	37.0 ± 4.5			
Relative Energy Expenditure (kcals/kg)					0.615	<0.001	0.389
Exercise	4.05 ± 0.41	3.88 ± 0.41	4.13 ± 0.49	4.14 ± 0.32			
Rest	0.51 ± 0.08	0.53 ± 0.08	0.52 ± 0.08	0.49 ± 0.10			
Respiratory Exchange Ratio					0.381	0.003	0.842
Exercise	0.82 ± 0.05	0.84 ± 0.04	0.81 ± 0.05	0.81 ± 0.05			
Rest	0.78 ± 0.05	0.80 ± 0.03	0.76 ± 0.06	0.77 ± 0.03			

4.3 ANALYSIS OF DATA BY SPECIFIC AIM

4.3.1 Specific Aim 1: Comparison of Ad-Libitum Energy Intake Following the Resting and Exercise Experimental Sessions

The primary aim of this study was to examine if a single bout of moderate-intensity exercise influenced ad-libitum energy intake during the 1-2 hour period post-exercise compared to a resting condition. Separate analyses were performed with energy intake expressed in 3 ways: 1) absolute energy intake (kcals), 2) energy intake relative to body weight (kcal/kg), 3) relative energy intake (REI) computed as the difference between energy intake and the energy expenditure during the experimental session (energy intake minus energy expenditure). These data are presented in Table 4.

Overall, there was no significant difference between energy intake 1-2 hours following exercise (551.5 ± 245.1 kcals) compared to the resting condition (548.7 ± 286.9 kcals). Furthermore, there was no difference in absolute energy intake between BMI groups. Percent body fat, REE, fitness, RER, cognitive restraint, and disinhibition were not significantly correlated with the difference in energy intake between conditions (Table 3) and thus were not included as covariates in the analyses. Individual responses are shown in Figure 6.

When energy intake was expressed relative to body weight (kcals/kg body weight), there was no difference between experimental conditions (exercise: 6.5 ± 2.9 kcals/kg vs. rest: 6.5 ± 3.5 kcals/kg; p=0.846). However, there was a significant BMI group effect with the overweight subjects having a higher energy intake (9.137 kcals/kg) compared to the Class II obese individuals (4.696 kcals/kg; p=0.033) when averaged across conditions. Additionally, there was a trend towards a significant BMI group x experimental condition interaction effect (p=0.099; Figure 7) with energy intake (kcals/kg) being significantly higher following the resting condition compared to the exercise condition in the overweight subjects (p<0.05) but there was no difference between conditions for the Class I and Class II obese individuals. Additional analyses revealed that % body fat (r=-0.523), fitness (r=0.708), and cognitive restraint (r=-0.616) were all significantly correlated with the difference in energy intake (kcals/kg) between conditions (p<0.05, Table 3). Thus, those subjects who had a higher percent body fat and higher cognitive restraint score had a lower energy intake post-rest compared to post-exercise while those who had higher fitness levels consumed more in the post-rest

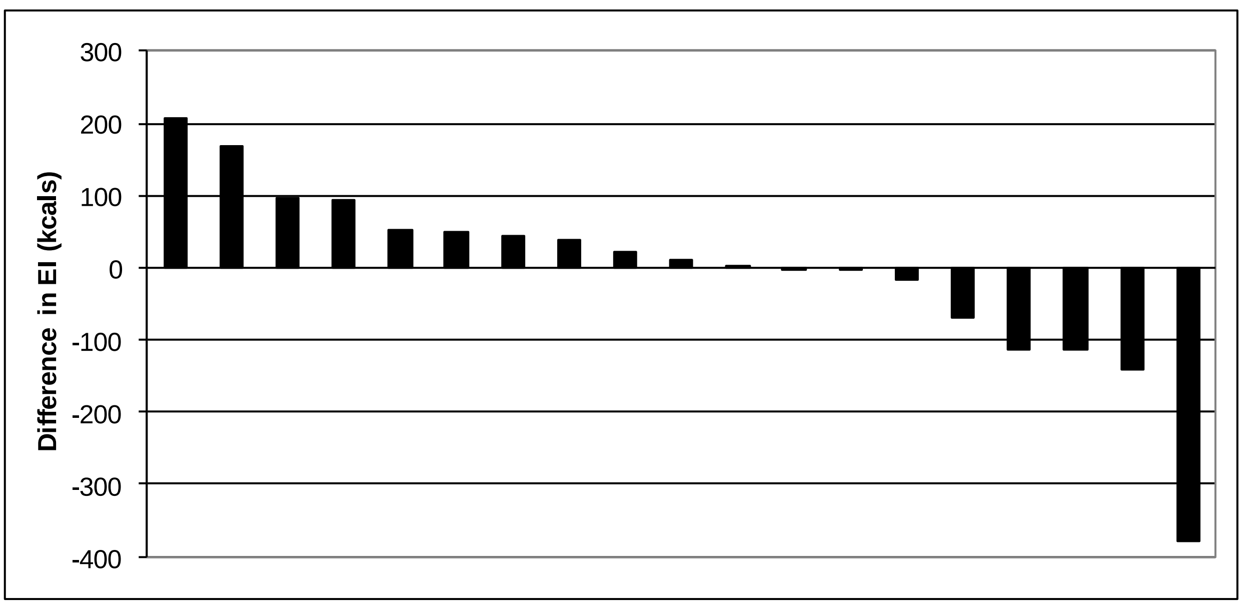

Figure 6: Individual differences in energy intake between exercise and resting sessions*

*Difference was calculated as the energy intake for the resting session minus the energy intake for the exercise session. A positive number indicates energy intake was higher following the resting session compared to the resting session.

compared to post-exercise condition. When these variables were included as covariates in the analyses, the non-significant condition effect (p=0.846) that was previously reported for energy intake relative to body weight became significant (p<0.001).

When relative energy intake (REI) was calculated as the ad-libitum energy intake minus the energy expenditure of the testing session, REI was significantly lower in the exercise condition (197.8 ± 256.6 kcals) compared to the resting condition (504.3 ± 290.1 kcals) when averaged across BMI groups (p<0.001; effect size: 0.856; Table 4). There was no difference in REI between BMI classifications and the difference in REI was not significantly correlated with % body fat, REE, fitness, RER, cognitive restraint, and disinhibition (Table 3).

Table 3: Correlational matrix for the difference in energy intake between testing conditions and descriptive variables

	Difference in energy intake (rest – exercise)	**Difference in energy intake (kcals/kg) (rest-exercise)**	**Difference in REI (rest-exercise)**
BMI	-0.385	-0.406	0.069
% Body fat	-0.295	-0.523 *	0.172
REE	-0.208	-0.270	0.073
Fitness	0.211	0.708 **	-0.170
RER	0.036	0.310	0.197
Cognitive restraint	-0.151	-0.616 *	0.349
Disinhibition	0.303	-0.249	-0.084

* p<0.05 ** p<0.001

Table 4: Ad-libitum energy intake 1-2 hours post-testing and fluid intake throughout the testing period

	All groups (n=19)	Overweight (n=6)	Class I (n=7)	Class II (n=6)	BMI Group	Testing Condition	Condition x Group
Absolute Energy Intake (kcals)					0.558	0.960	0.195
Post-exercise	551.5 ± 245.1	606.7 ± 205.4	542.9 ± 195.1	506.2 ± 349.6			
Post-rest	548.7 ± 286.9	681.4 ± 182.6	514.2 ± 213.0	455.9 ± 418.8			
Relative Energy Intake (kcals/kg)					.028	.846	0.099
Post-exercise	6.5 ± 2.9	8.6 ± 2.7	6.0 ± 2.1	5.0 ± 3.2			
Post-rest	6.5 ± 3.5	9.7 ± 2.3	5.7 ± 2.3	4.4 ± 3.7			
Relative Energy Intake (EI – EE)					0.295	<0.001	0.978
Post-exercise	197.8 ± 256.5	333.1 ± 224.0	169.5 ± 169.2	95.6 ± 341.6			
Post-rest	504.3 ± 290.1	644.1 ±176.9	467.4 ± 212.9	407.6 ± 425.2			
Fluid Intake (mL)					0.819	0.027	0.257
Post-exercise *	672.8 ± 616.7	688.2 ± 374.9	823.1 ± 952.3	482.2 ± 254.6			
Post-rest *	438.7 ± 336.3	647.8 ± 244.6	402.5 ± 430.8	271.8 ± 192.5			

* Subjects were provided with water immediately post-exercise or rest. The quantity of water consumed immediately post-testing up until 120-minutes post-testing was added to the amount of coffee or tea consumed during the ad-libitum feeding period to quantify the total fluid intake for the testing session

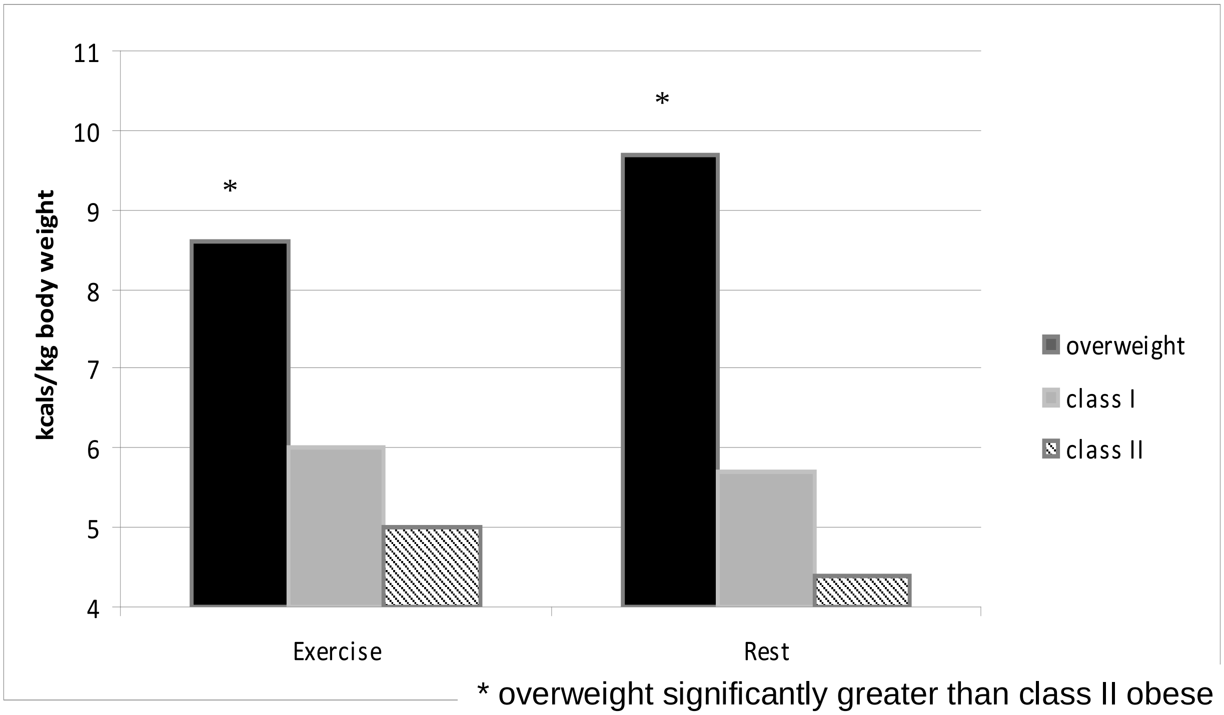

Figure 7: Energy intake expressed relative to body weight (kcals/kg) for BMI groups

Fluid intake was measured for two-hours following the experimental testing sessions and was significantly greater in the exercise condition (672.8 ± 616.7 mL) compared to the resting condition (438.7 ± 336.3 mL; p=0.027). As a result of this difference, fluid intake was controlled for in the statistical analyses that compared ad-libitum energy intake between experimental conditions and this covariate did not alter the findings. Additionally, there was no difference in fluid intake between exercise and resting sessions between BMI groups (Table 4).

Subjects were also queried after completing both experimental sessions about whether they were aware that food intake was being monitored during the 60-minute ad-libitum feeding period. Thirty-seven percent of the participants (n=7) believed that food intake was being monitored while at the facility, forty-two percent (n=8) were unsure, and 21% (n=4) did not believe that food intake was being measured. When this was considered in the analysis this did not influence the findings presented above. Lastly, energy intake post-exercise was not correlated with the energy expenditure of the exercise bout (r=-0.016, p=0.949).

4.3.2 Specific Aim 2: Comparison of Acylated Ghrelin Concentrations Following Exercise and Rest

This study sought to examine how a bout of moderate-intensity exercise affects acylated ghrelin concentrations compared to a resting condition. Four separate comparisons between conditions were made: 1) at pre-testing in order to ensure that there were no differences between testing days, 2) from pre-testing to immediately post-testing to examine the effect of the exercise bout, 3) from immediate post-testing to 60-minutes post-testing to examine the influence of exercise in the short-term, and 4) during the ad-libitum feeding period 1-2 hours following the cessation of the exercise or resting bout.

Pre-testing acylated ghrelin concentrations were not different between exercise (94.6 ± 61.7 pg/mL) and resting (91.5 ± 50.3 pg/mL) conditions (p=0.726). Furthermore, the change in hormone concentrations from pre-testing to immediately post-testing were similar between conditions (exercise: 3.47 ± 25.6 pg/mL vs. rest: -3.07 ± 45.8 pg/mL; p=0.608) although there was a slight increase in ghrelin immediately post-exercise and a slight decrease in ghrelin immediately post-rest. Acylated ghrelin was significantly decreased from immediately post-testing to 60-minutes post testing in the exercise condition (p=0.028) but not the resting condition (p=0.086). However, this decrease was not different between conditions (exercise: -27.0 ± 49.1 pg/mL vs. resting: -19.4 ± 45.2 pg/mL; p=0.549). Lastly, the non-signficant decrease in acylated ghrelin over the feeding period (1-2 hours post-tesing) was not different between testing days (exercise: -13.7 ± 39.2 pg/mL vs. rest: -4.3 ± 73.8 pg/mL; p=0.520).

To examine the pattern of difference between the groups over the entire 2.5 to 3 hour testing period, a 5 x 2 (time x testing condition) ANOVA was performed. Acylated ghrelin concentrations were not altered by a bout of moderate intensity exercise compared to a resting condition (Table 5). The time x testing condition interaction effect and the main effect of condition were not significant (p=0.163 and p=0.619 respectively). There was a significant main effect of time (p=0.007), but post-hoc tests using the Bonferroni adjustment revealed that there was no difference in ghrelin concentrations between any of the time points. The assumption of normality was checked using the Shapiro-Wilk test and was violated at several time points. However, the skewness within each group was in the same direction and ANOVA should therefore be robust against a violation of this assumption. Sphericity was measured using Mauchly's test and was violated for the main effect of time, thus the Greenhouse-Geisser adjustment was used. Acylated ghrelin concentrations plotted over time are shown in Figure 8 and individual data are shown in Appendix J.

Exploratory analyses were performed to examine the influence of BMI on acylated ghrelin concentrations between conditions. A 5 x 2 x 3 ANOVA (time x testing condition x BMI group) revealed that there were no differences between BMI groups (p=0.536). The inclusion of BMI in the model did not alter the previously stated findings nor were any of the interaction effects involving BMI significant (data not shown).

It should be noted that while processing the blood samples for the first 3 subjects, 10 µL of HCl was added to each milliliter of plasma, which was less than what the protocol stated (100 µL/mL plasma). The dilution factor only plays a small role in the assay and thus it appears that this deviation from the protocol did not impact the findings. However, the statistical analyses for acylated ghrelin were performed excluding these 3 subjects (n=16) and the results were unaltered. Thus, all 19 subjects were included in the data presented. However, for one subject, the post-testing sample for the resting condition was lost during processing, thus at that time point, only data from 18 subjects were used.

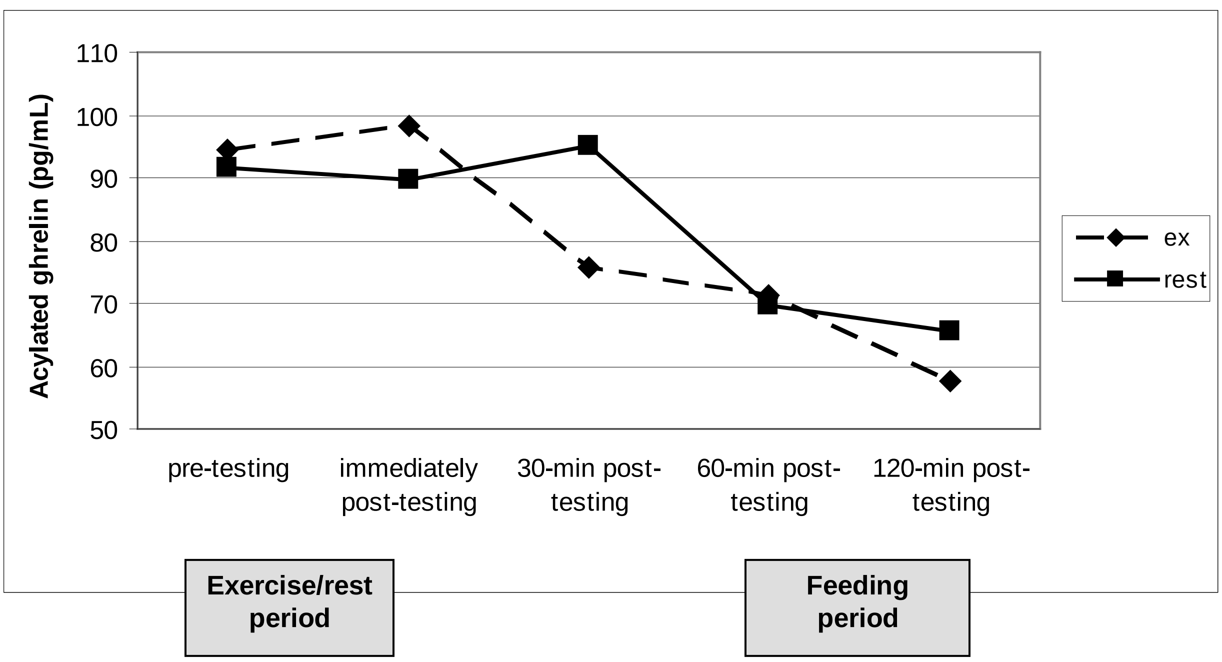

Figure 8: Change in acylated ghrelin concentrations over time for testing conditions

Table 5: Changes in appetite-regulating hormone concentrations throughout the testing days

	Pre-testing	Immediately Post-testing	30-min post-testing	60-min post-testing (before feeding)	120-min post-testing (after feeding)	Time	Testing Condition	Condition x Time
Ghrelin (pg/mL) (n=19)						0.007	0.558	0.198
Exercise	94.6 ± 61.7	98.2 ± 68.8	75.6 ± 37.5	71.2 ± 38.0	57.5 ± 35.0			
Rest	91.5 ± 50.3	89.8 ± 37.6	95.0 ± 52.4	69.8 ± 40.2	65.5 ± 53.2			
Total GLP-1 (ng/mL) (n=18)						0.418	0.059	0.420
Exercise	2.53 ± 0.8	2.56 ± 0.7	2.48 ± 0.6	2.38 ± 0.5	2.51 ± 0.7			
Rest	2.60 ± 0.8	2.55 ± 0.8	2.58 ± 0.6	2.62 ± 0.7	2.72 ± 0.6			

4.3.3 Specific Aim 3: Comparison of GLP-1 Concentrations Following Exercise and Resting Conditions

A primary aim of this study was to examine the influence of a moderate-intensity bout of exercise on GLP-1 concentrations compared to a resting condition. In order to thoroughly answer this research question, four separate comparisons between testing conditions were made. Paired samples t-tests were performed to examine: 1) whether pre-testing GLP-1 levels were different between testing days, 2) the influence of exercise on GLP-1 immediately post-testing, 3) the short-tem effect of the exercise session on GLP-1 (immediately post-testing to 60-minutes post-testing), and 4) the influence of feeding (1-2 hours post-testing) on GLP-1 concentrations.

Prior to testing, there was no difference in GLP-1 between testing days (ex: 2.53 ± 0.8 ng/mL vs. rest: 2.60 ± 0.8 ng/mL; $p=0.407$). The change in GLP-1 from pre-testing to immediately post-testing was not different between the exercise (increased 0.03 ± 0.3 ng/mL) and resting (decreased 0.05 ± 0.5 ng/mL) conditions. GLP-1 significantly decreased from post-testing to 60-minutes post-testing ($p=0.047$) in the exercise session but not in the resting condition ($p=0.578$). However, the change in GLP-1 over this time period was not different between testing conditions (exercise: -0.17 ± 0.3 ng/mL vs. rest: 0.07 ± 0.5 ng/mL; $p=0.153$). Lastly, there was a non-significant increase in GLP-1 from 60-minutes post-testing (immediately prior to feeding) to 120-minutes post-testing (following the feeding period) with no difference between conditions (exercise: 0.13 ± 0.3 ng/mL vs. resting: 0.10 ± 0.4 ng/mL; $p=0.770$).

A 5 x 2 (time x testing condition) ANOVA was performed to examine the pattern of difference between groups over the entire testing visit for GLP-1 levels. The main effect of time was not significant (p=0.418), the testing condition x time interaction effect was not significant (p=0.420), but there was a trend toward a significant condition effect (p=0.059; see Table 5) with GLP-1 being higher on the resting day compared to the exercise testing day. All data were both normally distributed or positively skewed and the assumption of sphericity was met. When checking the assumptions, it was determined that an outlier was present at the 60-min post-rest time point (32.8 ng/mL). Thus, this subject was excluded from the analyses and the previously stated findings related to GLP-1 are based on a sample size of 18. GLP-1 concentrations are plotted versus time in Figure 9 and individual data are shown in Appendix J.

Additional exploratory analyses were performed to examine the influence of BMI on acylated ghrelin concentrations between conditions and over time. A 5 x 2 x 3 ANOVA (time x testing condition x BMI group) revealed that the inclusion of BMI in the model had no impact on the previously mentioned findings. Additionally, the main effect of BMI group was not significant nor were the interaction effects involving BMI significant (data not shown).

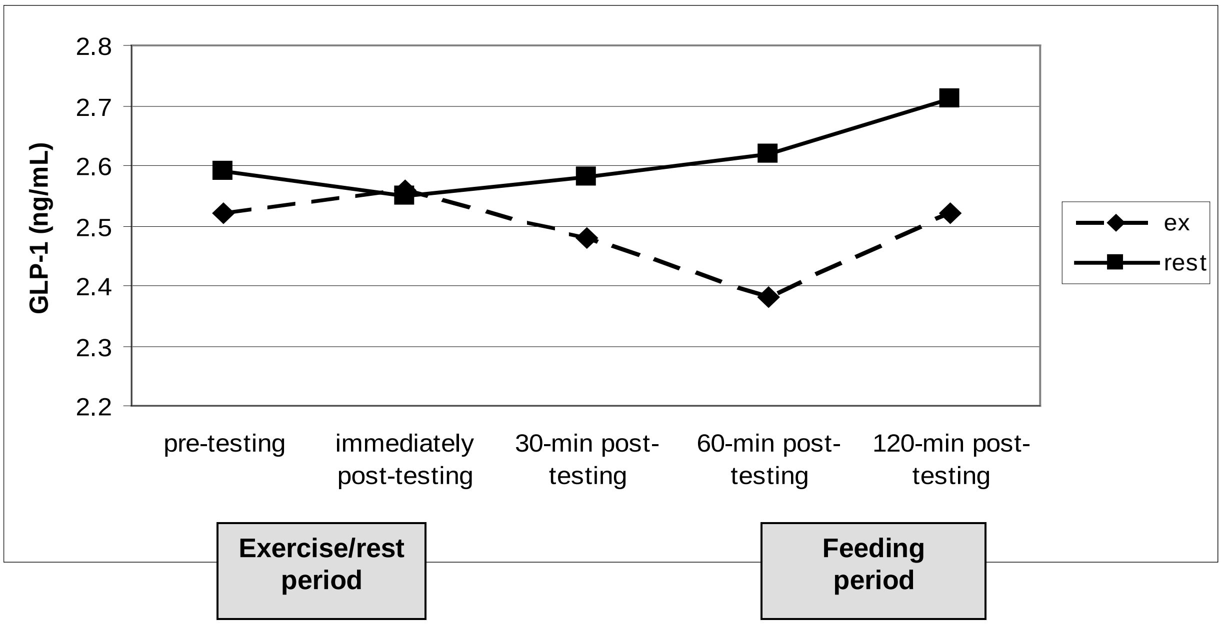

Figure 9: Change in total GLP-1 concentration over time for testing conditions

4.3.4 Specific Aim 4: The Effect of Exercise on Subjective Feelings of Hunger

The final primary aim of this study was to examine if subjective feelings of hunger changed over time in response to a bout of moderate-intensity exercise when compared to a resting condition. In order to thoroughly answer this research question, four separate comparisons were made between testing sessions: 1) to determine whether pre-test hunger ratings were different between testing days, 2) to examine the influence of exercise on hunger ratings from pre-testing to immediately post-testing, 3) to examine if the change in hunger immediately post-testing to 60-minutes post-testing was different between conditions, and 4) to determine the influence of feeding (1-2 hours post-testing) on hunger scores.

Overall, subjects were asked to report their hunger levels in two ways: using a visual analogue scale (VAS), and using a Likert-type scale (LTS) at five separate time points. Sixteen of the 19 subjects had complete VAS hunger data and all 19 subjects had complete LTS data. Therefore, since a fewer number of subjects had complete VAS data and the findings were similar between measurement types, only LTS hunger findings will be reported in the following text. Both LTS and VAS data are shown in Table 6.

There was no difference in baseline hunger levels between testing conditions (exercise: 1.8 ± 1.3 vs. rest: 2.1 ± 1.7; p=0.392). There was a trend (p=0.095) for hunger scores to increase more in the exercise condition compared to the resting condition from pre-testing to immediately post-testing (exercise: 1.16 ± 1.2 vs. rest: 0.42 ± 1.4). However, by 30 minutes post-testing, the change in hunger from baseline was no longer different between conditions (p=0.601). When examining the change in hunger from immediately post-testing to 60-minutes post-testing, there was a significant difference between groups (p=0.035) with hunger scores rising in the resting condition (0.68 ± 0.7) but not in the exercise condition (-0.05 ± 1.4). However, hunger scores were similar between groups at the 60-minute post-testing time point (exercise: 3.0 ± 1.6 vs. rest: 3.2 ± 1.5; p=0.507). Finally, there was a significant reduction in hunger from 60-minutes post-testing to the end of the feeding period (120-minutes post-testing); however, this change was not different between conditions (p=0.163).

A 5 x 2 (time x testing condition) ANOVA was performed to examine the pattern of difference in hunger ratings between experimental conditions over time. The condition x time interaction effect was not significant ($p=0.303$), nor was there a difference in hunger levels between conditions when averaged across time ($p = 0.959$). However, there was a significant main effect of time ($p<0.001$) with post-hoc tests using the Bonferroni adjustment revealing that hunger levels were significantly higher immediately post-test, 30-min post, and 60-min post and significantly lower at 120-min post-testing compared to pre-testing hunger scores ($p<0.05$). Additionally, hunger levels at each time point measured were significantly different from the 120-min post-testing time point ($p<0.05$; Table 6). The change in hunger ratings over time is shown graphically in Figure 10.

Exploratory analyses were performed to determine the influence of BMI on subjective feelings of hunger. A 5 x 2 x 3 (time x testing condition x BMI group) mixed ANOVA revealed that the inclusion of BMI into the model did not significantly alter the previously stated findings and none of the interaction effects that included BMI were significant (data not shown).

Subjects were also queried after completing both experimental sessions about whether they believe that exercise influences their feelings of hunger. Overall, 53% of the participants (n=10) reported that exercise typically increases their hunger, 31% (n=6) stated that they believe that exercise decreases their hunger, and 16% (n=3) thought that exercise had no effect on their feelings of hunger. When this factor was considered in the above analyses, the findings previously presented were not altered.

Also, when individuals were grouped into categories based upon their beliefs about the influence of exercise on hunger, there was no significant difference in measured subjective feelings of hunger or energy intake between groups (data not shown).

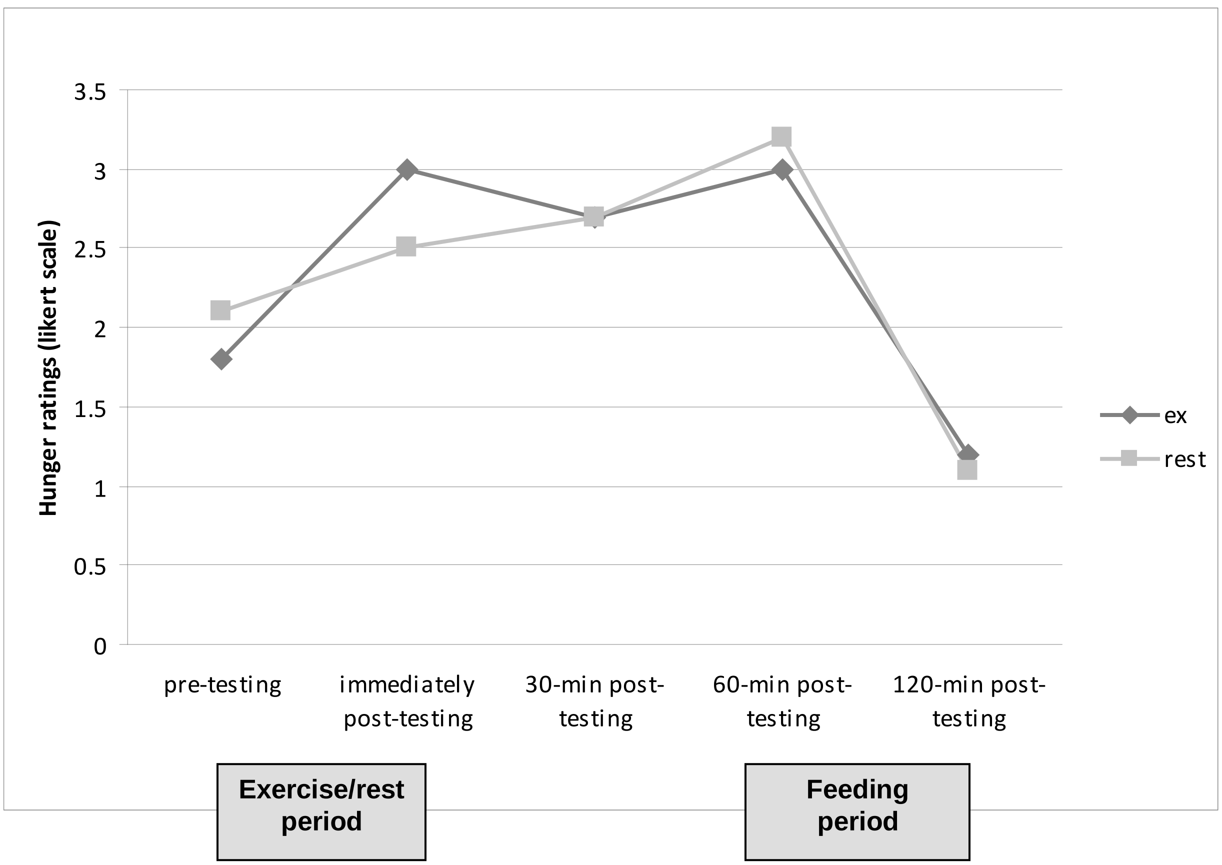

Figure 10: Change in subjective feelings of hunger over time for testing conditions

Table 6: Change in hunger ratings throughout the exercise and resting testing days

	Pre-testing	Immediately Post-testing	30-min post-testing	60-min post-testing (before feeding)	120-min post-testing (after feeding)	Time	Testing Condition	Condition x Time
Hunger (VAS) (n=16)						<0.001	0.577	0.540
Exercise	2.2 ± 2.1	3.1 ± 2.4	2.9 ± 2.1	3.6 ± 2.4	0.4 ± 0.6			
Rest	1.8 ± 2.2	2.4 ± 2.4	3.2 ± 3.0	3.6 ± 2.8	0.2 ± 0.4			
Hunger (Likert) (n=19)						<0.001	0.959	0.303
Exercise	1.8 ± 1.3	3.0 ± 1.5	2.7 ± 1.3	3.0 ± 1.6	1.2 ± 0.4			
Rest	2.1 ± 1.7	2.5 ± 1.5	2.7 ± 1.7	3.2 ± 1.5	1.1 ± 0.2			

4.4 EXPLORATORY ANALYSES

The primary purpose of this study was to examine whether a bout of moderate intensity exercise had an acute influence on energy intake and to explore potential physiological mechanisms that could be mediating this relationship. However, in addition to the physiological drive to eat, it is well established that there is also a psychological component to feeding. Thus, as part of the current study, various measures of mood were collected throughout the experimental testing session and the data were analyzed to determine whether any of these psychological variables influenced energy intake.

4.4.1 Mood

Constructs of positive well-being, psychological distress, and fatigue were measured at 5 separate time points (pre-testing, immediately post-testing, 30-minutes post-testing, 60-minutes post-testing, and 120-minutes post-testing) using the Subjective Exercise Experience Scale (SEES). A 5 x 2 (time x testing condition) within-subjects ANOVA was performed on each of these constructs of mood. Additionally, data on positive and negative affect were collected at 4 separate time points throughout the experimental testing day (pre-testing, immediately post-testing, 60-minutes post-testing, and 120 minutes post-testing) using the Positive and Negative Affect Schedule (PANAS). Separate 4 x 2 (time x testing condition) ANOVAs were performed on positive affect and negative affect. For those variables that were not normally distributed (psychological distress, fatigue, and negative affect), the skewness was in the same direction and thus, ANOVA is robust against this assumption. When the assumption of sphericity was not met, the Greenhouse-Geisser adjustment was used.

4.4.1.1 Comparison of Mood Constructs Between Experimental Testing Sessions

A 5 x 2 (time x testing condition) within-subjects ANOVA revealed that the condition x time interaction effect for the positive well-being construct of the SEES questionnaire was not significant ($p=0.819$). The main effect of condition was significant ($p<0.001$) with higher positive well-being scores seen in the exercise condition compared to the resting condition. The main effect of time ($p=0.007$) was also significant and scores of positive well-being were reduced over time (Table 7). For the psychological distress construct, neither the time x condition interaction effect ($p=0.870$) nor the main effect of condition ($p=0.395$) was significant. However, there was a main effect of time ($p=0.038$) but post-hoc tests using a Bonferroni adjustment revealed that there were no significant differences in psychological distress between any of the time points. Lastly, none of the effects examined for the fatigue construct of the SEES questionnaire were significant (Table 7).

Table 7: Changes in mood across the exercise and resting conditions

	Pre-testing	Immediately post-testing	30-min post-testing	60-min post-testing	120-min post-testing	Time	Testing Condition	Condition x Time
Positive Well Being (SEES)						0.007	<0.001	0.819
Exercise	20.2 ± 3.2	19.6 ± 5.5	19.6 ± 4.6	17.9 ± 5.5	19.2 ± 5.3			
Rest	18.5 ± 4.8	17.2 ± 4.8	17.1 ± 5.1	16.3 ± 5.4	17.3 ± 5.7			
Psychological Distress (SEES)						0.038	0.395	0.870
Exercise	5.4 ± 2.3	4.8 ± 2.2	4.4 ± 0.8	4.8 ± 2.7	4.3 ± 0.9			
Rest	5.4 ± 2.5	4.5 ± 1.1	4.2 ± 0.5	4.1 ± 0.3	4.2 ± 0.5			
Fatigue (SEES)						0.511	0.471	0.261
Exercise	8.6 ± 3.0	9.3 ± 4.8	7.3 ± 3.4	7.6 ± 4.1	8.6 ± 5.1			
Rest	8.4 ± 5.6	7.6 ± 3.9	7.7 ± 3.9	7.6 ± 4.7	7.2 ± 4.3			
Positive Affect (PANAS)						0.063	0.610	0.031
Exercise	28.8 ± 6.4	30.1 ± 7.3	N/A	25.5 ± 7.8	26.3 ± 8.1			
Rest	28.0 ± 7.4	26.3 ± 7.1	N/A	26.4 ± 8.3	27.2 ± 8.6			
Negative Affect (PANAS)						0.479	0.189	0.296
Exercise	10.7 ± 1.1	10.8 ± 1.5	N/A	11.7 ± 5.4	10.2 ± 0.5			
Rest	10.6 ± 0.5	10.3 ± 0.5	N/A	10.2 ± 0.5	10.4 ± 1.0			

A 4 x 2 (time x testing condition) ANOVA revealed that there was no difference over time or between testing conditions for the negative affect subscale of the PANAS questionnaire. However, there was a significant time x condition interaction effect for the positive affect subscale ($p<0.05$;Figure 11). There was a non-significant increase in positive affect from pre-testing to immediately post-testing (1.26 ± 6.9; $p=0.434$) in the exercise condition and a non-significant decrease over the same time period in resting condition (-1.68 ± 3.8; $p=0.069$). Paired samples t-tests revealed that the change in positive affect from pre-testing to immediately post-testing was not significantly different between groups ($p=0.114$). Additionally, the change in positive affect from immediately post-testing to 60-minutes post-testing was significantly different between groups ($p=0.020$); positive affect decreased in the exercise condition (-4.6 ± 8.4) and was relatively unaltered in the resting condition (0.11 ± 3.2). By 60-minutes post-testing, positive affect was significantly reduced compared to pre-testing scores in the exercise condition ($p=0.013$), and there was a similar trend seen in the resting condition ($p=0.070$). However, there was no difference between testing conditions at this time point ($p=0.583$).

Exploratory analyses were performed in order to determine if the inclusion of BMI categories into the model would alter the findings. Two separate 4 x 2 x 3 (time x testing condition x BMI group) mixed ANOVAs were performed on positive and negative affect and three separate 5 x 2 x 3 (time x testing condition x BMI group) mixed ANOVAs were performed using the positive well-being, psychological distress, and fatigue constructs of the SEES questionnaires. The time x condition x BMI category interaction effect was not significant, nor were there any significant time x BMI category interaction effects or condition x BMI category interaction effects for any of the mood constructs (data not shown).

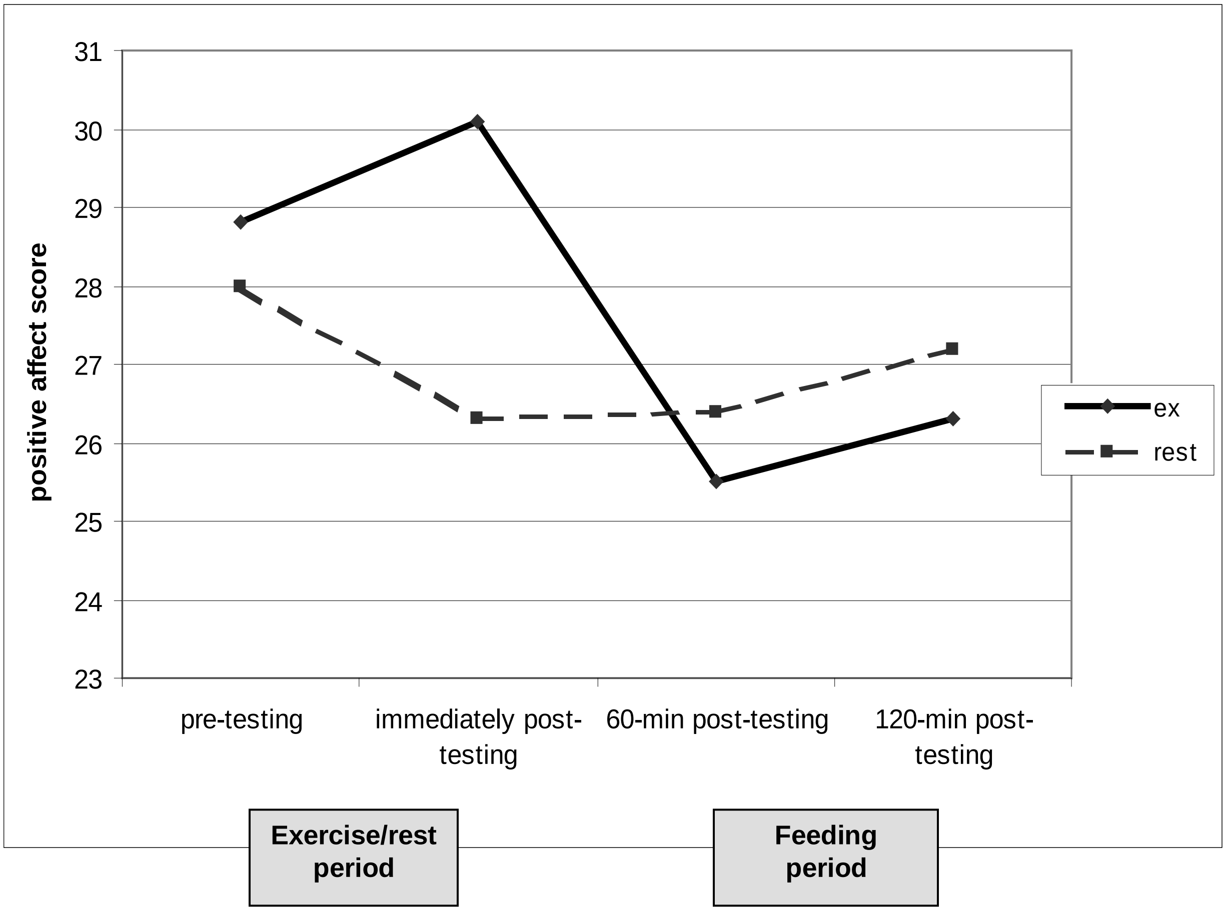

Figure 11: Changes in positive affect over time for testing conditions

4.4.1.2 Influence of Positive Affect on Energy Intake

As a result of the significant interaction effect for positive affect, exploratory analyses were performed to examine the impact of changes in positive affect on absolute and relative energy intake. Subjects were grouped into one of two categories based upon their change in mood from baseline to post-testing in the exercise condition: improved positive affect (IPA) and worsened positive affect (WPA). Any individual with a change from baseline to post-testing that was less than +2 points was categorized into the WPA group.

A 2 x 2 mixed ANOVA was performed as a function of condition (exercise and rest) and mood state (IPA and WPA). When individuals were grouped according to whether they had improved or worsened positive affect from baseline to post-exercise there was a trend towards a significant condition by mood state interaction effect ($p=0.077$; Figure 12). Those individuals who reported a decrease in positive affect ($n=8$) from baseline to post-exercise had a higher absolute energy intake following exercise (588.0 ± 233.7 kcals) compared to rest (524.6 ± 281.7 kcals), although this was not significant ($p=0.267$). Those subjects who reported an improvement in positive affect from baseline to post-exercise ($n=11$) had a lower energy intake following exercise (524.9 ± 260.9) compared to rest (566.1 ± 303.0 kcals). However, this was not significant (0.177). When the change in mood from baseline to 60-minutes post-exercise was calculated and subjects were coded in the same manner as described above there were no differences found between groups. There were no significant findings found when REI was used in place of energy intake.

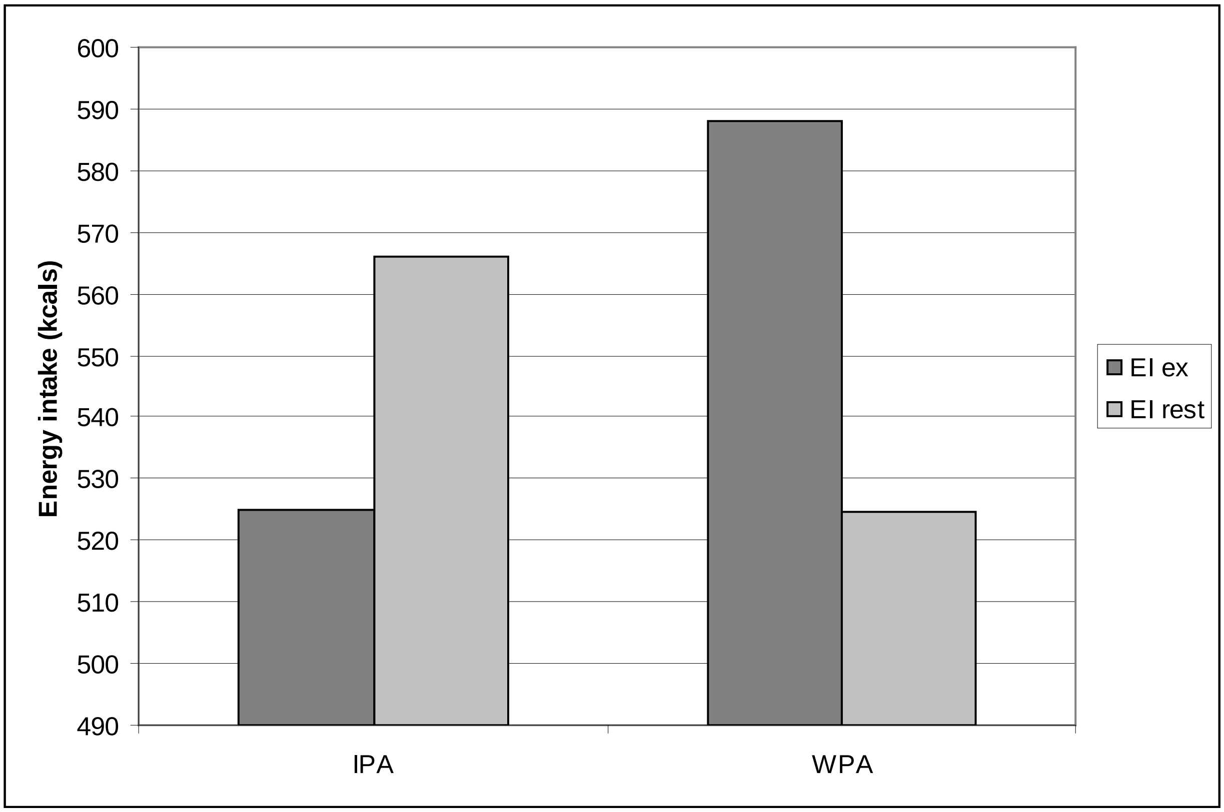

Figure 12: Energy intake for exercise and resting conditions for those with improved and worsened positive affect

4.4.2 Correlational Analyses

Correlational analyses were performed to examine whether significant relationships existed between the numerous variables examined in the current study. The following relationships were considered: 1) hunger and energy intake, 2) hunger and GLP-1 and acylated ghrelin concentrations, 3) energy intake and acylated ghrelin and GLP-1, 4) pre-testing GLP-1 and acylated ghrelin with subject descriptives (ie-body weight, percent body fat, age, and resting energy expenditure).

Energy intake following exercise was significantly correlated with hunger ratings immediately post-exercise (r=0.709, p=0.001), 30-minutes post (r=0.682, p=0.001), but not 60-minutes post-exercise (r=0.393, p=0.096). Energy intake following rest was significantly correlated with hunger ratings at each of the time points (immediately post-testing: r=0.760, 30-minutes post-testing: r=0.749, 60-minutes post-testing: r=0.682; p<0.001). Similar correlations were seen when REI was used in place of energy intake.

Energy intake and subjective feelings of hunger were not significantly correlated with GLP-1 or acylated ghrelin concentrations at any time point for either experimental condition. Pre-testing GLP-1 concentrations were significantly correlated with age (r=0.504, p=0.028), but not weight, BMI, percent body fat, or REE. Pre-testing acylated ghrelin concentrations were significantly correlated with REE (r=-0.476, p=0.039), but not age, body weight, BMI, or percent body fat.

5.0 DISCUSSION

5.1 INTRODUCTION

Obesity is a major public health concern in the United States [1]. As a result of the numerous health risks associated with excess body weight [2, 4, 8, 10], it is necessary to better understand the various factors involved in the regulation of energy balance. A typical weight loss prescription includes recommendations to decrease caloric intake and increase energy expenditure through exercise [12]. However, the contribution of exercise in the regulation of body weight is not fully understood [16].

It is possible that when exercise is used as part of a weight loss prescription, the sole contribution of that bout of exercise to weight loss is the increased energy expenditure gained through the exercise bout. However, there is some evidence to suggest that exercise may play a role in appetite regulation [104]. It is currently unknown whether a single bout of exercise acutely alters hunger levels and subsequent energy intake. Additionally, if there is an acute relationship seen between exercise and food intake, the mechanism driving this relationship needs to be established.

To date, no study has examined this association between exercise and energy intake while simultaneously exploring potential physiological and psychological mechanisms that may be mediating this relationship, in a group of overweight/obese adults. Thus, the purpose of this study was to examine how a single bout of moderate intensity exercise influenced food intake, subjective feelings of hunger, GLP-1 and acylated ghrelin concentrations, and various psychological parameters in a group of sedentary, overweight and obese women.

5.2 SUMMARY OF MAJOR FINDINGS

5.2.1 The Influence of Exercise on Energy Intake

Absolute ad-libitum energy intake was unchanged following a bout of moderate intensity exercise compared to a resting condition (551.5 ± 245.1 kcals vs. 548.7 ± 286.9 kcals). These findings are similar to previous research that has been conducted in a lean population and have reported a loose coupling between exercise and energy intake [19, 22, 32, 51, 52, 61]. However, this is one of the few studies to demonstrate this in an overweight/obese population. To date, only three studies have thoroughly compared food intake following exercise and rest in this population [31, 38, 105]. In a small sample of males, Westerterp-Plantenga and colleagues [38] reported energy intake to be lower following exercise (120 minute cycling bout at 60% maximal workload) compared to a resting condition (549 ± 48 kcals vs. 740 ± 71 kcals; Cohen's d = 3.15). However, the design of this study was quite different from the current study in the mode of exercise employed, duration of the exercise bout, and subject population and sample size. The second study conducted by Ueda and colleagues [105] found that a 60 minute cycling bout at 50% VO_{2max} elicited a decrease in both absolute energy intake and relative energy intake compared to a resting session in a group of males. Similar to Westerterp-Plantenga, overweight males were observed in this study and despite the shorter exercise duration, similar results were found.

In contrast to Westerterp-Plantenga [38] and Ueda [105], Kissileff and colleagues [31] found energy intake to be unaltered following a 40-minute bout of either moderate-intensity (30W) or vigorous intensity (90W) cycling compared to a resting condition in 9 sedentary, overweight/obese women. The design of this study was different from the current study in that the feeding period started 15 minutes post-exercise or rest, and subjects were not provided with a buffet-style meal, rather they were given a strawberry yogurt shake. Additionally, the mode of exercise was cycling while treadmill walking was employed in the current study. Despite these methodological differences between our study and Kissiliff's, both studies reported no difference in energy intake following exercise and rest, which is in apparent contrast to the Westerterp-Plantenga [38] and Ueda [105] studies.

Due to the limited number of studies conducted in this population, it is difficult to draw definitive conclusions regarding the effect of exercise on energy intake. However, these four studies suggest that variables such as gender or the duration of the exercise bout may influence feeding post-exercise. Thus, future studies should seek to confirm these theories by examining differences in energy intake following exercise between males and females, across different BMI groups and under different exercise paradigms, to gain a better understanding of whether these factors may influence appetite regulation post-exercise.

Implications for Weight Control

Although exercise did not result in a reduction in food intake, it is important to note that food intake was not increased in response to the exercise session. These findings further expand the literature, suggesting that exercise can play a primary role in regulating body weight and may do so by allowing an energy deficit created through an exercise session to persist following exercise. For example, in the current study, when the energy expenditure of the exercise bout was taken into consideration and the relative energy intake was calculated, a larger energy deficit was created following exercise compared to rest (see Table 4). This suggests that an individual does not compensate for the energy cost of the exercise within two hours of the exercise session. If an individual does not compensate later in the day, the negative energy balance created through exercise can significantly impact body weight.

One limitation of previous studies in this area of research is that the energy expenditure of the exercise session is not often accounted for and relative energy intake is not considered. Thus, the authors of these studies suggest that exercise has no impact on energy intake. However, caution should be taken when interpreting these findings regarding the role of exercise in appetite regulation. As demonstrated in the current study, an energy deficit was created and persisted 2-hours post-exercise, which may indicate that exercise has the ability to create a negative energy balance, at least in the short-term. Additionally, unlike food restriction that results in an increase in food intake to restore energy homeostasis, the current study demonstrates that exercise does not appear to create a similar compensatory response, thus favoring weight loss. These findings are similar to that of Hubert and colleagues [19] and suggest that these two dissimilar methods of creating a negative energy balance (calorie restriction and exercise) have noticeably different effects on appetite. Thus, future studies should continue to explore how exercise and calorie restriction differentially impact appetite and possible mechanisms contributing to appetite regulation. Furthermore, studies should account for the energy cost of the exercise session when explaining the effect of exercise on daily energy balance.

Individual differences in energy intake following exercise compared to rest

Although there was no difference in the mean absolute energy intake between conditions, caution should be taken when making generalizations surrounding these findings. In the current study, 5 subjects consumed > 50 kcals less in the post-exercise condition compared to the post-resting condition, 9 reported no change (post-exercise was within ± 50 kcals of the post-resting session), and 5 reported an increase in food intake post-exercise compared to post-rest. These individual differences are not reflected when the data is analyzed as a group since some individuals had a higher food intake following exercise while others had a higher food intake following rest, thus negating the fact that differences between conditions existed for approximately half of the subjects. The cause of these individual differences is not known; however it is likely that these differences may be the result of a physiological or psychological response to the exercise bout. In the current study, acylated ghrelin and GLP-1 were not correlated with the difference in energy intake between exercise and resting conditions. Additionally, when subjects were grouped into one of three categories based upon the difference in energy intake between the exercise and resting conditions (Group 1: energy intake higher post-exercise compared to post-rest, Group 2: energy intake higher post-rest compared to post-exercise, and Group 3: less than a ± 50 kcal difference in energy intake between conditions), acylated ghrelin, GLP-1, and subjective feelings of hunger over the entire testing sessions were not different between these groups. Therefore, these factors do not explain the individual differences seen in the current study.

Future studies should consider individual data and seek to examine why this inter-individual variability exists before drawing conclusions about the efficacy of exercise on energy balance. Perhaps this would provide insight into the mechanism through which exercise assists in the regulation of food intake and to understand why this may vary between persons. Lastly, it is unknown whether the results of the current study would be replicated had additional trials of each testing day been performed. Thus, future studies should also examine the intra-individual variability between testing days when examining the acute relationship between exercise and food intake.

<u>The influence of BMI on energy intake</u>

One factor that may explain some of the individual variance in energy intake is BMI. Although there was no difference in ad-libitum energy intake or relative energy intake post-exercise between BMI groups, this was the first study to examine energy intake relative to body weight. Although underpowered, there was a trend toward a significant BMI group x testing condition interaction effect present in the current study. This reflected a modest increase in food intake (kcals/kg) post-exercise compared to post-rest in the Class I and Class II obese subjects and a modest decrease in post-exercise food intake (kcals/kg) compared to a post-resting condition in the overweight subjects (see Table 4 & Figure 7). These data suggest that exercise may alter some physiological or psychological parameter that reduces feeding signals post-exercise more in overweight individuals compared to obese subjects.

A potential mechanistic pathway that may explain these findings is the respiratory exchange ratio (RER). Previous research has shown that the RER of an exercise bout may predict the quantity and quality of food consumed post-exercise [23]. For example, Almeras et al. [23] reported that men with a low RER during exercise (high fat oxidation) had a reduction in post-exercise energy intake (relative to the energy cost of exercise) compared to those with a high RER. However, in the current study there was no difference in exercise RER between BMI groups, which suggests that this would not explain the modest increase in energy intake (kcals/kg) that was observed in the obese subjects and not the overweight subjects.

Another possible explanation as to why differences in energy intake relative to body weight were observed between BMI groups could be attributed to the total energy cost of the exercise bout. The energy expenditure of the exercise session was calculated based upon body weight (3.0 kcals/kg/body weight), and thus the absolute energy expenditure during the exercise session was lowest in the overweight subjects compared to the obese subjects. Although purely speculative, it is possible that an absolute energy expenditure threshold exists, that any energy expenditure above that threshold would result in an increase in food intake post-exercise. It is possible that the Class I and Class II obese subjects exceeded that threshold, explaining the increase in food intake observed post-exercise. Future studies in overweight/obese patients should seek to determine if an energy expenditure threshold exists, and to determine the most optimal energy expenditure of an exercise session that favors the creation of a negative energy balance in the short-term post-exercise.

Overall, findings from the current study demonstrate a need for additional studies to examine the influence of BMI on energy intake post-exercise and explore possible reasons explaining why subjects with a higher BMI increased food intake post-exercise while overweight subjects decreased food intake post-exercise. Our data suggest that the higher one's body weight, the more difficult it may be for an individual to sustain an energy deficit that is created by an exercise bout which can have profound implications for weight control.

In addition to the trend towards a significant BMI group x testing condition interaction effect in the current study, there was also a BMI group effect for energy intake relative to body weight. Energy intake (kcals/kg) was higher in the overweight subjects compared to the class II obese subjects (see Table 4). Since the class II obese subjects had higher levels of dietary restraint compared to the overweight subjects, it is possible that they were more consciously aware of their food intake and/or were concerned about being watched while feeding. Another possible explanation as to why differences in energy intake relative to body weight were seen between BMI groups could be the result of the pre-load breakfast meal that was consumed by the subjects 2.5 hours prior to the start of testing. The pre-load breakfast was standardized so that each subject received the same calorie intake relative to their resting energy expenditure (15% REE). Thus, the overweight subjects consumed significantly fewer absolute calories compared to the Class II obese subjects prior to testing, possibly contributing to greater food consumption relative to their body weight during both testing sessions. These hypotheses need to be further investigated.

5.2.2 The effect of Exercise on Acylated Ghrelin Concentrations

Ghrelin is an orexigenic hormone that is produced primarily in the stomach and is involved in appetite regulation [67]. Typically, levels rise immediately prior to feeding and fall following a meal [67]. However, recently it was discovered that the inactive form of ghrelin (non-acylated) did not play a role in the regulation of energy homeostasis and only the active form of ghrelin (acylated ghrelin) was responsible for this action [76]. Thus, when exploring a potential mechanism mediating a relationship between exercise and food intake, acylated ghrelin concentrations were measured in the current study.

Findings from the current study indicate that a bout of moderate-intensity exercise does not alter acylated ghrelin concentrations from pre-exercise to immediately post-exercise, or from immediately post-exercise to 60-minutes post-exercise compared to a resting condition. Very few studies have sought to examine how ghrelin responds acutely to exercise and only one study has included overweight/obese subjects. In contrast to the current study, Marzullo and colleagues [48] found acylated ghrelin to be reduced immediately following a VO_{2max} test compared to pre-testing values in a group of obese subjects (BMI: 33.7 ± 1.5 kg/m^2). The difference in the findings of the study conducted by Marzullo et al. and the current study may be due to a number of factors.

First, the Marzullo et al. study did not have a resting control condition, making it difficult to determine if the reduction in acylated ghrelin was the result of the exercise bout, or the time that had elapsed from pre-exercise to post-exercise. Marzullo et al. also examined the change in acylated ghrelin following a bout of peak exercise whereas the current study used a bout of moderate-intensity exercise. Additionally, the authors did not note whether the suppression in acylated ghrelin persisted post-exercise nor did they examine whether a reduction in acylated ghrelin influenced hunger or food intake.

The only other studies that have examined the influence of exercise on acylated ghrelin have utilized an active and lean population [47, 106]. One study found acylated ghrelin concentrations to be suppressed 30 minutes into a 60-minute exercise bout, but there was no significant difference between the exercise and resting condition immediately post-exercise or at any of the other follow-up time points [47]. However, it should be noted that there was a trend for acylated ghrelin to be suppressed for up to 8 hours post-exercise and it is possible that this may have reached significance had the sample size been larger than 9 subjects. A second study found acylated ghrelin to be lower post-exercise, but levels had returned to normal 1-hour post-exercise [106].

Besides the different populations utilized, there were two main differences between these studies compared to the current study. First, subjects in the previous studies were in a fasted state, while subjects in the current study were fed 2.5 hours prior to testing. Second, the exercise bout in the previous study was 60 minutes, while the average treadmill time in the current study was 42 minutes. It is possible that this additional 18 minutes of exercise accounted for the different responses in acylated ghrelin concentrations between the two studies.

Overall, the current study contributes to the existing literature that has examined the change in acylated ghrelin concentrations in response to exercise. Due to a limited number of studies in this area and the contradictory findings, additional studies should be conducted to better understand if changes in ghrelin could explain the attenuation in food consumption in response to a bout of exercise.

Mean acylated ghrelin concentrations at baseline

Mean acylated ghrelin concentrations in the current study were 93.07 ± 52.9 pg/ml at baseline in this group of overweight/obese women (BMI of 32.5 ± 4.3 kg/m^2). These values are lower than those found by Marzullo et al. [107] who reported mean acylated ghrelin concentrations to be 180.4 ± 18.5 pg/mL in obese subjects (BMI: 32.4 ± 1.6 kg/m^2) and 411.8 ± 57.4 pg/mL in lean subjects. Similarly, another study by Marzullo and colleagues [48] reported acylated ghrelin to be 290 ± 43 pg/mL in a group of obese subjects (BMI: 33.7 ± 1.5 kg/m^2). One difference between the current study and these two studies is that acylated ghrelin was measured under fasting conditions in the previous studies, while in the current study, subjects were fed 2.5 hours prior to measurement. This could explain the lower values seen in the current study since ghrelin is typically higher in a fasted state [67].

The influence of body weight on acylated ghrelin concentrations

In the current study, acylated ghrelin was not correlated with BMI, body weight, percent body fat or resting energy expenditure. This is in contrast to previous studies that have reported an inverse relationship between total and acylated ghrelin and these variables [77, 107]. In fact, Marzullo and colleagues [107] have shown that the entire ghrelin system is impaired with obesity. Thus, under resting conditions, leaner individuals would have higher ghrelin concentrations compared to those with excess body weight [77]. Since ghrelin assists with satiety, it could be hypothesized that overweight individuals have consistently higher hunger levels compared to their leaner counterparts. Fortunately, ghrelin is sensitive to changes in body weight and has been shown to increase following weight loss [108]. However, since a relationship between ghrelin and body weight was not found in the current study, these findings may suggest that once an individual is overweight, the degree of impairment in the ghrelin system is no longer dependent upon body weight. It is hypothesized that there may be a certain body weight threshold, that once reached, results in an impairment in the ghrelin system. Future studies should seek to determine if and why this threshold exists.

The influence of feeding on acylated ghrelin

Interestingly, in the current study, there were no significant differences in acylated ghrelin concentrations between any time points. There was a trend for acylated ghrelin to be lower at 120-minutes post-exercise (following feeding) compared to the other time points, but this was not statistically significant. This finding is surprising since previous research has indicated that ghrelin levels typically fall following a meal, serving as a signal to stop eating [67, 109]. However, English and colleagues [110] recently demonstrated that the decline in ghrelin that is often seen following a meal in lean subjects is not present in obese individuals. This is in accord to what was found in the current study. The lack of suppression in acylated ghrelin following feeding has the potential to lead to an increase in food consumption beyond that time point, which can be detrimental in the weight control efforts of obese individuals. Although this was not a primary aim of the current study, it is an important finding nonetheless and future studies should continue to examine differences in ghrelin concentrations between lean and obese subjects at rest, following exercise, and following feeding.

5.2.3 The Effect of Exercise on GLP-1

Glucagon-like peptide 1 is an anorexigenic gastrointestinal hormone which has been shown to increase satiety in both lean [39, 78] and obese subjects [40, 79] when administered peripherally. The release of GLP-1 appears to be dependent upon body weight, with obese individuals reporting an attenuated response during feeding compared to lean subjects [83]. It has been suggested that GLP-1 release is regulated by the autonomic nervous system [111] thus it is hypothesized that the stimulation of this system during exercise will enhance GLP-1 secretion.

Overall, findings from the current study do not substantiate this claim and GLP-1 was unaltered immediately post-exercise and 60-minutes post-exercise compared to a resting condition. The body of literature examining the effect of a bout of exercise on GLP-1 is sparse [20, 41, 42, 66, 82, 105]. The majority of these studies have been conducted in lean subjects and have found that GLP-1 is increased during exercise and immediately post-exercise [30, 66, 82] thus favoring a decrease in food intake. However, only two studies have examined the response of GLP-1 to exercise in an overweight/obese population and both studies made the comparison between lean and obese subjects [42, 105]. Ueda and colleagues [105] reported that GLP-1 was increased following a 60-minute bout of cycling at 50% VO_{2max} in a group of obese males (BMI: 30.0 ± 3.1 kg/m^2). Furthermore, this increase in GLP-1 was sustained into the recovery period, until feeding at 60-minutes post-exercise. The mean values of GLP-1 were not different between normal weight and obese subjects. Energy intake was lower following the exercise condition compared to the resting session despite hunger ratings being unaltered by the exercise bout. These results are in contrast to the current study and could be the result of the gender of the subjects or the duration, intensity, and/or mode of the exercise session. Similar to the current study, subjects in this study did exercise in a fed state.

Following a 60-minute low-intensity cycling bout (25% of maximal power), Adam et al. [42] found GLP-1 concentrations to be significantly higher in the normal weight subjects, but not overweight/obese subjects (BMI: 30.9 ± 2.7 kg/m^2). However, hunger was not assessed nor was energy intake monitored. Subjects in this study exercised in a fasted state and at a lower intensity than the current study, yet reported similar findings in the obese subjects. One interesting finding by Adam et al. [42] was that following weight loss, the impairment in GLP-1 in response to exercise was diminished and GLP-1 was significantly higher following exercise compared to rest, suggesting that GLP-1 release may be dependent upon body weight. Overall, the paucity of data in this area of research warrants the need for future studies to thoroughly examine how GLP-1 is affected by a bout of exercise and the subsequent implications on food intake post-exercise in overweight/obese subjects.

The influence of body weight on GLP-1 concentrations

In the current study, GLP-1 was not correlated with either BMI or body weight at any time point. This was unexpected since previous studies have demonstrated that excess body weight is associated with lower GLP-1 levels [83, 112], which is suggestive of lower feelings of satiety in overweight subjects in response to feeding. Although subjects in the current study were not compared to normal weight controls, our data suggest that there may be a BMI threshold at which an impairment in GLP-1 secretion occurs and it is possible that all subjects in this study were above that threshold. This could explain why a relationship between body weight and GLP-1 was not seen. However, in the current study GLP-1 was correlated with percent body fat; thus future studies should seek to explore the influence of body fat on GLP-1 secretion and whether this relationship exists independent of body weight.

5.2.4 The Effect of Exercise on Subjective Feelings of Hunger

In the current study there was a trend for subjective feelings of hunger to increase more in the exercise condition compared to the resting condition from pre-testing to immediately post-testing. However, this effect was short-lived and there was no difference between conditions at 30-minutes or 60-minutes post-testing. Although hunger ratings were not different between resting and exercise conditions, hunger did increase over time (up until feeding at 60-minutes post-testing) and subjective feelings of hunger at the 3 time points measured post-testing were related to the amount of food consumed during the ad-libitum feeding period.

The result in the current study that hunger was unaltered by a bout of exercise is contrary to previous research in an overweight population. An earlier study reported hunger levels to be suppressed following 2 hours of moderate intensity cycling compared to a similar length resting session in overweight males [38]. This suppression in hunger translated into a reduction in food intake post-exercise. This is suggestive of exercise-induced anorexia, which has previously been reported following high-intensity exercise [32]. However, it should be noted that feeding began 10 minutes post-exercise and thus it is not known how long this suppression in hunger would have remained if the feeding period were delayed. In contrast, Kissliff et al. [31] found hunger to be transiently increased following a 40-minute bout of moderate-intensity exercise but not vigorous intensity exercise, compared a non-exercise condition in obese women. Interestingly, higher hunger levels following exercise did not translate into an increase in food consumption 15 minutes post-exercise, suggesting that there may have been other outside factors (ie – physiological, psychological, or environmental parameters) involved in the regulation of energy intake.

The apparent differences between the above mentioned studies, and the length of follow-up post-exercise make it difficult to draw conclusions about the acute effect of exercise on subjective feelings of hunger. From the limited data available in an overweight/obese population, it appears data are inconclusive on whether exercise alters feelings of hunger. Although there are other factors that regulate food intake besides hunger, the fact that exercise does not appear to increase hunger has promising implications for the role of exercise in weight control. Future studies should be conducted in the overweight/obese population to confirm the findings from the current study.

Relationship between subjective feelings of hunger and appetite-regulating hormone concentrations

Interestingly, hunger was not correlated with either GLP-1 or acylated ghrelin at respective time points for either the exercise or resting condition. Considering that both of these hormones are thought to be involved in the regulation of appetite, it is surprising that subjective feelings of hunger were not associated with hormone concentrations. Previously, Martins et al. [30] reported an inverse temporal pattern between plasma levels of certain gut peptides (PYY, GLP-1, and PP) and hunger ratings during the 1-hour exercise/rest period, speculating that as levels of these hormones rose, satiety also rose, thereby driving down hunger ratings. However, in that study, correlations between hunger and appetite-regulating hormones were not reported, making it difficult to discern whether or not there was a relationship present between these two variables.

Future studies that examine the hormonal response to a bout of exercise should consider correlational analyses to better understand the relationship between changes in appetite-regulating hormone concentrations during exercise and subjective feelings of hunger. This relationship has not been clearly established when physiological changes in hormone concentrations are observed. Moreover, the majority of the studies that have reported an association between GLP-1 or acylated ghrelin and hunger have used intravenous infusion to determine the influence of the hormones on feelings of hunger

[39, 40, 78, 79]. However, the magnitude of hormone infused is typically much higher than what is seen at physiological levels. As suggested by Martins [30], it is possible that the satiety effects of these hormones are only present at levels induced through infusion, not physiological levels resulting from exercise. This warrants further investigation.

5.2.5 Energy Expenditure of the Exercise Bout

The exercise protocol in the current study sought to create a similar relative energy deficit between individuals with different body weights. Thus, neither the duration of the exercise session, nor the total energy expenditure of the session were held constant among subjects. Instead, the duration of the exercise bout varied between individuals and was the length of time needed to elicit an energy expenditure of 3.0 kcal/kg/body weight, which was determined based upon the ACSM prediction equations for walking. During the testing session, the incline of the treadmill was adjusted whenever a subject's heart rate fell outside of the predetermined heart rate range (70-75% of age-predicted maximal HR). Similarly, the energy cost of the particular walking speed and grade was calculated throughout the testing session so that the duration of exercise bout could be adjusted accordingly. However, the energy expenditure measured through indirect calorimetry (4.05 ± 0.4 kcals/kg) was significantly greater than the predicted energy expenditure (3.0 kcal/kg). Thus, the energy cost of the exercise session was actually much larger than what the study protocol originally stated.

There are several possible explanations that could explain these differences between the actual and predicted energy expenditures. First, it is possible that the

ACSM prediction equations for walking may underestimate energy expenditure in an overweight/obese population, indicating that sedentary, overweight/obese individuals may be less efficient while walking on a treadmill compared to the population utilized in validating the prediction equations. Second, previous research has shown that over time, VO_2 has a tendency to drift upward with heavy exercise [113] and the prediction equations may not take this into account. Lastly, the error associated with measured oxygen consumption via indirect calorimetry cannot be discounted.

Although there were discrepancies between the measured oxygen consumption and the predicted VO_2, the finding that exercise has no influence on food intake remains. In actuality, the higher than expected energy cost of the exercise bout strengthens the current findings since the energy deficit created through exercise was even greater than anticipated by the protocol, and still there was no difference in post-testing food intake between conditions. These findings have promising implications for weight control, suggesting that large energy deficits can be created through exercise without a subsequent rise in energy intake to account for this deficit. Future studies should determine whether there is an upper cut-off point for energy expenditure, which would result in an increase in food consumption, overriding the attenuated need to restore energy homeostasis post-exercise.

5.2.6 Psychological Parameters

It is well accepted that there is both a physiological and psychological component to feeding. Although a physiological mechanism was not identified in the current study, it is possible that other biomarkers, or interactions between different hormones may be responsible for attenuating the need to restore energy balance following the energy deficit created by an exercise bout. However, it is also plausible that even if a physiological mechanism was identified, that a psychological component may override the physiological drive to eat. Thus, levels of dietary restraint and disinhibition are important to examine when discussing appetite regulation.

Relationship between exercise, dietary restraint, and body weight

In the current study, there was a positive relationship between dietary restraint and body weight, but this association was not observed with disinhibition. Class II obese subjects had significantly higher restraint scores than the overweight individuals. Dietary restraint was not associated with absolute food intake but there was a positive relationship between restraint scores and food intake post-exercise but not post-rest, when energy intake was expressed relative to body weight ($r=0.552$, $p=0.017$). These findings suggest that following exercise, those with higher restraint scores (also those with a higher BMI), may consciously increase food intake due to their awareness of the energy cost of the exercise bout. Future studies should continue to explore the relationships between dietary restraint, BMI, and food intake post-exercise. However, based upon the current findings, individuals seeking weight loss should be informed about the true energy cost of an exercise bout to ensure that they do not over compensate (through feeding) for the energy expended during exercise, thus hindering their weight loss efforts.

The effect of exercise on measures of mood

Exercise can elicit changes in mood, which may be dependent upon the type and/or intensity of the exercise employed and can vary between persons. Previous studies have found that acutely, exercise can have mood-enhancing effects [114] and also mood deteriorating effects [102] in sedentary individuals. However, the sedentary and overweight/obese population has not been thoroughly studied. Furthermore, the relationship between changes in mood from pre to post-exercise and food intake is not well established. The current study found a trend towards a significant interaction effect between mood state and food intake. Those subjects who reported a decrease in positive affect following exercise had a tendency to increase food consumption post-exercise compared to rest. Conversely, those subjects who reported an improvement in positive affect following exercise consumed less following the exercise bout. This would suggest that if exercise has the ability to alter positive affect this could provide another avenue through which exercise can assist in weight control efforts. Additionally, this interaction between mood and energy intake may account for some of the individual differences seen in food consumption post-exercise compared to rest.

Contrary to the current findings, a previous study that sought to examine the interaction between mood and post-exercise energy intake [115] found that changes in positive affect from pre to post-exercise did not influence feeding. However, these researchers did report a significant interaction effect between negative affect and testing condition, which was not seen in the current study. A possible explanation for these differences between this study and the current study could be due to the exercise bout itself. Schneider and colleagues [115] had subjects perform step-ups for 3-minutes while in the current study the exercise duration was much longer. Considering that approximately 58% of individuals reported an improvement in positive affect immediately following the exercise bout in the current study, these results are promising and thus warrant future research.

5.3 LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Only overweight/obese, sedentary and otherwise healthy females between the ages of 18 and 45 were eligible to participate in this study. Thus, caution should be taken before generalizing these findings to other groups of individuals. Furthermore, the current study was limited by several factors that may have contributed to the observed findings. The following is a list of possible limitations of the current study:

1) This study was underpowered to detect a significant difference in food intake or hormone concentrations between subjects in each of the three BMI categories. In the research design, an appropriate sample size was determined to be 21, one that would detect a significant difference between testing conditions on food intake, not differences between BMI groupings. Despite a small sample size, there was a non-significant trend towards a condition x BMI group interaction effect when examining the influence of exercise on relative energy intake (kcals/kg body weight). These findings suggest that individual body weight may be an influential factor in appetite regulation post-exercise and thus future studies

should be powered appropriately to detect possible differences that may be attributed to variations in BMI.

2) One of the goals of the present study was to blind subjects to the fact that food intake was being monitored while at the facility. However, 15 of the 19 subjects reported an awareness or sense that energy intake was being monitored during the testing session. The effect that this had on food consumption is not known; however it is possible that an awareness of the measurement of food intake could impact food consumption. Thus, future studies should utilize better methods to ensure that subjects are kept blinded to the measurement of energy intake.

3) The order of the testing sessions was randomly assigned. Thus, the treadmill walking time had to be estimated prior to the testing session and was done so using regression equations. As discussed in the results section, the exercise session was on average 7 minutes longer than the resting session, which was most often due to an upward drift in heart rate over time. Thus the discrepancy in testing times is a limitation to this study.

4) Blood was drawn via a needle stick at five time points over the course of the testing session. In some subjects, multiple attempts were necessary thus prolonging a particular blood draw, which could have impacted the results. Therefore, an angiocatheter may be a more viable method of drawing blood in

future studies. Additionally, the influence of a needle stick on measures of positive and negative affect should not be overlooked. Questionnaires were administered prior to drawing blood; however an individuals' anticipation of the blood draw may have confounded the mood-related findings. Again, the insertion of an angiocather would assist to minimize some of anticipatory influence of the blood draw on measures of affect.

5) This study was not powered to detect differences in hormone concentrations between resting and exercise conditions. Large variability was seen in hormone levels, specifically with acylated ghrelin. Future studies should take into consideration the large inter-individual variability that exists and adjust the sample size accordingly. Additionally, studies should seek to understand why such large variability is present and to determine if there are any factors that predict individual differences.

6) When exploring a potential physiological mechanism mediating the findings related to effect of exercise on food intake, the current study only measured acylated ghrelin and GLP-1 concentrations. However, there are many other hormones or physiological factors involved in the regulation of appetite that should also be considered in future studies. These factors include, but are not limited to, cortisol, neuropeptide-Y, cholecystokinin, peptide-YY, corticotropin-releasing hormone, leptin and insulin.

7) In the current study, acylated ghrelin and GLP-1 concentrations were not altered by a bout of exercise. It is possible that our findings differed from the existing literature due to the fact that our subjects were in a fed state while previous studies have tested subjects under fasting conditions. Therefore, future studies should compare responses to exercise under fasted and fed conditions on ghrelin and GLP-1.

5.4 CONCLUSIONS

The role of exercise in weight control is controversial due to the potential influence of exercise on appetite regulation. Studies conducted in this area of research have mainly utilized a lean and active population; therefore it is unknown how overweight individuals acutely respond to an exercise bout. Whether exercise may hinder one's weight loss efforts due to an increase in food intake following exercise is questionable. Findings from the current study indicate that overall, exercise does not acutely influence food intake in an overweight population, thus making it a valuable component for managing body weight. However, large inter-individual variability was seen in the current study with some individuals increasing food intake post-exercise while others decreased food intake in response to an exercise bout. Explanations for this variability need to be further investigated and caution should be taken when interpreting these results and generalizing these findings.

Acylated ghrelin and GLP-1 were not altered in response to a bout of exercise in the current study and did not explain the individual variation in post-exercise energy intake seen. Therefore, the effect of exercise on additional biomarkers should be considered in future studies. Although exploratory in nature, we found that improvements in positive affect following exercise resulted in a suppression in food intake compared to a resting condition, suggesting that a psychological component may be influencing feeding post-exercise. Additional studies should be conducted to confirm these findings.

APPENDIX A

INFORMED CONSENT DOCUMENT

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CONSENT TO ACT AS A SUBJECT IN A RESEARCH STUDY

TITLE: The Acute Effect of Exercise on Biomarkers

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SOURCE OF SUPPORT: School of Education, University of Pittsburgh

 Participant's Initials: _________

DESCRIPTION:

Excess body weight is associated with a number of adverse health consequences including an increased risk for developing heart disease, diabetes, and certain forms of cancer. Previous research indicates that physical activity can greatly improve the abovementioned conditions and also assist in the regulation of body weight. The purpose of this study is to examine the effect of a moderate intensity bout of exercise on various physiological (this is how your body reacts to exercise) and psychological (this is how exercise makes you think about things that affect your behavior) parameters in a group of overweight women to better understand the role of physical activity in weight control.

You are invited to take part in this research study because you are within the weight range for this study, and do not have any medical conditions that would prohibit you from participating in moderate intensity exercise. Moderate activity is defined as an activity similar to brisk walking where you can also have a conversation. People invited into this study are women between 18-45 years of age. This study is being performed on a total of 21 individuals and will be conducted at a University of Pittsburgh facility.

If you decide to participate in this research study, you will undergo the following procedures that are not part of your standard medical care:

Screening Procedures:

Procedures to determine if you are eligible to take part in a research study are called "screening procedures". For this research study, the screening procedures include:

You will complete a physical activity readiness questionnaire (PAR-Q), and this will take approximately 5 minutes to complete. You will also complete a detailed medical history, and this will take approximately 20 minutes to complete. These questionnaires will allow the investigators to determine if you have any significant medical condition that would indicate that exercise is unsafe for you. You will also be required to provide medical clearance from your personal physician before starting this study. Participants cannot be pregnant, and you will be required to accurately report whether you are pregnant to the investigators prior to beginning this study and during the study if your status should change. Additionally, you will need to provide clearance from your personal physician prior to participation in this program.

Experimental Procedures:

If you qualify to take part in this research study, you will undergo the following experimental procedures:

You will first be asked to undergo assessments of height, weight, blood pressure, body composition, resting energy expenditure, physical fitness, and level of physical activity. These assessments will take place at the Physical Activity and Weight Management Research Center in Birmingham Towers (South Side of Pittsburgh) at the University of Pittsburgh, and these assessments will be completed in approximately 120 minutes (2 hours). A brief description of these assessments follows.

A. Body Weight and Height (5 minutes): Your body weight will be measured using a standard medical scale. Your height will be measured with a ruler that is attached to a flat wall. These will be measured at your assessment visit and just prior to the two testing sessions.

B. Body Composition (20 minutes): Your body composition is the amount of fat weight and lean weight (muscle and bone) that you have on your body. Your body composition will be measured using a technique known as Bioelectrical Impedance Analysis (BIA). This procedure requires that a small electrode be placed on your hand, wrist, ankle, and foot. A low-level signal that is not harmful to you and that you will not feel is transmitted between the electrodes.

C. Blood Pressure (5 minutes): Your blood pressure will be measured using a standard blood pressure cuff and will follow standard measurement procedures.

D. Resting Energy Expenditure (50 minutes): Your resting energy expenditure, or the number of calories that you burn at rest, will be measured during your assessment visit. For this procedure, you will be asked to lie quietly in a room for approximately 30 minutes to ensure that your body is in a rested state. Then, a plastic canopy will be placed over your upper body, and the air that your breath in and out will be analyzed for a period of 20 minutes.

E. Cardiorespiratory Fitness (30 minutes): Measurement of your cardiorespiratory fitness will provide information about how fit your heart and lungs are to perform exercise. Your fitness will be estimated by having you walk on a treadmill. The speed of the treadmill will be kept at 3.0 mph (a brisk pace), however the grade of the treadmill will increase 2.5% every 3 minutes so that it feels like you are walking up a hill. As you are walking, your heart rate, blood pressure, and perception of physical exertion will be measured. Your heart rate will be measured using an electrocardiogram, which is also known as an ECG. The ECG will require that electrodes be placed on the chest and abdomen areas of your body. You will continue to walk on this treadmill until you reach a heart rate that is 85 percent of your maximal capacity, which is the highest heart rate you can achieve and is estimated by subtracting your age from 220 beats per minute, and then the test will be stopped. A staff member who is certified as an Exercise Specialist by the American College of Sports Medicine and at least one additional staff member will conduct this test. No other study participants will be in the testing room during this assessment. If during this test it is determined that you have a medical condition that makes it unsafe for you to exercise, you will no longer be permitted to participate in this study, and you will be referred to your primary care physician for medical follow-up.

F. <u>Physical Activity Levels (10 minutes):</u> You will also be asked to participate in a brief, structured interview to determine your daily physical activity patterns. A staff member at the facility will ask you a series of questions related to your physical activity levels over the past week.

If you are currently a subject in another ongoing research study at the Physical Activity and Weight Management Research Center and you have already completed assessments of height, weight, blood pressure, body composition, fitness, and physical activity levels within 4 weeks of signing this consent document, and if those tests utilized the same procedures as described above, you grant the investigators permission to use the results from the previously completed tests so that you do not need to perform these tests again for this current study.

<u>Rest and Exercise Testing Sessions:</u>
After completing your assessments, you will be asked to schedule two visits to the Physical Activity and Weight Management Research Center. One visit will require you to exercise and the other will not require you to exercise. These visits will be separated by at least 2 days and be within days 7 and 21 of your menstrual cycle. The order in which these visits take place will be randomly determined using a method similar to flipping a coin. A more detailed description of each of these visits is shown below.

A. <u>Exercise Condition:</u> Prior to this visit, you will be asked to consume a liquid meal replacement, which will be in the form of a commercially available shake, at home 90 minutes prior to arriving at the research facility. This shake will be provided to you on the day of your assessment visit. Upon arrival, all testing procedures will be reviewed and the following will occur, and these procedures will take approximately a total of 3 hours to complete.
   a. You will then be asked to complete questionnaires related to your exercise, eating behaviors, mood, and other factors related to how you feel on that day.
   b. Blood will be taken 5 times throughout the course of the testing session via a needle stick. A sample will be taken immediately prior to the start of exercise, immediately following the exercise session, and at 30, 60 and 120 minutes after you stop the exercise session. Each blood sample will be approximately 1 tablespoon of blood, with a total of approximately

5 tablespoons of blood being collected during this entire session. Your blood will then be analyzed to measure levels of ghrelin, cortisol, neuropetide-Y, insulin, leptin, glucagon-like peptide-1, cholecystokinin, and peptide-YY, which are biomarkers of stress, metabolism, and digestion that are thought to be involved in weight control. Blood samples will be obtained by a trained phlebotomist or medical technician.

c. During the exercise session, you will walk on a treadmill at a speed of 3.0 mph (which is similar to brisk walking) and at an incline that will put your heart rate between 70-75% of your age-predicted maximal heart rate, which is considered to be a moderate intensity. While you are walking, your heart rate will be monitored continuously using a strap that is attached to your chest just below your breast bone. You will also wear a facemask that allows for the collection of the air that you breathe in and out, and this will allow for the determination of how many calories you are burning during this exercise session. Your airflow will not be obstructed using this procedure. You will walk on the treadmill for approximately 30-40 minutes, with the actual time determined by how long it takes you to burn a predetermined number of calories. Every 15 minutes while you are walking, you will be asked to rate how hard you think the exercise feels and be asked questions related to how you feel at the moment.
d. Upon completion of the exercise, you will again be asked a series of questions related to mood, hunger, and energy levels. Then, you will be brought to a separate room in which you will be asked to rest quietly in a seated position for 60 minutes. During this time, a video will be provided for you to watch and blood samples will be collected following 30 and 60 minutes of rest. After 60 minutes, you will be asked to again complete a series of questionnaires related to your mood, hunger, and energy levels.
e. After the 60 minutes of rest in a seated position you will be allowed to eat, and a variety of snack foods to choose from will be provided to you by the investigators. You will remain at the research center for 60 additional minutes to allow the investigators to collect the final blood sample at 120 minutes (2 hours) after you completed the exercise session.
f. You will also complete questionnaires to assess mood, hunger, and energy levels following this 2-hour period after completing your exercise session. It is estimated that it will take you approximately 15 minutes to complete these questionnaires.

B. Resting Condition: Prior to this visit, you will be asked to consume a liquid meal replacement, which will be in the form of a commercially available shake, at home 90 minutes prior to arriving at the research facility. This shake will be provided to you on the day of your assessment visit. Upon arrival, all testing procedures will be reviewed and the following will occur, and these procedures will take approximately a total of 3 hours to complete.

a. You will then be asked to complete questionnaires related to your exercise, eating behaviors, mood, and other factors related to how you feel on that day.
b. Blood will be taken 5 times throughout the course of the testing session via a needle stick. A sample will be taken immediately prior to the start of resting, immediately following the resting session, and at 30, 60 and 120 minutes after you stop the resting session. Each blood sample will be approximately 1 tablespoon of blood, with a total of approximately 5 tablespoons of blood being collected during this entire session. Your blood will then be analyzed to measure levels of ghrelin, cortisol, neuropetide-Y, insulin, leptin, glucagon-like peptide-1, cholecystokinin, and peptide-YY, which are biomarkers of stress, metabolism, and digestion that are thought to be involved in weight control. Blood samples will be obtained by a trained phlebotomist or medical technician.
c. During this rest session, you will remain in a seated position for approximately 40 minutes. While you are seated, your heart rate will be monitored continuously using a strap that is attached to your chest just below your breast bone. You will also wear a facemask that allows for the collection of the air that you breathe in and out, and this will allow for the determination of how many calories you are burning during this session. Your airflow will not be obstructed using this procedure. Every 15 minutes while you are seated, you will be asked questions related to how you feel at the moment.
d. Upon completion of the 40 minute seated rest session, you will again be asked a series of questions related to hunger, mood, and energy levels. Then, you will be brought to a separate room in which you will be asked to rest quietly in a seated position for 60 minutes. During this time, a video will be provided for you to watch and blood samples will be collected following 30 and 60 minutes of rest. After 60 minutes, you will be asked again to complete a series of questionnaires related to your mood, hunger, and energy levels.
e. After the 60 minutes of rest in a seated position you will be allowed to eat, and a variety of snack foods to choose from will be provided to you by the investigators. You will remain at the research center for 60 additional minutes to allow the investigators to collect the final blood sample at 120 minutes (2 hours) after you completed the initial seated rest session.
f. You will also complete questionnaires to assess mood, hunger, and energy levels following this 2-hour period after completing your initial seated rest session. It is estimated that it will take you approximately 15 minutes to complete these questionnaires.

RISKS and BENEFITS:

The possible risks of this research study may be due to the exercise that you will be performing and the assessments that will be performed.

Risks

A. Risks of Exercise: There are moderate risks associated with participating in an exercise test. During exercise, you may experience a serious cardiac (affecting your heart) event, an arrhythmia (your heart beats at a pace that is not normal), or chest pain. An example of a cardiac event would be a heart attack or another medical condition that causes damage to your heart or cardiovascular system. The possibility of experiencing a serious cardiac event has been estimated to be approximately 6 per 10,000 in exercising adults. Therefore, the risk is of this happening to you is rare, because it occurs in less than 1% of people (less than 1 out of 100 people). In addition, during exercise, you may experience an increase in heart rate, an increase in blood pressure, shortness of breath, general fatigue, and in some cases muscle soreness. The risk of this happening to you is likely because these occur in more that 25% of people (more than 25 out of 100 people). In the event that you experience a serious medical condition during your exercise session, the session will be stopped and appropriate emergency medical care will be provided. This may include providing CPR until Paramedics or other appropriate medical personnel arrive.

B. Risk of having the air that you breathe in and out measured by a metabolic cart: When measuring the air that you breathe in and out during exercise, you may experience a dry mouth. The risk of this happening to you is likely because this occurs in more than 25% of people (more than 25 out of 100 people).

C. Risk of Electrocardiogram (ECG): You may experience skin irritation or skin redness from electrodes being placed on your skin. The risk of this happening to you is likely because this occurs in more than 25% of people (more than 25 out of 100 people).

D. Risk of Bioelectrical Impedance Analysis (BIA): You may experience skin irritation or skin redness from electrodes being placed on your skin. The risk of this happening to you is likely because this occurs in more than 25% of people (more than 25 out of 100 people).

E. Risk of Measuring Resting Energy Expenditure: The measurement of resting energy expenditure will be done using a ventilated hood. The only adverse factor associated with this is that you may experience a feeling of claustrophobia. The risk of this happening to you is infrequent because it occurs in less than 1-10% of people (1 to 10 out of 100 people). A person will be bedside at all times and check to see if you are comfortable. The transparent hood can be easily removed if necessary.

F. Risk Associated with Completion of Questionnaires: You may experience non-physical risks such as boredom, frustration, stress, and time constraints when completing the questionnaires. The risk of this happening to you is likely because this occurs in more than 25% of people (more than 25 out of 100 people).

G. Risk of Drawing Blood: The risks of drawing blood from a vein include discomfort at the site of puncture and possible bruising and swelling around the puncture site. The risks of this happening to you are likely because they occur in more than 25% of people (more than 25 out of 100 people). Although rare, it is possible that you may develop an infection or experience faintness from the procedure. The risk of an infection or fainting occurring is rare because they occur in less than 1% of people (less than 1 out of 100 people).

H. Risk of Consuming a Liquid Meal Replacement: As described above, you will be provided with meal replacements to consume on the morning of testing. There is a possibility that you may not like the taste of the meal replacement. Also, consuming it may result in bloating, gas and indigestion. This is rare and occurs in less than 1% of people (less than 1 out of 100 people).

Benefits:

There are no direct benefits that you will receive from participating in this study.

If we should find out about a medical condition you were unaware of, with your written permission, this information will be shared with the doctor of your choice.

NEW INFORMATION:

You will be promptly notified if any new information develops during the conduct of this research study, which may cause you to change your mind about continuing to participate.

COSTS and PAYMENTS:

Neither you, nor your insurance provider, will be charged for the costs of any of the procedures performed for the purpose of this research study. These costs will be paid by the sponsor of this research study.

You will be paid $300 upon completion of all testing procedures which include the initial assessment visit, the exercise testing session, and the resting testing session described above. Thus, a total of $300 can be earned for your participation in this study.

COMPENSATION FOR INJURY:

University of Pittsburgh researchers and their associates who provide services at the University of Pittsburgh Medical Center (UPMC) recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator listed on the first page of this form.

Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. It is possible that UPMC may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care unless otherwise specifically stated below. There is no plan for monetary compensation. You do not, however, waive any legal rights by signing this form.

CONFIDENTIALITY:

Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. In addition, all research databases will have password controlled access, and this will be controlled by the researchers. Only the researchers listed on the first page of this form and their staff will have access to your research records. You will not be identified by name in any publication of research results unless you sign a separate form giving your permission (release).

This research study will involve the recording of current and/or future identifiable medical information from your hospital and/or other (e.g. physician office) records. The information that will be recorded will be limited to information concerning medical clearance from your physician to participate in this research study. This may include information related to coronary heart disease risk factors such as blood pressure, blood cholesterol, or other medical conditions that may increase the risk of heart disease and/or indicate that exercise participation may be unsafe for you. This information will be used to determine whether it is safe for you to participate in this research study.

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information related to your participation in this research study:

> Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical record information) for the purpose of monitoring the appropriate conduct of this research study.
>
> In unusual cases, your research records may be required to release identifiable information (which may include your identifiable medical record information) related to your participation in this research study in response to an order from a court of law. If the researchers learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.
>
> A cardiologist in the University of Pittsburgh School of Medicine and UPMC will review the exercise tests that are completed as part of your participation in this study, and he/she will have access to your identifiable medical information.

The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical record information) related to your participation in this research study for 6 years following the completion of this study, as per University policy, or when such is approved by the sponsor of this study, whichever should occur last.

RIGHT TO PARTICIPATE or WITHDRAW FROM PARTICIPATION:

Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed, in general, to participate in the research study.) Whether or not you provide your consent for participation in this research study will have no affect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no affect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study.) Any identifiable research information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for participation in this research study will have no affect on your current or future relationship with the University of Pittsburgh. Your decision to withdraw your consent for participation in this research study will have no affect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

It is possible that you may be removed from the research study by the researchers if, for example, your health status changes and it does not appear that it is safe for you to continue to reduce your food intake, exercise, or lose weight. You will also be removed if you should become pregnant during this study.
****

****

VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research study during the course of this study, and that such future questions, concerns or complaints will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed a listed investigator. I understand that I may contact the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable.

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

______________________________ ________________

Participant's Signature Date

CERTIFICATION OF INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual, and I have discussed the potential benefits and possible risks of study participation. Any question the individual has about this study have been answered, and we will always be available to address future questions, concerns or complaints as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

______________________________ ________________

Printed Name of Person Obtaining Consent Role in Research Study

______________________________ ________________

Signature of Person Obtaining Consent Date

APPENDIX B

PHYSICIAN CONSENT DOCUMENT

PHYSICIAN CONSENT TO PARTICIPATE IN AN EXERCISE STUDY AT THE UNIVERSITY OF PITTSBURGH

TO:	RETURN TO: (envelope provided)
______________________ **Physician's Name**	University of Pittsburgh Department of Health and Physical Activity
______________________ **Address**	Physical Activity and Weight Management Research Center Attention: Jess 2100 Wharton Street, Suite 600 Pittsburgh, PA 15203
______________________ **City** **State** **Zip**	Telephone: (412) 488-4184 FAX: (412) 488-4174
( ) ______________________ **Telephone Number**	

Your patient ______________________________ has asked to participate in an exercise study at the University of Pittsburgh. The purpose of this study is to examine the effect of exercise on various physiological and psychological parameters in sedentary, overweight women. This study will involve the following:

1. Assessments of body weight, body composition, physical fitness, and resting energy expenditure.
2. A graded exercise test which involves walking on a motorized treadmill, with the workload gradually increasing every 3 minutes. The test will be terminated when the patient achieves 85% of their age-predicted maximal heart rate, or prior to this level if the individual experiences signs or symptoms that would indicate that exercise is contraindicated. Both blood pressure and heart rate will be monitored continuously. The ACSM Guidelines for Exercise Testing will be followed.
3. Two testing sessions. One in which the subject will exercise between 30-45 minutes at 70-75% of her age-predicted maximal heart rate and the other in which she will rest for a similar duration of time. Blood will be drawn 5 times throughout each testing session and the amount of blood drawn for an entire testing session is equivalent to 2 tablespoons.
4. A list of additional factors that are exclusionary criteria for this study that you should consider are listed on the attached sheet.

****

Please indicate below if this study seems appropriate for your patient or if you see any contraindications for her participation (*please check the appropriate box below).*

☐ I know of no contraindications to this patient participating in any of the above components of the program.

☐ I feel that this program would not be appropriate for this patient for the following reason(s):

____

____ ____________________

Signature of Physician Date

Please consider the following Inclusion and Exclusion Criteria as you evaluate whether your patient is capable of safely participating in the exercise research study at the University of Pittsburgh.

Inclusion Criteria:	Exclusion Criteria:
• Female • 18-45 years of age • BMI = 25-39.9 kg/m^2 • Ability to provide informed consent. • Ability to provide consent from their personal physician to participate in this study.	• Reporting regular exercise participation of at least 20 minutes per day on at least 3 days per week during the previous six months. (*This study is designed to recruit relatively sedentary adults.)* • Weight loss of >5% of body weight within the previous 6 months. • Women who are currently pregnant, pregnant within the previous six months, or planning on becoming pregnant within the next month. (Pregnancy during initial screening will be based on self-report and will be included on the detailed medical history that is completed by subjects) • Diabetes, hypothyroidism, or other medical conditions which would affect energy metabolism. • History of myocardial infarction or valvular disease. • Non-medicated resting systolic blood pressure ≥140 mmHg or non-medicated resting diastolic blood pressure ≥90 mmHg, or taking medication that would affect blood pressure. • Taking medication that would affect resting heart rate or the heart rate response during exercise (e.g., beta blockade). • Arrhythmia on resting or exercise electrocardiogram that would indicate that vigorous exercise was contraindicated. • Taking medication that could affect metabolism and/or contribute to change in body weight. • Being treated by a therapist for psychological issues or problems, taking psychotropic medications, or receiving treatment with psychotropic medications with the previous 6 months. • History of orthopedic complications that would prevent optimal participation in exercise (e.g., heel spurs, severe arthritis). • Post-menopausal women or those reporting irregular menses

APPENDIX C

CONFIRMATION OF ASSESSMENT VISIT

Confirmation of Assessment Visit

Name: ____

When: Day: _______________ Date: _____________ Time: ____________

Where: Physical Activity and Weight Management Research Center
University of Pittsburgh
Birmingham Towers, Suite 600
2100 Wharton Street (South Side)
Pittsburgh, PA 15203

ON THE DAY OF YOUR ASSESSMENT VISIT THE FOLLOWING WILL OCCUR:

- Your height and weight will be assessed
- Your body composition will be measured
- Your resting metabolic rate will be measured
- Your fitness will be assessed while walking on the treadmill
- You will be asked to complete a few brief questionnaires

PLEASE FOLLOW THE INSTRUCTIONS BELOW PRIOR TO YOUR SESSION:

1. Do not eat or drink (with the exception of water) for a minimum of 12 hours prior to your assessment
2. Avoid consuming any over-the-counter medications on the day of testing
3. Abstain from all vigorous physical activity for at least 24 hours prior to testing
4. Transport yourself to our facility in a vehicle and refrain from excessive walking on the morning of testing.
5. You will be walking on the treadmill so be sure to wear lightweight, comfortable clothing and tennis shoes or sneakers.

It is very important that you adhere to these guidelines so we ask that you make a strong effort to do so. Should you have any questions about any testing guidelines or procedures, do no hesitate to call **Jess Unick at 412-488-4170.** If you are unable to make this visit, please call the main number at 412-488-4184 to reschedule. Thank you again for your participation in this study!

APPENDIX D

ASSESSMENT VISIT DATA COLLECTION FORM

IRB #PRO08110427: The Acute Effect of exercise on Biomarkers
PI: Jessica Unick, MS

Subject ID_________________________ Date consent signed__________________

Baseline Study Procedures	**Performed per Protocol and Documented?**	**Y/N**
Telephone screening	Date______________ By________________	
Orientation session Date___________		
Physician's clearance provided Visit to lab: Confirm fasted for 12 hrs, no vigorous physical activity day before, no OTC medications & arrived via vehicle Height, Weight, B/P	PCP clearance: __________ PAR-Q __________ Criteria approved: ___Yes ____No Arrived by__________________ Hgt__________ Wgt__________ BMI_______ B/P_______________ Age _________	
Bioelectric Impedance Analysis (BIA)	Resistance: __________ Reactance: ____________	
Resting energy expenditure (7:00-11:00 AM)	Rested for 30 mins prior ________ 20-minute steady state measurement under canopy: Start time_____________ End time_____________ REE: ___________	
Physical fitness assessment Target HR: (220-age) x 85%=____________	Walk on treadmill @ 3miles/hour; grade increased by 2.5% every 3 minutes EKG leads applied Total Time on TM: _____________ Final RPE: ______________	
Food Preference Survey		
Physical Activity Levels (structured interview)		
Prep for next visit: • Liquid meal • List of guidelines • Food diary for 2 days prior to next session	Reviewed with subject _____YES 15% of REE: ______________ Grams of SF given: ___________ Comments:	
Schedule next visit within 7-21 days of onset of menstrual cycle	LMP___________Testing window: ______________ Randomized to ____Exercise *or* ____Sedentary first	

Signature____**Date**_______________________

APPENDIX E

RESTING SESSION DATA COLLECTION FORM

IRB #PRO08110427: The Acute Effect of exercise on Biomarkers
PI: Jessica Unick, MS

Subject ID_______________________ Date consent signed________________

Resting Test Session **DATE:**________________	**Performed per Protocol and Documented?**	**Y/N**
Confirm: Abstained from any exercise for 2 days Detailed food record for 2 days before Consumed liquid meal that morning No other food or beverages that morning	Liquid meal consumed at___________(**must be 1.5 hrs before start of session)**	
Questionnaires: • Subjective Exercise Experience Scale (SEES) • Positive Affect Negative Affect Schedule (PANAS) • Profile of Mood States (POMS)	Questionnaires: _____________am	
HR and oxygen monitors set up & weight	Wgt____________	
Initial blood draw	Collected @____________ By________________________	
Resting quietly in seated, upright position for a predetermined time Time required______________ Blood samples collected at end of rest period	Start time___________ End time____________ Samples collected @________________ By____________________________	
Questionnaires: SEES, PANAS, POMS	SEES: __________am PANAS: ________am POMS: _________am	
Rest quietly for next 2 hours with standardized video provided for **first hour** Blood draw at 30 and 60 minutes	Resting started_________ End __________ Blood samples Questionnaires: 30 mins____________ ___________am 60 mins____________ ___________am	
Given meal then observed for 60 minutes SEES, PANAS, POMS questionnaires Collect blood sample at end of eating period	Ate meal at_________ Total EI: ___________ Questionnaires: _______________am Blood collected at__________________	

Status at end of session:

Comments:
Signature____**Date**_______________

APPENDIX F

EXERCISE SESSION DATA COLLECTION FORM

IRB #PRO08110427: The Acute Effect of exercise on Biomarkers
PI: Jessica Unick, MS

Subject ID________________________ Date consent signed_________________

Exercise Testing Session **Date**____________	**Performed Per Protocol and Documented?**	**Y/N**
Confirm: Abstained from any form of exercise for 2 days Detailed food record for 2 days before No other food or beverages that morning Consumed liquid meal that morning	Liquid meal consumed at___________(**must be 1.5 hrs before start of session)**	
Heart rate monitor set up Weight	Wgt______________	
Questionnaires: • Subjective Exercise Experience Scale (SEES) • Positive Affect Negative Affect Schedule (PANAS) • Profile of Mood States (POMS)	Questionnaires: _________am	
Initial blood draw	Collected @____________ By_________________________	
Exercise Testing (treadmill at 3 miles/hr): **Goal to reach 70-75% of age-predicted maximum HR** **Target HR=**______________	Time on TM: ____________ Total EE: ______________ Avg RER: ______________ Blood sample collected at__________ By______________________________	
Questionnaires: SEES, PANAS, POMS	SEES: __________am PANAS: ________am POMS: _________am	
Rest quietly for next 2 hours with standardized video provided for first hour Blood sample @ 30 and 60 mins post exercise SEES and PANAS **prior to** each sample	Resting started __________ End__________ Blood samples: Questionnaires: 30 mins_________ ____________am 60 mins_________ ____________am Collected by__________________________	
Given meal and told to eat for period of 60 min SEES, PANAS and POMS questionnaires Blood sample collected at end of eating session	Ate meal at________________ Total EI: ______________ Questionnaires: ____________am Sample collected at________________ By______________________________	

Status at end of session:
Comments:

Signature_______________________________________ **Date**_________________

APPENDIX G

POST-TESTING QUESTIONNAIRE

Post-Testing Questionnaire

<u>Likeability of test meal</u> *(circle appropriate answer)*

How did you like the cereal?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the Nutrigrain bar?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the Nature Valley trail mix bar?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the Nature Valley yogurt granola bar?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the bagels?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the cream cheese?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the butter?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the peanut butter?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the yogurt?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the donuts?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the fruit?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the milk?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the coffee creamer?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the coffee?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How did you like the tea?

1	2	3	4	5	☐ did not eat
Not at All		Somewhat liked it		Really liked it	

How many calories do you think you consumed while at our facility today?

____

How many calories do you think you burned while walking on the treadmill during your testing visit when blood was drawn before and after exercise?

____

In general, which of the following is true? (*circle your answer)*

1) I believe that exercise increases my hunger within a few hours post-exercise
2) I believe that exercise decreases my hunger within a few hours post-exercise
3) I believe that exercise has no impact on my hunger within a few hours post-exercise

Did you believe that your food intake was being monitored while you were at our facility?

Yes No Not sure

APPENDIX H

DEBRIEFING SCRIPT

As part of this study, we wanted to look at the effect of exercise on levels of various appetite-regulating hormones in your blood. Additionally, we wanted to examine the impact that exercise has on food intake. Thus, we wanted to see whether exercise increases or decreases your hunger levels and food intake compared to a resting condition. In order to get the most accurate results, we did not previously inform you that food intake was going to be monitored because we didn't want your knowledge of this to impact the types or quantity of foods that you consumed. However, now that you have completed all testing procedures, we wanted to inform you about some of the additional things that we are going to examine in this study.

The manner in which your food intake was measured was through weighing all of the foods prior to placing them in front of you and then again weighing these foods following your last blood draw. From that information we are then able to calculate the amount of food that you consumed or total calories as well as the macronutrient composition of your food choices. We believe that by examining why some people increase food intake following exercise and why others decrease food intake or do not alter food intake following exercise, will help us to better understand the role of exercise in weight control.

Thank you again for deciding to participate in this study and we hope to gain some valuable information from the data we collected from you as well as other individuals who participated in this study. If there are any questions that you have about the study, we would be more than willing to answer them. Thank you again for your participation!!

APPENDIX I

FOOD INTAKE DATA COLLECTION FORM

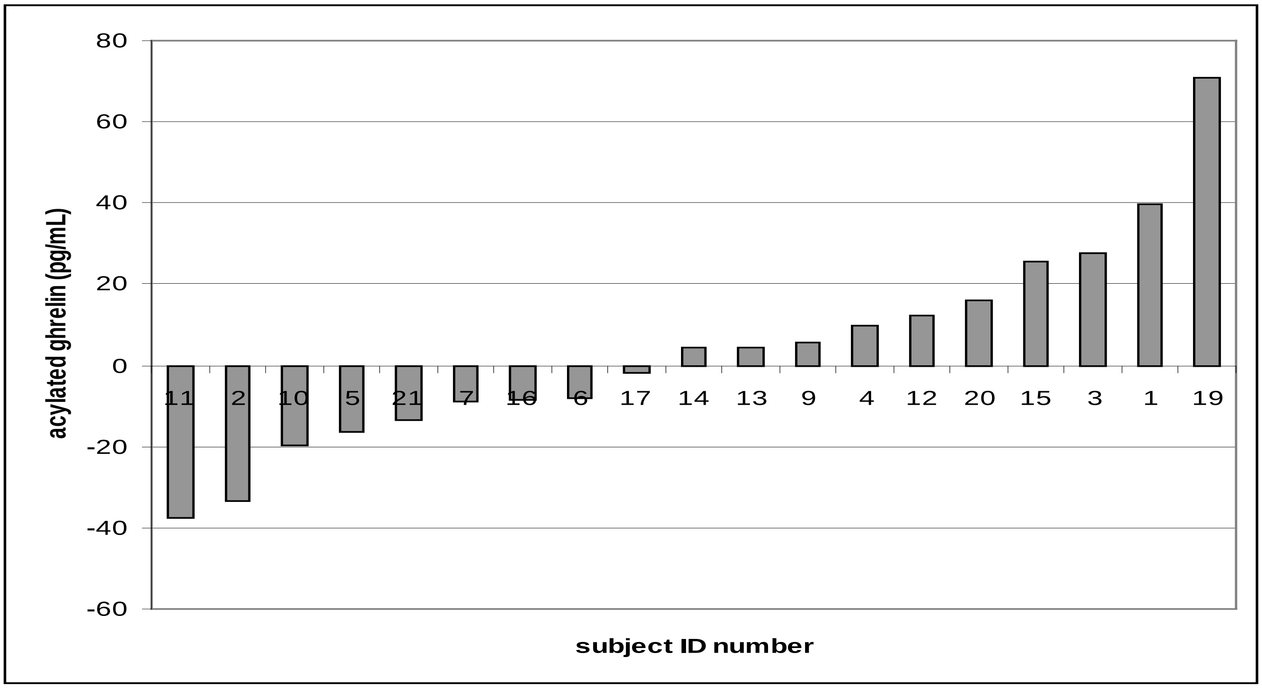
Subject ID: ___________________ Visit # ________ Time of feeding period: _________________

A	B	C	D	E	F
Food (kcals/g)	**Weight of food provided + container (g)**	**Weight of food & container (g) left over after feeding**	**Total grams consumed (Column B-C)**	**Kcals/g**	**Total calories consumed (kcals)**
Cherrios				3.571	
Frosted Flakes				3.667	
Granola				4.364	
Nutrigrain bar				3.514	
Trail mix bar				4.000	
Nature Valley yogurt bar				4.000	
Raisin bagel				2.626	
Plain bagel				2.626	
Mixed fruit				0.500	
Choco donut				4.400	
Powdered donut				3.934	

A	B	C	D	E	F
Food (kcals/g)	**Weight of food provided + container (g)**	**Weight of food & container (g) left over after feeding**	**Total grams consumed (Column B-C)**	**Kcals/g**	**Total calories consumed (kcals)**
Regular yogurt (Dannon vanilla)				0.881	
Strawberry yogurt (Dannon Light/Fit)				0.485	
Skim milk				0.375	
2% milk				0.542	
Butter (Land O lakes)				7.143	
Light cream cheese				2.258	
Reg. cream cheese				2.813	
Peanut butter				5.938	
Coffee creamer ½ & ½				1.333	
Sugar 1 packet = 15 kcal				3.750	
Coffee					
Tea					
Water					
TOTAL					

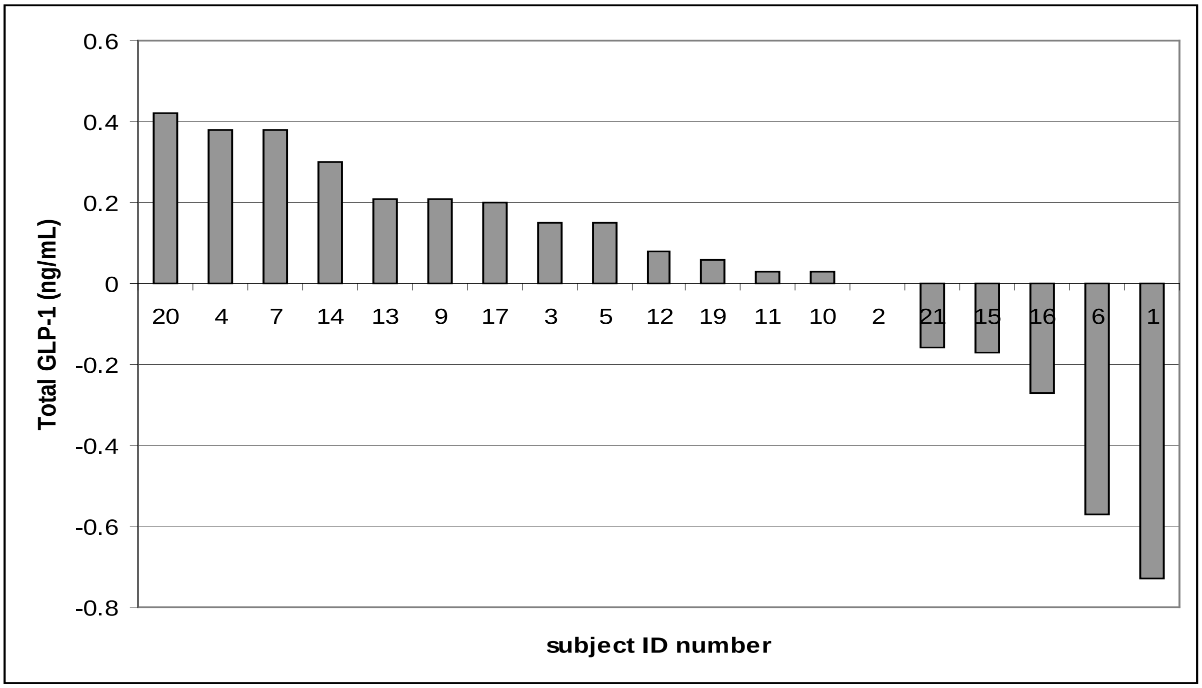
APPENDIX J

INDIVIDUAL HORMONE DATA



Changes in acylated ghrelin from pre-exercise to post-exercise for individual subjects *

*negative number indicative of a decrease in acylated ghrelin post-exercise compared to pre-exercise



Changes in GLP-1 from pre-exercise to post-exercise for individual subjects *

*negative number indicative of a decrease in GLP-1 post-exercise compared to pre-exercise

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