Spring 5-13-2016

Effect of Acute High Intensity Interval Exercise and Energy Balance on Plasma Acylated Ghrelin Concentrations

Calvin L. Cole

ACCEPTANCE

This dissertation, EFFECT OF ACUTE HIGH INTENSITY INTERVAL EXERCISE AND ENERGY BALANCE ON PLASMA ACYLATED GHRELIN CONCENTRATIONS, by CALVIN L. COLE, was prepared under the direction of the candidate's Dissertation Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree, Doctor of Philosophy, in the College of Education and Human Development, Georgia State University.

The Dissertation Advisory Committee and the student's Department Chairperson, as representatives of the faculty, certify that this dissertation has met all standards of excellence and scholarship as determined by the faculty.

_____
Walter R. Thompson, Ph.D.
Committee Chair

_____ _____
Leslie J. Brandon, Ph.D. Jeffrey Otis, Ph.D.
Committee Member Committee Member

_____ _____
Dan Benardot, Ph.D. Robert Hendrick, Ph.D.
Committee Member Committee Member

_____
Date

_____
Mark D. Geil, Ph.D.
Chairperson, Department of Kinesiology and Health

_____
Paul A. Alberto, Ph.D.
Dean, College of Education & Human Development

AUTHOR'S STATEMENT

By presenting this dissertation as a partial fulfillment of the requirements for the advanced degree from Georgia State University, I agree that the library of Georgia State University shall make it available for inspection and circulation in accordance with its regulations governing materials of this type. I agree that permission to quote, to copy from, or to publish this dissertation may be granted by the professor under whose direction it was written, by the College of Education and Human Development's Director of Graduate Studies, or by me. Such quoting, copying, or publishing must be solely for scholarly purposes and will not involve potential financial gain. It is understood that any copying from or publication of this dissertation which involves potential financial gain will not be allowed without my written permission.

Calvin L. Cole

_____

Name

NOTICE TO BORROWERS

All dissertations deposited in the Georgia State University library must be used in accordance with the stipulations prescribed by the author in the preceding statement. The author of this dissertation is:

Calvin Lloyd Cole
1505 Paxford Overlook,
Marietta, GA. 30066

The director of this dissertation is:

Walter R. Thompson Ph.D.
Department of Kinesiology and Health
College of Education and Human Development

Georgia State University
Atlanta, GA 30303

CURRICULUM VITAE

Calvin L. Cole

1505 Paxford Overlook, Marietta Ga. 30066
Phone: 404-931-9549
Email: ccole13@gsu.edu

EDUCATION

Georgia State University **May 2016**
Atlanta, Georgia
Ph.D. Kinesiology and Health

Dissertation Topic
Effect of Energy Balance and Acute High Intensity Exercise on Plasma Acylated Ghrelin Concentrations

Advisors: Dr. Walt R. Thompson & Dr. Leslie J. Brandon

Georgia State University **May 2011**
Atlanta, Georgia
Masters of Science, Exercise Science

University of Central Arkansas **May 2002**
Conway, Arkansas
Bachelor of Science, Business Administration

PROFESSIONAL CERTIFICATIONS & AFFILIATIONS

- Urban Fellow (2012-2014)
- Kappa Delta PI (International Honor Society in Education)
- International Sport Science Association (ISSA) Certified (February, 2004 to present)
- American Heart Association Instructor certified (September, 2013 to present)
- American Heart Association CPR Certified (May, 2002 to present)
- USA Weightlifting Certified Sports Coach (November, 2010 to present)

PROFESSIONAL EXPERIENCE

Georgia State University **August 2012-Present**
Instructor
Department of Kinesiology and Health
Anatomy/Principles of Physical Activity and Fitness/ Graduate Exercise Physiology Lab

Georgia Perimeter College **January 2012-Present**
Adjunct Professor *Dunwoody, GA*
Instruct Personal and Community Health, First Aid Safety and CPR, and Weight Training. Developed comprehensive syllabi with measurable outcomes. Designed assignments that enable students to master concepts. Created and administered assessments in a clear and effective manner. Graded assessments and provided necessary feedback to students.

Concourse Athletic Club **August 2011- Present**
Personal Trainer *Atlanta, GA*
Design and facilitate fitness regimens for clients that maximize their fitness potential. Discuss and coordinate health and fitness goals for clients and re-evaluate those health and fitness goals periodically at scheduled times to gauge the attainment of the previously established goals. As part of the responsibilities at Concourse, researched already developed techniques that assist in the rehabilitation and prevention of injury.

Georgia Tech Athletics **August 2010 – May 2011**
Intern *Atlanta, GA*
Assisted the Director and Assistant Director of Strength & Conditioning with eight collegiate sports. Completed daily clean up and maintenance of the facility and all its equipment; setup and break down weight room and/or field for workouts/conditioning. Assisted with program design, organization, implementation, and supervision of training sessions.

Corporate Sports Unlimited, Inc. **March 2008 – March 2012**
Personal Trainer *Atlanta, GA*
Designed and facilitated fitness regimens for clients that maximized their fitness potential. Discussed and coordinated health and fitness goals for clients and re-evaluated those health and fitness goals periodically at scheduled times to gauge the attainment of the goals set. Also, reviewed client's nutritional habits and offered advice upon request.

Body of Change **February 2004 – August 2009**
Personal Trainer *Atlanta, GA*
Designed and facilitated fitness regimens for clients that maximized their fitness potential. Discussed and coordinated health and fitness goals for clients and re-evaluated those health and fitness goals periodically at scheduled times to gauge the attainment of the goals set. Also, reviewed client's nutritional habits and offered advice upon request.

PROFESSIONAL PUBLICATIONS

Brandon LJ., Proctor L., Cole CL., Comparison of Obesity Assessments and Cardiometabolic risks in African and European American Women, *Ethnicity & Diseases*. 2014, 24, 475-480.

Cole CL, Brandon LJ, Benardot D, & Thompson W. Relationships among energy balance, time of day, and obesity prevalence. Medicine & Science in Sports & Exercise 2015; 47(5S): 637-641. (Refereed Journal Research Abstract)

PROFESSIONAL PAPERS IN PREPRATION

Cole CL., Benardot D., Thompson WT., Otis J., Brandon LJ., The Impact Of Acute High Intensity Interval Exercise And Energy Balance On Plasma Acylated Ghrelin Levels In Obese And Non-Obese Men (Dissertation)

Cole CL., Benardot D., Morris DJ., Thompson WT., Brandon LJ., Hourly Energy Balance is a Factor in Body Composition and Obesity

Spicer B., Cole CL., Brandon LJ., Impact of body composition on Division I Women Basketball Players Prior, during and After the season

Effect of Acute High Intensity Interval Exercise and Energy Balance on Plasma Acylated Ghrelin Concentrations

By

Calvin L. Cole

Under the Direction of Dr. Walter R. Thompson

ABSTRACT

Ghrelin is an appetite-stimulating hormone produced mainly in the stomach and duodenum. Poor ghrelin control is often caused by obesity-related hyperinsulinemia, which fails to suppress ghrelin and results in excess appetite and higher body fat storage that perpetuates even greater fat accumulation. High intensity exercise has been shown to acutely decrease plasma acylated ghrelin concentrations in healthy weight individuals. However, the evidence for how exercise affects ghrelin in obese individuals is currently lacking. **PURPOSE:** To compare the effects of high intensity interval exercise on acute plasma acylated ghrelin levels in obese and non-obese males. **METHODS:** Eighteen subjects with a mean age of 29.8 yr. (± 7.6) were assessed for body fat percent (BF%), acylated ghrelin and hunger. Subjects included 9 non- obese men (BF% mean= 13.7 ± 3.6) and 9 obese men (BF% mean = 31.7 ± 4.7) who agreed to participate in this study. Using a crossover design, participants were randomly assigned to an exercise or control condition, with each subject acting as their own control. The exercise trial consisted of participants cycling at high intensity intervals for 20 minutes (not including the 5 minute warmup and cool down) at a rate of 65% to 85% of their heart rate reserve on a cycle ergometer followed by 60 minutes of rest. The control trial consisted of 90 minutes of rest. Blood samples (3-4ml) were collected at baseline, 0.5, 1, and 1.5 hours post-intervention. Acylated ghrelin concentrations were determined from plasma. Hunger was assessed using a 10-point Likert-type scale while blood samples were being drawn. Group means for plasma ghrelin concentrations between groups were analyzed using an independent t-test. The effect of exercise on ghrelin was analyzed using paired t-test. The relationship between perceived hunger and ghrelin was assessed using Pearson correlations. **RESULTS:** Baseline plasma ghrelin levels were significantly higher in the non-obese group when compared to the obese group ($t = 3.43$, $p =$

.036). While exercise was effective in reducing plasma acylated ghrelin levels in the non-obese group ($t = 2.34$, $p = .047$), no significant changes were found in acylated ghrelin in the obese group between baseline or any time point following the exercise intervention. **CONCLUSIONS:** The low resting levels of plasma ghrelin concentrations exhibited by the obese subjects, when compared to non-obese subjects, may result in long fasting periods that lead to hypoglycemia and a hyperinsulinemic response at the next eating opportunity. Furthermore, the lack of reduction in ghrelin following exercise may result in an overconsumption of energy. Both the sustained ghrelin with associated excess energy intake and the hyperinsulinemia may result in sustained or increased fat storage in obese individuals.

INDEX WORDS: Ghrelin, Obesity, Exercise

Effect of Acute High Intensity Interval Exercise and Energy Balance on Plasma Acylated Ghrelin Concentrations

By

Calvin L. Cole

A Dissertation

Presented in Partial Fulfillment of Requirements for the

Degree of

Doctor of Philosophy

in

Exercise Physiology

in

The Department of Kinesiology and Health

in

The College of Education and Human Development
Georgia State University

Atlanta, GA
2016

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Effect of Acute High Intensity Interval Exercise and Energy Balance on Plasma Acylated Ghrelin Concentrations

Chapter 1

Introduction

Worldwide obesity rates have nearly doubled since 1980 (43). In 2008, more than 1.4 billion adults, 20 years and older, were considered overweight. Of these, over 200 million men and nearly 300 million women were obese (43). This creates a public health crisis as overweight and obesity are leading risks for global premature deaths and disease. In addition to being a severe health risk itself, being overweight or obese also increases the risk for cardiovascular disease (coronary artery disease and stroke), diabetes, osteoarthritis, and cancers such as; endometrial, breast and colon. Although the causes of overweight and obesity are multifaceted, one of the primary factors is directly related to energy imbalance between calories consumed and expended (43). This discrepancy in energy balance is associated with an increase in energy intake and a concomitant decrease in physical activity (43). A report from the World Health Organization points to a decrease in energy intake and an increase in physical activity as the two primary alternatives that individuals can use to reduce bodyweight and begin to resolve the epidemic of overweight and obesity (43).

Research completed over the last 15 years has discovered a number of potentially important causes of the complex condition that is obesity. Ghrelin is one of several peptide hormones secreted by the gastrointestinal tract and has received significant attention from the research community as a potential target to regulate body weight (9, 19, 21, 41, 48). After its discovery as an endogenous ligand to the hypothalamic growth hormone (GH), researchers soon found that exogenous administration of ghrelin in rodents increased food intake that caused fat

and weight gain. This effect of ghrelin has highlighted its role in weight regulation (19, 41, 47, 53).

Ghrelin is synthesized as a preprohormone, which is proteolytically processed to a 28 amino acid peptide (3, 28). Ghrelin circulates in both venous and arterial blood in two different forms: acylated (or n-octanoylated, AG) and unacylated (or des-octanoylated or des-acylated, UAG or dAG) (31, 47). AG uniquely features a posttranslational esterification of a fatty acid on the serine residue at position 3. Ghrelin acylation is considered necessary for its actions as an endogenous ligand to the growth-hormone secretagogue receptor (GHSR) in which it stimulates the release of growth hormone (GH). Under homeostatic conditions, AG accounts for approximately 10% of total circulating ghrelin. Although UAG does not affect GH release, it is not biologically inactive. For example, UAG binds to other unknown receptors and have effects that are inconclusive in the research (27, 47).

Ghrelin is secreted mostly by stomach fundus, but also by the placenta, and in small amounts by the kidney, the pituitary and the hypothalamus (53). Ghrelin's ability to regulate appetite is independent of its role in GH stimulation (19). Research suggests that the hypothalamic hormones neuropeptide Y (NPY) and agouti growth-related peptide (AGRP) are intermediaries to the orexigenic effects of ghrelin. In these animal studies, the administration of ghrelin caused an increase in hypothalamic NPY and AGRP mRNA levels and the orexigenic effect was maintained even in mice that lacked NPY (19).

In addition, ghrelin has been shown to activate growth hormone secretagogue receptors (GHSRs) located on the pituitary and GH releasing hormone (GHRH) containing neurons in the hypothalamic arcuate nucleus, which stimulate GH release. In parallel, the activation of GHSRs by ghrelin on NPY/AGRP producing neurons located in the arcuate nucleus stimulates food

intake. Ghrelin increases fat tissue by decreasing fat oxidation and stimulates gastric motility and emptying (41). Studies on rodents and humans have established ghrelin as a food intake initiator and as a regulator of energy homeostasis (18, 52). Also, it is the first discovered food intake stimulating signal secreted by the stomach (41).

In adults, plasma ghrelin concentrations have been shown to increase prior to a meal and during fasting and to decrease within one hour after a meal no matter the macronutrient content. Ghrelin levels are increased under conditions of prolonged negative energy balance (i.e., low calorie diets, chronic exercise and/or anorexia nervosa) (41). In contrast, under obese conditions (chronic positive energy balance), pre-prandial ghrelin concentrations are typically low when compared to non-obese subjects. This trend in low pre-prandial ghrelin concentrations may be related to an over consumption of energy by this population, that results in excessive adipose deposits. However, a reduction in body weight in obese patients has been shown to result in an increase in ghrelin concentration (60).

During the past decade, the effect of exercise on ghrelin and energy intake has been studied extensively. Research to date has been inconclusive; however there is evidence suggesting that in non-obese populations, concentrations of plasma acylated ghrelin are suppressed after strenuous endurance exercise (55, 57), while concentrations of anorexigenic hormones (i.e., GLP-1, PYY, and PP) are increased (48) (see figure 1). These alterations in plasma ghrelin concentrations are shown to have a linear relationship with appetite at rest (as assessed using subjective visual analogue scales for feelings such as hunger, fullness, satisfaction and prospective food consumption) and may provide a potential mechanism for alterations of appetite or food and beverage intake post-exercise (5, 8, 22).

Unfortunately, published studies on this topic have differed in mode, intensity and/or duration of exercise making it difficult to define an advantageous form of exercise that will decrease plasma acylated ghrelin levels. In the majority of studies that have used non-obese subjects of both genders, with similar mode, duration and intensity the results indicate a reduction in plasma acylated ghrelin levels following exercise (48). In contrast, the results of the few studies that have attempted to compare the effects of acute exercise on plasma ghrelin levels in obese subjects remain inconclusive (26, 37). Research that compares these two populations have yet to come to a consensus on the mode, duration and intensity that is most viable for overweight and obese populations. Because individuals who are obese may not be accustomed to exercise, research done on this population should include exercises that match intensity, mode and duration of exercise with the physical capabilities of members of this group. Furthermore, exercise prescription for this population should be set to a level that is safe and attainable for their fitness level. Therefore, it is important to investigate the relationship between an acute bout of short-term high intensity interval training on plasma ghrelin concentrations in a population of healthy weight and obese males. Based on the efficacy of strenuous exercise to decrease plasma acylated ghrelin levels and increase anorexigenic hormones, we hypothesize that the intermittent high intensity bouts of exercise, performed in set time intervals will be effective in reducing acylated ghrelin levels in obese subjects to the same degree as healthy weight subjects.

Ghrelin and Energy Balance

The balancing of energy intake and expenditure involves multiple, complex systems. For example, when considering energy intake; the meal size, meal frequency, diet quality, and net absorption should be quantified. In addition, energy expenditure involves the consideration of several variables, including diet quality, metabolism, physical activity, meal frequency, and the

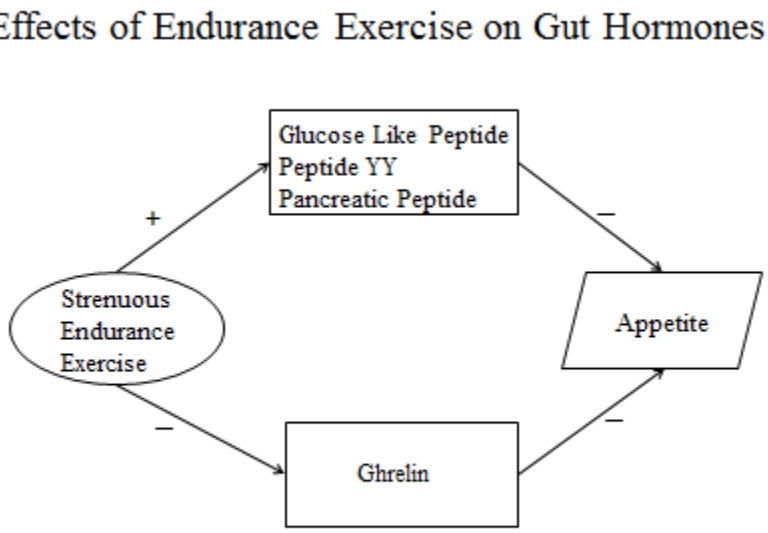


Figure 1. Endurance exercise leads to an increase in anorexigenic hormones and a decrease in orexigenic hormones and both hormones lead to a reduction in appetite

thermic effect of food (4). Once all of these factors are considered, the impact of ghrelin and other peptide hormones must also be taken into account.

Short term studies have highlighted ghrelin's role in energy balance. In rodents, exogenous ghrelin has been shown to signal meal initiation and cause a significant and dose related increase in cumulative food intake in rats (49). In humans, ghrelin levels are increased in response to a short-term fast and then decrease post-prandially (21). Ghrelin's role as a long-term regulator of energy balance and weight regulation is highlighted by the fact that plasma ghrelin levels fluctuate up and down in response to energy expenditure and intake, respectively. However, studies have shown that this action of ghrelin may be unique to lean subjects; it seems that obese subjects do not experience this same reduction in plasma ghrelin in response to feeding (44, 50), which may result in an over consumption of energy. This dysregulation of ghrelin production may, in part, contribute to weight gain in the obese population (19). The observation that ghrelin is further decreased in cases of abnormally high energy intake is evidence to the relationship between ghrelin activity and energy balance in obese subjects (38).

Ghrelin and Growth Hormone

Growth Hormone is a peptide hormone secreted from the anterior pituitary gland that stimulates overall body and cell reproduction and regeneration. Research has shown that GH release is regulated by two mechanisms: it is stimulated by hypothalamic GHRH and it is inhibited by somatostatin (32). However, recent research has revealed that ghrelin also activates the release of GH by binding to GHSRs (32).

Specifically, ghrelin stimulates GH release from primary pituitary cells in a dose-dependent manner by traveling through the bloodstream from the stomach to the pituitary (32).

Furthermore, intravenous injection of ghrelin induces potent GH release 5-15 minutes after injection before returning to basal levels approximately one hour later (13).

Although ghrelin has been found to stimulate both GH secretion and appetite, ones action is not dependent on the other. For example, the exogenous administration of ghrelin caused an increase in food intake in growth hormone-deficient spontaneous dwarf rats, suggesting that the feeding behavior function of ghrelin is independent of its GH stimulating function (49). These findings suggest that ghrelin acts on a site other than the GHSR to exert its affect as an appetite enhancer. This distinction is important if the blockade of ghrelin is to be used therapeutically to reduce body fat and weight. Because ghrelin stimulates GH the use of an antagonist to block its effects might have an adverse impact on growth and therefore, should not be used. However, because the effect of ghrelin on feeding have been found to be unrelated to its impact on GH secretion, research that focuses on locating the receptor that it interacts with to initiate appetite will go a long way in determining ghrelin's efficacy as a weight loss therapy.

Effect of Ghrelin on Glucose and Insulin

Ghrelin is also expressed in the β-cells of the pancreas (10, 14, 23, 56, 58) suggesting a possible relationship with insulin. Insulin is released from β-cells in the pancreas in response to high plasma glucose concentrations. It regulates the metabolism of carbohydrates and fats by promoting the absorption of glucose from the blood to skeletal muscles and fat tissue and by causing fat to be stored rather than used for energy. The first indications that there was an interaction between ghrelin and glucose metabolism resulted from the discovery that subcutaneous ghrelin injections induced an increase of the respiratory quotient (RQ). To researchers, the augmented utilization of carbohydrate and reduced utilization of fat to meet energy requirements was consistent with the observed increase in body fat (52). Numerous other

studies since 2000 have suggested that ghrelin has an important role in modulating β-cell function and glucose homeostasis (15, 34, 39, 40).

Many studies have investigated the short-term and long-term effects of ghrelin; however, effects on glucose and insulin homeostasis are still being debated. When administered acutely ghrelin induced hyperglycemia and reduced insulin secretion in healthy weight, healthy humans (6). In contrast, when the experiments were carried out in obese patients, there were no differences in glucose or insulin levels following ghrelin administration (1). Other studies have concluded that prolonged ghrelin infusion lowered insulin sensitivity (45). Together these results suggest that the short-term effects of ghrelin induce hyperglycemia, hypoinsulinemia, and insulin resistance in healthy, healthy weight humans. Yet, Heppner et al. (27) found that central infusion of AG and dAG increased insulin in the presence of glucose. In this study the effect of either type of ghrelin isoform caused glucose stimulated hyperinsulinemia when administered intracerebroventricularly. However, AG and dAG are blunted and nullified, respectively, when delivered subcutaneously (27). This demonstrates that the GHSR in the central nervous system play a critical role in insulin handling.

Blockade of ghrelin significantly lowers fasting glucose concentrations and enhances the insulin response (17). In obese, insulin resistant rodents, Esler et al. (20) found that short-term blockade of endogenous ghrelin had no noticeable effect on insulin sensitivity but did improve glucose tolerance by stimulating insulin secretion. However, ghrelin was blocked for several days in the same population of mice and plasma glucose levels diminished. This reduction in glucose levels was accompanied by a moderate decrease in serum insulin levels, suggesting that the insulin resistance was improved in the long-term (2). The data obtained in this research

suggests that acute and chronic reductions in ghrelin may improve glucose tolerance and improve insulin resistance in obese, insulin resistant populations.

Ghrelin and Adiposity

Ghrelin has been found to be produced in the region of the brain involved in the control of energy balance (11). Studies have found that peripheral and central administration of ghrelin caused weight and fat gain by reducing fat utilization. For example, Heppner et al. (27) found that central administration of both AG and dAG increased adiposity. AG reduced locomotor activity (LA) and increased the respiratory exchange ratio (RER) (as measured by direct calorimetry). However, when AG and dAG were injected peripherally the increase in adiposity was blunted and ablated, respectively. Both AG and dAG increased feeding, but the effect of increased adiposity was maintained even when feeding was not increased. The increase in fat mass in response to ghrelin injections is somewhat surprising when considering the lipolytic effect of GH, yet the appetite stimulating effect of ghrelin has been found to be independent of its effect on GH secretion (53). An increase in energy intake in mice might be the primary cause of an increase in adiposity in response to an elevation in plasma ghrelin concentrations. Wren et al. (59) observed that ghrelin administered either centrally or peripherally, dramatically increased food intake in rats exposed to ad libitum feeding. Shintani et al. (49) concluded that ghrelin reversed the leptin-induced inhibition of food intake and decreased the hypothalamic neuropeptide Y expression. NPY is thought to cause leptin's satiety effect. This might suggest that ghrelin is an antagonist to leptin (42). Other research suggests that the orexigenic potency of ghrelin is caused by the activation of hypothalamic NPY and Y1 receptors along with a subsequent increase in gastric emptying (3). This finding has challenged research that contends that the most important hypothalamic target of ghrelin is AGRP (29).

Studies have shown a dramatic pre-prandial rise and postprandial fall in circulating ghrelin levels, thereby substantiating ghrelin as a physiological meal initiator (12). Research has also found that obese subjects have lower plasma ghrelin concentrations than that of age-matched, lean controls. It has been suggested that chronic positive energy balance in the obese population may cause a down-regulation of plasma ghrelin concentrations. Although ghrelin has been shown to increase body weight and fat, its role in human obesity has been challenged. Studies that utilized clamped energy balance conditions suggest that ghrelin was not related to the magnitude of body weight changes in either overfeeding or negative energy balance (46).

Taken together the data from the research completed on ghrelin and adiposity suggests that it may increase adiposity, by increasing feeding, decreasing LA and increasing RER. However, there has yet to be any understanding of the mechanisms that reduce plasma ghrelin concentrations in the obese and increase it under extreme low body weight conditions. Nevertheless, ghrelin's role as a regulator of energy balance has been hypothesized, such that it is secreted in times of negative energy balance to signal the hypothalamus when an increase in metabolic efficiency is needed and is not secreted during times of positive energy balance as to prevent the consumption of additional energy.

Effect of Macronutrients on Ghrelin

Research has shown that digestion of protein, carbohydrates and fat effect plasma ghrelin levels in different capacities. In studies completed on healthy weight subjects the results suggest that protein has the greatest impact on long-term satiety and lowering of plasma ghrelin concentrations. Foster-Schubert et al. (21) used isoenergy; isovolemic beverages composed primarily of carbohydrates, proteins or lipids as their intervention and found that protein had the highest magnitude of ghrelin suppression followed by carbohydrates and lipids. Although

carbohydrates had the fastest ghrelin lowering effect, three hours after ingestion ghrelin levels rose above pre-prandial values. Data from a study investigating the correlations between macronutrient intake and human brain and gut hormone responses concluded that although there were no differences in brain responses when the diverse macronutrients were consumed, ghrelin levels were decreased to a higher degree in response to protein consumption when compared to carbohydrates and fats (35). These data suggest that in healthy weight individuals, protein is the optimal macronutrient for long term satiety and lower ghrelin levels. However, the same is not true for obese individuals.

It has been well documented that body mass index (BMI), body fat and indices of central fat distribution are inversely associated with fasting plasma ghrelin concentrations (33). Positive energy balance observed in obese individuals may suppress maximal circulating ghrelin to limit energy consumption. The impaired cholinergic (vagal) regulation of postprandial drop in ghrelin concentrations might be also responsible for the dysregulated ghrelin control in obese subjects (36). Although obese subjects have low pre-prandial ghrelin levels their postprandial ghrelin secretion is not sufficiently suppressed to reduce appetite, which causes continued hunger after a meal (33). In a study on ghrelin responses to high fat and high protein iso-energy meals in a group of obese women, Pavlatos et al. (44) determined that neither macronutrient elicited a significant acute ghrelin response. In support of these findings, Tentolouris et al. (50) reported that a high carbohydrate meal was also incapable of inducing a decrease in postprandial ghrelin levels in a group of obese women. In addition, leaner individuals had higher fasting ghrelin levels and faster declines of postprandial ghrelin. It seems, then, that leaner people feel hungrier before a meal but become satiated faster than an obese individual. Some factors that may explain the blunted postprandial response in obese subjects might be the impaired post-meal

elevation of gastrointestinal hormones with anorexigenic and insulinomimetic properties, such as glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and peptide YY (PYY) (24). Taken together, the data from these experiments show a vast difference exists in the ability of an obese individual to secret and uptake ghrelin when compared to a healthy weight individual.

Effect of Acute Exercise on Ghrelin

Evidence has suggested that concentrations of plasma acylated ghrelin are suppressed after strenuous endurance exercise in non-obese, healthy subjects. Reductions in ghrelin levels have been detected along with a concomitant decrease in appetite at rest and may provide a potential mechanism for alterations of appetite or food beverage intake post-exercise (5, 8, 22), especially in obese populations.

Past studies related to the effects of exercise on plasma ghrelin levels have utilized a variety of modes, intensities and durations of exercise. Typically, moderate to vigorous cycling or running at intensities ranging from 50% to 75% of maximal oxygen uptake ($\dot{V}O_{2max}$) and lasting approximately one hour have been used (21). In a study completed by Vatansever-Ozen et al. (55), 10 healthy male subjects ran for 105 minutes at an intensity of 50% of $\dot{V}O_{2max}$ and 15 minutes at 70% of $\dot{V}O_{2max}$ (for a total of 120 minutes). The results of this study concluded that this mode and intensity of exercise caused a reduction in AG levels that correlated with a reduction in appetite. In contrast to these findings, data from Burns et al. (9) suggests that an acute bout of treadmill running has no effect on total plasma ghrelin levels. The differences in the findings may result from the fact that one study measured AG and the other measured total ghrelin. It is still being debated whether or not dAG has the same effects on the human body as AG. Some studies suggest that AG and dAG have different actions (48), while others have

found that they exhibit similar effects on adiposity and glucose homeostasis (27). The fact that Burns et al. (9) used both males and females in their subject pool may have also altered their results as differences between appetite and energy intake may be dissimilar between males and females. Hagobian and colleagues (25) evaluated sex differences in hormones and energy intake in response to control conditions with four days of exercise with energy replacement (to maintain energy balance) and four days of exercise without energy replacement (energy deficit). In response to a meal tolerance test after four days in balance or deficit, women had markedly higher AG concentrations compared with baseline, while no significant difference existed in men.

Although some of the research investigating the effects of exercise on plasma ghrelin concentrations has utilized obese female and male subjects (26, 37, 51, 54), most research has been centered on healthy weight females and males (5, 7, 8, 9, 16, 23, 30, 37, 55, 57). Research involving obese subjects is lacking therefore there is no agreement in the literature in regards to an appropriate mode, duration or intensity of exercise that may be beneficial in reducing plasma ghrelin levels in this population. Furthermore, the issue on whether acute exercise impacts ghrelin levels in this population is still inconclusive. Using a population of overweight women, Tiryaki-Sonmez et al. (51) found that an acute bout of running at an intensity of 50% of $\dot{V}O_{2max}$ significantly reduced AG levels in this group. An earlier study concluded that walking at an intensity of 70% to 75% of age-predicted maximal heart rate elicited no effect on plasma AG (54). Many differences in methodology could account for the difference in results between these experiments. In the group that observed a change, the subjects were younger and although they were overweight they were not obese, as were the group of women in which no significant difference was found. Also, the intensity of exercise was more appropriately defined in the

group for which exercise significantly reduced AG. Exercise using age predicted maximal heart rate does not take the fitness level of the participant into account; therefore the exercise may not produce the desired result. Because these studies utilized female subjects, the question exists whether or not these results would be the same using a male population with similar body compositions.

In addition to the studies on obese individuals as a whole lacking in the scientific literature, research comparing healthy weight to obese individuals (using the same mode, duration and intensity of exercise) are almost void in the literature. The research that does exist either utilizes a mixture of males and females at a moderate intensity and duration (26) or they do not provide the distribution of the population used nor the duration or intensity of exercise (37). It would be very difficult to extrapolate meaningful interventions from these data.

Summary

The research on ghrelin is conclusive on its role to regulate energy balance by stimulating feeding during times of negative energy balance and decreasing feeding during times of positive energy balance. In addition, research has shown that plasma ghrelin levels are reduced post-prandially faster in healthy weight than in the overweight or obese. Increases in plasma ghrelin concentrations have also been associated with reductions in fat metabolism. These mechanisms might contribute to preventing weight gain in healthy weight individuals and stimulate additional weight gain in the overweight or obese. Thus, interventions that target ghrelin might aid in weight reduction in overweight or obese populations. Exercise has been proposed as one method of modulating ghrelin levels in these individuals. Many forms of acute endurance exercise have been shown to reduce plasma AG concentrations in healthy weight individuals. These reductions in AG levels have been observed with a concomitant decrease in appetite and increase in the

secretion of anorexigenic hormones (i.e., GLP-1, PYY, and PP) (see figure 2). However, the effect of exercise on plasma ghrelin levels and anorexigenic hormones in the obese is inconclusive. The research studies that have been completed on this topic have differed in their results, mode, intensity and duration of exercise. Also, studies have failed to investigate an intensity of exercise that is feasible for this population.

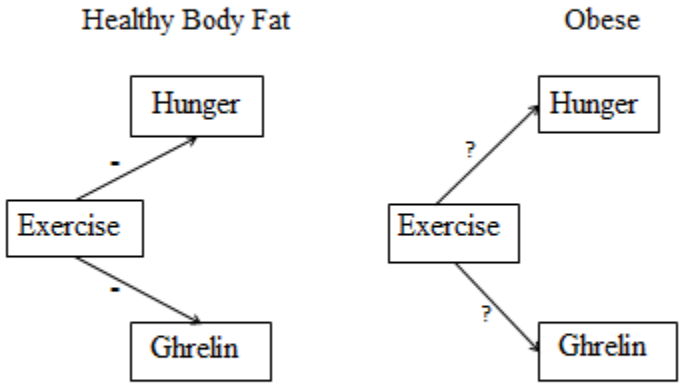


Figure 2. Shows the effects of exercise in normal weight people and the lack of evidence on the effects of exercise in overweight/obese people

REFERENCES

1. Alvarez-Castro P, Isidro ML, García-Buela J, Dieguez C, Casanueva FF, Cordido F. Effect of acute ghrelin administration on glycaemia and insulin levels in obese patients. *Diabetes, Obesity and Metabolism*. 2006;8(5):555-60.
2. Asakawa A, Inui A, Kaga T, et al. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut*. 2003;52(7):947-52.
3. Asakawa A, Inui A, Kaga O, et al. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology*. 2001;120(2):337-45.
4. Benardot D. Energy Thermodynamics Revisited: Energy intake strategies for optimizing athlete body composition and performance. *PENSAR EN MOVIMIENTO: Revista de Ciencias del Ejercicio y la Salud* (EISSN 1659-4436)2013;11(2):1-13
5. Bilski J, Teleglów A, Zahradnik-Bilska J, Dembiński A, Warzecha Z. Effects of exercise on appetite and food intake regulation. *Medicina Sportiva*. 2009;13(2):82-94.
6. Broglio F, Arvat E, Benso A, et al. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *The Journal of Clinical Endocrinology & Metabolism*. 2001;86(10):5083-86.
7. Broom D, Batterham RL, King JA, Stensel DJ. Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *Am J Physiol Regul Integr Comp Physiol*. 2009; 296:R29–R35
8. Broom DR, Stensel DJ, Bishop NC, Burns SF, Miyashita M. Exercise-induced suppression of acylated ghrelin in humans. *Journal of applied physiology*. 2007;102(6):2165-71.

9. Burns SF, Broom DR, Miyashita M, Mundy C, Stensel DJ. A single session of treadmill running has no effect on plasma total ghrelin concentrations. *Journal of sports sciences*. 2007;25(6):635-42.

10. Colombo M, Gregersen S, Xiao J, Hermansen K. Effects of ghrelin and other neuropeptides (CART, MCH, orexin A and B, and GLP-1) on the release of insulin from isolated rat islets. *Pancreas*. 2003;27(2):161-66.

11. Cowley MA, Smith RG, Diano S, et al. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron.* 2003;37(4):649-61.

12. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*. 2001;50(8):1714-19.

13. Date Y, Murakami N, Kojima M, et al. Central effects of a novel acylated peptide, ghrelin, on growth hormone release in rats. *Biochemical and biophysical research communications*. 2000;275(2):477-80.

14. Date Y, Nakazato M, Hashiguchi S, et al. Ghrelin is present in pancreatic α-cells of humans and rats and stimulates insulin secretion. *Diabetes.* 2002;51(1):124-129.

15. Date Y, Nakazato M, Murakami N, Kojima M, Kangawa K, Matsukura S. Ghrelin acts in the central nervous system to stimulate gastric acid secretion. *Biochemical and biophysical research communications*. 2001;280(3):904-07.

16. Deighton K, Barry R, Connon CE, Stensel DJ. Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl Physiol.* 2013; 113:1147–56

17. Dezaki K, Hosoda H, Kakei M, et al. Endogenous Ghrelin in Pancreatic Islets Restricts Insulin Release by Attenuating Ca2+ Signaling in β-Cells Implication in the Glycemic Control in Rodents. *Diabetes*. 2004;53(12):3142-51.

18. Drazen DL, Woods SC. Peripheral signals in the control of satiety and hunger. *Current Opinion in Clinical Nutrition & Metabolic Care*. 2003;6(6):621-29.

19. Eisenstein J, Greenberg A. Ghrelin: update 2003. *Nutrition reviews*.2003;61(3):101-4.

20. Esler WP, Rudolph J, Claus TH, et al. Small-molecule ghrelin receptor antagonists improve glucose tolerance, suppress appetite, and promote weight loss. *Endocrinology*. 2007;148(11):5175-85.

21. Foster-Schubert KE, Overduin J, Prudom CE, et al., Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. *The Journal of Clinical Endocrinology & Metabolism*.2008;93(5):1971-79.

22. Gibbons C, Caudwell P, Finlayson G, Webb DL, Hellström PM, Näslund E, Blundell JE. Comparison of postprandial profiles of ghrelin, active GLP-1, and total PYY to meals varying in fat and carbohydrate and their association with hunger and the phases of satiety. *The Journal of Clinical Endocrinology & Metabolism*. 2013;98(5): E847-55.

23. Gnanapavan S, Kola B, Bustin SA, et al. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *The Journal of Clinical Endocrinology & Metabolism*. 2002;87(6):2988.

24. Hagemann D, Holstb JJ, Gethmanna A, Banascha M, Schmidta W, Meiera JJ. Glucagon-like peptide 1 (GLP-1) suppresses ghrelin levels in humans via increased insulin secretion. *Regulatory peptides*. 2007;143(1):64-68.

25. Hagobian TA, Sharoff CG, Stephens BR, et al. Effects of exercise on energy-regulating hormones and appetite in men and women. *American Journal of Physiology-Regulatory. Integrative and Comparative Physiology*. 2009;296(2):R233-42.

26. Heden TD, Liu Y, Park Y, Dellsperger KC, Kanaley JA. Acute aerobic exercise differentially alters acylated ghrelin and perceived fullness in normal-weight and obese individuals. *Journal of Applied Physiology*. 2013;115(5):680-87.

27. Heppner KM, Piechowski CL, Müller A, et al. Both acyl and des-acyl ghrelin regulate adiposity and glucose metabolism via central nervous system ghrelin receptors. *Diabetes*. 2014;63(1):122-31.

28. Hosoda H, Kojima M, Mizushima T, Shimizu S, Kangawa K. Structural divergence of human ghrelin Identification of multiple ghrelin-derived molecules produced by post-translational processing. *Journal of Biological Chemistry*. 2003;278(1):64-70.

29. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology*. 2000;141(12):4797-00.

30. King JA, Wasse LK, Ewens J, et al. Differential acylated ghrelin, peptide YY3-36, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *J Clin Endocrinol Metab*. 2011;96: 1114–21

31. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999;402(6762):656-60.

32. Kojima M, Hosoda H, Kangawa K. Purification and distribution of ghrelin: the natural endogenous ligand for the growth hormone secretagogue receptor. *Hormone Research in Paediatrics*. 2004;56(Suppl. 1):93-97.

33. Koliaki C, Kokkinos A, Tentolouris N, Katsilambros N. The effect of ingested macronutrients on postprandial ghrelin response: a critical review of existing literature data. *International journal of peptides*. 2010;2010.

34. Latour MG, Lautt WW. The hepatic vagus nerve in the control of insulin sensitivity in the rat. *Autonomic Neuroscience*. 2002;95(1):125-30.

35. Li J, An R, Zhang Y, Li X, Wang S. Correlations of macronutrient-induced functional magnetic resonance imaging signal changes in human brain and gut hormone responses. *The American journal of clinical nutrition*. 2012;96(2):275-82.

36. Maier C, Riedl M, Vila G, et al. Cholinergic regulation of ghrelin and peptide YY release may be impaired in obesity. *Diabetes.* 2008;57(9):2332-40.

37. Marzullo P, Salvadori A, Brunani A, et al. Acylated ghrelin decreases during acute exercise in the lean and obese state. *Clinical endocrinology*. 2008;69(6):970-71.

38. Marzullo P, Verti B, Savia G, et al. The relationship between active ghrelin levels and human obesity involves alterations in resting energy expenditure. *The Journal of Clinical Endocrinology & Metabolism*. 2004;89(2):936-39.

39. Masuda Y, Tanaka T, Inomata N, et al. Ghrelin stimulates gastric acid secretion and motility in rats. *Biochemical and biophysical research communications*. 2000;276(3): 905-08.

40. Matsuhisa M, Yamasaki Y, Shiba Y, et al. Important role of the hepatic vagus nerve in glucose uptake and production by the liver. *Metabolis.,* 2000;49(1):11-16.

41. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clinical Chemistry*.2004;50(9):1511-25.

42. Nakazato M, Murakami N, Date Y, et al. A role for ghrelin in the central regulation of feeding. *Nature*. 2001;409(6817):194-98.

43. Organization, W.H., *Obesity and Overweight.* World Organization, 2015. http://www.who.int/mediacentre/factsheets/fs311/en/(MediaCentre (Fact Sheet)): p. N311.

44. Pavlatos S, Kokkinos A, Tentolouris N, Doupis J, Kyriaki D, Katsilambros N. Acute effects of high-protein and high-fat isoenergetic meals on total ghrelin plasma concentrations in lean and obese women. *Hormone and metabolic research*. 2005;37(12): 773-75.

45. Perseghin G, Caumo A, Caloni M, Testolin G, Luzi L. Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. *The Journal of Clinical Endocrinology & Metabolism*. 2001;86(10):4776-81.

46. Ravussin E, Tschöp M, Morales S, Bouchard C, Heiman ML. Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins. *The Journal of Clinical Endocrinology & Metabolism*. 2001;86(9):4547-51.

47. Sangiao-Alvarellos S. Cordido F. Effect of ghrelin on glucose-insulin homeostasis: therapeutic implications. *International journal of peptides*. 2010;2010.

48. Schubert MM, Sabapathy S, Leveritt M, Desbrow B. Acute exercise and hormones related to appetite regulation: a meta-analysis. *Sports Medicine*. 2014;44(3):387-403.

49. Shintani M, Ogawa Y, Ebihara K, et al. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the

activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes*. 2001;50(2):227-32.

50. Tentolouris N, Kokkinos A, Tsigos C, et al. Differential effects of high-fat and high-carbohydrate content isoenergetic meals on plasma active ghrelin concentrations in lean and obese women. *Hormone and metabolic research*. 2004;36(08):559-63.

51. Tiryaki-Sonmez G, Ozen S, Bugdayci G, et al. Effect Of Exercise On Appetite-Regulating Hormones In Overweight Women. *Biology of sport*. 2013;30(2):75.

52. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature*. 2000;407(6806):908-13.

53. Ukkola O, Pöykkö S. Ghrelin, growth and obesity. *Annals of medicine*. 2002;34(2):102-08.

54. Unick JL, Otto AD, Goodpaster BH, Helsel DL, Pellegrini CA, Jakicic JM. Acute effect of walking on energy intake in obese women. *Appetite*. 2010;55(3):413-19.

55. Vatansever-Ozen S, Tiryaki-Sonmez G, Bugdayci G, Ozen G. The effects of exercise on food intake and hunger: Relationship with acylated ghrelin and leptin. *Journal of sports science & medicine*. 2011;10(2):283.

56. Volante M, Allìa E, Gugliotta P, et al. Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. *The Journal of Clinical Endocrinology & Metabolism*. 2002;87(3):1300-08.

57. Wasse LK, Sunderland C, King JA, Miyashita M, Stensel DJ. The influence of vigorous running and cycling exercise on hunger perceptions and plasma acylated ghrelin concentrations in lean young men. *Applied Physiology, Nutrition, and Metabolism*. 2012;38(999):1-6.

58. Wierup N, Yang S, McEvilly RJ, Mulder H, Sundler F. Ghrelin is expressed in a novel endocrine cell type in developing rat islets and inhibits insulin secretion from INS-1 (832/13) cells. *Journal of Histochemistry & Cytochemistry*. 2004;52(3):301-10.

59. Wren A, Small CJ, Ward HL, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology*. 2000;141(11):4325-28.

60. Zigman JM, Elmquist JK. Minireview: from anorexia to obesity—the yin and yang of body weight control. *Endocrinology*. 2003;144(9):3749-56.

Effect of Acute High Intensity Interval Exercise and Energy Balance on Plasma Acylated Ghrelin Concentrations

Chapter 2

Obesity has become a public health crisis that is a leading risk factor for global premature deaths and disease. Although the causes of obesity are multifaceted, one of the primary factors is directly related to energy imbalance between calories consumed and expended (22). This discrepancy in energy balance is associated with an increase in energy intake and a concomitant decrease in physical activity (22). The report from the World Health Organization points to a decrease in energy intake and an increase in physical activity as the two primary alternatives that individuals can use to reduce body fat and begin to resolve the epidemic of obesity.

Research completed over the last 15 years has discovered a number of potentially important causes of the complex condition that is obesity. Ghrelin is one of several peptide hormones secreted by the gastrointestinal tract and has received significant attention from the research community as a potential target to regulate body weight (5, 8, 9, 20, 25). After its discovery as an endogenous ligand to the hypothalamic growth hormone (GH), researchers soon found that exogenous administration of ghrelin in rodents increased food intake and caused fat and weight gain. This effect of ghrelin has highlighted its role in weight regulation (8, 20, 24, 30).

Ghrelin is synthesized as a preprohormone, which is proteolytically processed to a 28 amino acid peptide (1, 15). Ghrelin circulates in both venous and arterial blood in two different forms: acylated (or n-octanoylated, AG) and unacylated (or des-octanoylated or des-acylated, UAG or dAG) (18, 24). AG uniquely features a posttranslational esterification of a fatty acid on the serine residue at position 3. Ghrelin acylation is considered necessary for its actions as an

endogenous ligand to the growth-hormone secretagogue receptor (GHSR) in which it stimulates the release of growth hormone (GH). Under homeostatic conditions, AG accounts for approximately 10% of total circulating ghrelin. Although UAG does not affect GH release, it is not biologically inactive. For example, UAG binds to other unknown receptors and have effects that are inconclusive in the research (11, 24).

Ghrelin is secreted mostly by stomach fundus, but also by the placenta, and in small amounts by the kidney, the pituitary and the hypothalamus (30). Ghrelin's ability to regulate feeding is independent of its role in GH stimulation (8). Research suggests that the hypothalamic hormones neuropeptide Y (NPY) and agouti growth-related peptide (AGRP) are intermediaries to the orexigenic effects of ghrelin. In these animal studies, the administration of ghrelin caused an increase in hypothalamic NPY and AGRP mRNA levels and the orexigenic effect was maintained even in mice that lacked NPY (8).

In adults, plasma ghrelin concentrations have been shown to increase prior to a meal and during fasting and to decrease within one hour after a meal no matter the macronutrient content. Ghrelin levels are increased under conditions of prolonged negative energy balance (i.e., low calorie diets, chronic exercise and/or anorexia nervosa) (20). In contrast, under obese conditions (chronic positive energy balance), ghrelin concentrations are typically low, which may be related to energy consumption patterns that result in excessive adipose deposits, whereas a reduction in body weight in obese patients results in an increase in ghrelin concentration (34).

During the past decade, the effect of exercise on ghrelin and energy intake has been studied extensively. Research to date has been inconclusive; however there is evidence suggesting that in non-obese populations, concentrations of plasma acylated ghrelin are suppressed after strenuous endurance exercise (32, 33), while concentrations of anorexigenic

hormones (i.e., GLP-1, PYY, and PP) are increased (25) (see figure 1). These alterations in plasma ghrelin concentrations are shown to have a linear relationship with appetite at rest (as assessed using subjective visual analogue scales for feelings such as hunger, fullness, satisfaction and prospective food consumption) and may provide a potential mechanism for alterations of appetite or food and beverage intake post-exercise (2, 3, 10).

The purpose of this study was to investigate the effect of a bout of short-term high intensity interval exercise on plasma acylated ghrelin (AG) levels in obese men when compared to non-obese men. Although the exercise method includes high intensity exercise the intensity was defined by each individual's pre-calculated heart rate reserve. The duration of each bout of high intensity exercise was 20 minutes not including the warm up and cool down period, after which the participant dismounted the cycle and rested in the seated position until having blood drawn. Based on the efficacy of moderate to vigorous exercise to decrease plasma ghrelin levels, we hypothesized that high intensity interval exercise would be effective in acutely reducing acylated ghrelin levels in obese subjects. However, the degree to which ghrelin concentrations are reduced will be significantly less in the obese group than in a non-obese group. This research project is designed to answer the question marks in figure 2.

Methods

Participants

All procedures were approved by the Georgia State University Institutional Review Board (IRB). Eighteen healthy men (9 obese and 9 non-obese) aged 28.9 ± 7.6 years participated in this study. Participants signed a written informed consent that detailed all of the procedures and risks involved in the experiment. The participants completed a health screen and

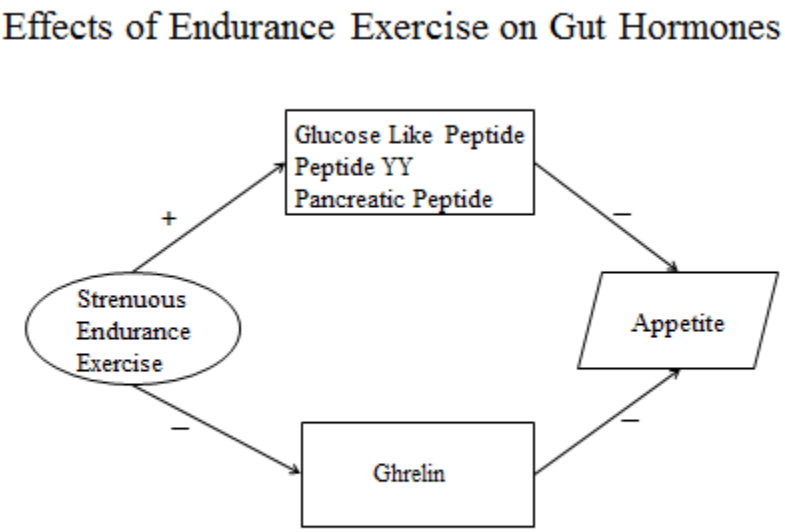


Figure 1. Endurance exercise leads to an increase in anorexigenic hormones and a decrease in orexigenic hormones and both hormones lead to a reduction in appetite

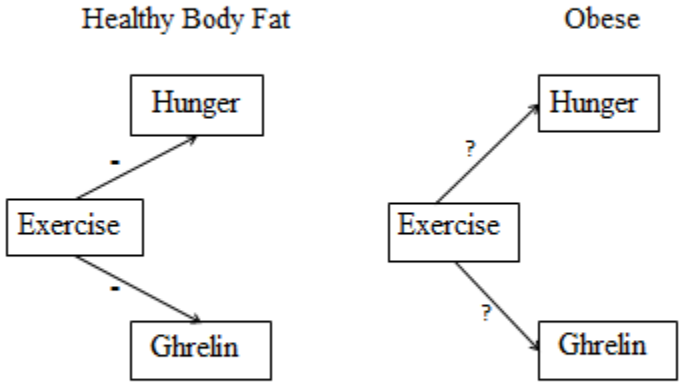


Figure 2. Shows the effects of exercise in normal weight people and the lack of evidence on the effects of exercise in overweight/obese people

physical activity questionnaire (Appendix A). All participants were non-smokers, not on a weight gain or weight loss diet at the time of the experiment and had not been on any such diet in the previous six months leading up to participation in the experiment. Each individual maintained a stable bodyweight (had not lost or gained more than 10% of their bodyweight) in the previous six months, had no gastric or digestive problems, no known history of cardiovascular disease, including: diabetes, stroke or heart attack and had a resting arterial blood pressure <140/90 mmHg. To gauge whether or not a prospective participant had incurred a weight change of 10% or more in the six months leading up to the experiment, they were asked, "if their clothing sizes had changed in the six months leading up to the experiment".

Preliminary Tests

Anthropometry

Height was measured to the nearest .25 cm using a standard wall stadiometer. Body mass was measured to the nearest 0.1 kg using a standard digital weighing scale (Tanita, WB – 110A Class III, USA). Fat mass and lean mass was determined using Dual-energy X-ray absorptiometry (DEXA) (GE Lunar Prodigy Primo Bone Densitometer).

$\dot{V}O_{2max}$ test

Between 5-7 days prior to initiation of the experimental protocol, $\dot{V}O_{2max}$ was determined on a cycle ergometer (Velotron RaceMate CompuTrainer, USA) using a standard testing protocol in accordance with ACSM guidelines (22). All participants began the $\dot{V}O_{2max}$ testing with a 5 minute warm up. After the warm up participants cycled at a preset resistance of 75 watts. The resistance was then increased every 2 minutes by 25 watts until the participant could no longer continue or until $\dot{V}O_2$ began to decline. At the 2 minute mark participants were asked to rate their exertion using a Borg rate of perceived exertion scale while their heart rate and $\dot{V}O_2$

were assessed. Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured throughout the entire exercise session using a True One 2400 metabolic measurement system (PAR Medics, Sandy, Utah, USA) which is a computer controlled breath-by-breath analyzer. The participants $\dot{V}O_{2max}$ was defined as the highest value for ($\dot{V}O_2$) achieved during the exercise test. The attainment of a valid $\dot{V}O_{2max}$ required that participants met the following criteria: a final respiratory exchange ratio (RER) > 1.0, ($\dot{V}O_2$) consumption increased by < 2 $ml \cdot kg^{-1} \cdot min^{-1}$ with an increase in exercise intensity and with attainment of >85% of age-predicted maximal heart rate and or voluntary termination of the test by the participant.

Heart Rate (HR) was measured continuously throughout the test using a commercially available Polar HR monitor (S610i Polar Electron, Finland). Typically the test was terminated when subjects demonstrated that they could longer continue with the maximum workload or if the participant's oxygen utilization failed to increase with an increase in exercise intensity or began to decline. Participants were allowed a 5 minute cool down period after the test was terminated.

Experimental Protocol and Main Trials

Subjects visited the laboratory on three separate occasions. All participants performed the $\dot{V}O_{2max}$ test on their initial visit. After the $\dot{V}O_{2max}$ test participants were randomly assigned to a control or experimental trial. Two main trials (exercise and control) were performed in a counterbalanced, randomized design. The interval between the two trials was at least three days. In addition, participants were allowed to begin one of the trial conditions three days after the $\dot{V}O_{2max}$ test was completed. This was done to allow time for each participant to track two days of feeding and physical activity before each trial. For each trial the participants reported to the laboratory after a 10-hour overnight fast. Upon arrival participants were allowed to rest in a

seated position for 10 to 15 minutes, after which an intravenous catheter was inserted into an antecubital vein. During this period, participants were asked to rate their hunger using a hunger scale (described below). During the control experiment, the participants continued to rest (reading, working quietly, or watching television) for approximately two hours. During the exercise trial, the participants cycled on a cycle ergometer for 30 minutes (including a five minute warmup and five minute cool down) and then rested for one and a half hours (see Table 1). The exercise trial consisted of 20 minutes of high intensity interval exercise on a cycle ergometer at 65%-85% of heart rate reserve (HRR). Subjects were allowed a 5 minute warmup period after which subjects were allowed 30 seconds to attain a heart rate of 85% of their HRR and had to maintain that pace for 30 seconds. After 30 seconds, subjects were allowed a one minute active recovery period in which subjects pedaled at a reduced pace to allow their heartrate to drop to 65% of HRR (this counted as one repetition). If during the active recovery period the subject's heart rate dropped below 65% of HRR before the 1- minute recovery period had elapsed they were required to reconvene the working phase of exercise.

Blood samples (~3 mL) were drawn into chilled tubes containing 1.25mg·ml^{-1} Na_2EDTA and 500 TIU·ml^{-1} aprotinin (Kallikrein Inhibitor Unit), Phoenix, Burlingame, USA at baseline, and at 0.5, 1 and 1.5 hours after baseline for the determination of acylated ghrelin. All samples were drawn by a licensed registered nurse. Participants were in a seated position for at least five minutes before blood was withdrawn. Water was available ad libitum during both trials and participants were encouraged to drink at least 20 ounces before reporting to the laboratory to assure that blood volume was sufficient. Hunger was assessed at each blood sampling point.

Table 1. Shows the activities of each subject on both exercise and control days.

Variable		Exercise 40 Minutes	Resting 40 Minutes	Hunger Rating
Prep 25 Minutes		Insert catheter	Insert catheter	
Baseline 10 Minutes		Blood draw	Blood draw	X
Post Exercise 30 minutes		Blood draw	Blood draw	X
Post Exercise 1 hour		Blood draw	Blood draw	X
Post Exercise 1.5 hours		Blood draw	Blood draw	X

Dietary Protocol and Energy Balance

Participants were asked to maintain their normal dietary and physical activity programs throughout the duration of the study. Subjects were also asked to refrain from physical exercise, alcohol and caffeine for 24 hours before testing. Because energy balance and macronutrient intake can influence plasma ghrelin levels, participants were asked to consume precisely the same diet for two days prior to control and testing day. In the 48 hours prior to testing, individuals were asked to record an hour-by-hour account of all physical activity performed over resting levels and provide a detailed description of all food and beverage types consumed and the amount (see Appendix B). This information was used to determine each individual's energy balance. Energy balance was determined using NutriTiming® (NutriTiming LLC). Subjects were advised to consume their last meal at least 10 hours prior to each experimental trial.

Hunger Scale

The hunger scores were determined using the satiety and hunger scale developed by Burgoon (see Appendix C) (25). Participants indicated their perceived level of hunger by circling the number that best represented how hungry they felt. The following phrases were included in the scale: "famished, starving", "not hungry, not full", and "bursting, painfully full".

Blood samples and hormone analysis

Blood samples were drawn by a licensed registered nurse via a catheter (Insyte™ Autoguard™) inserted into the antecubital vein of each subject. Blood was drawn into 3ml lithium heparin tubes (B-D Vacutainer). Immediately after collecting blood samples, the sample tubes were centrifuged (GS-6KR Centrifuge, Beckman Coulter) at 2002 x g for 15 minutes at 4°C AG. The obtained plasma samples were immediately aliquoted into 20µl tubes and stored at –80°C until assayed. Plasma acylated ghrelin assay was performed using a commercially

available ELISA (EIA-A05106; Biotech Centre, SPI Bio & Ellipse Pharmaceuticals; Bertin Pharma, Montigny le Bretonneux, France) with a detection limit of 1.5 pg · ml^{-1}.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 20. Descriptive statistics were used to report the statistics of the two samples, obese and non-obese subjects. Independent samples t-test were used to assess between group differences in baseline acylated ghrelin levels, $\dot{V}O_{2max}$, HR and work rate. Repeated-measures, two-factor ANOVA was used to examine differences between the two trials and over four timed occasions for acylated ghrelin and to measure within group differences at each time point. Where significant main and interaction effects were found, post-hoc analysis was performed using Bonferroni correction for multiple comparisons. Pearson correlation was used to determine the relationship between energy balance, hunger and ghrelin between trials and within the two groups across the timed occasions. Data were presented as appropriate with statistical significance set at $p < 0.05$.

Results

Exercise Protocol and Testing

The non-obese group's $\dot{V}O_{2max}$ was statistically significantly higher than that of the obese group ($p = .004$) (Table 2). Also, the max HR and work rate achieved by the non-obese group was significantly higher than the obese group ($p = .045$ and .005, respectively) (Table 2). Although there were no significant differences found between the groups in relation to max HR achieved during the exercise protocol and the duration of the exercise protocol, the non-obese absolute force output during the interval exercise was significantly higher than the obese group ($p = .007$). Rate of perceived exertion did not differ between the groups.

Plasma-acylated ghrelin concentrations

Independent samples t-test revealed a statistically significant difference in acylated ghrelin levels between the groups at baseline ($p = .004$) and 90 minutes post exercise ($p = .036$) (Figure 3) during the control trial with the non-obese group having higher acylated ghrelin levels than the obese group at both time points. However, no significant differences were found between the groups at any time point during the exercise trial (Figure 4).

Repeated measures ANOVA showed a statistically significant difference in acylated ghrelin in the non-obese group between baseline and 90 minutes ($p = .024$). No differences were found within the obese group at any time point during the control trial (Figure 5 and 6). Analysis during the exercise trial revealed a significant difference in the non-obese group across the four time intervals. Pairwise comparisons indicate that the significant differences occurred between baseline and 30 minutes ($p = .047$) and 30 minutes and 90 minutes ($p = .011$) (Figure 7). In the non-obese group plasma-acylated ghrelin concentrations declined sharply after exercise but rose to near baseline levels 60 minutes post-exercise. No significant differences were found between any time intervals for the obese group (Figure 8).

Plasma-acylated ghrelin and hunger

Independent samples t-test revealed no differences between groups in perceived hunger ratings at any time interval during either trial. Pearson correlations revealed no relationships between plasma-acylated ghrelin levels and hunger perceptions in either group or trial.

Plasma-acylated ghrelin and energy balance

Pearson Correlations revealed a positive relationship between hours spent in an optimal energy balance (OEB = +/- 400 kcal) range and baseline plasma-acylated ghrelin concentrations ($r = .89$, $p = .003$) (hours spent in OEB = 12.89 ± 4.3) in the obese group. No relationships were

Table 2. Participant Descriptive Data

Variable	Non-Obese N = 9 $\overline{X}$ ± SD	Obese N = 9 $\overline{X}$ ± SD	Probability
Age (Years)	26.1 ± 5.4	31.7 ± 8.8	.127
Height (m)	1.82 ± 0.08	1.77 ± 0.05	.123
Body Fat (%)	13.7 ± 3.6	31.7 ± 4.7	.000**
Weight (kg)	87.7 ± 14.8	100.1 ± 11.6	.067
BMI	26.2 ± 3.0	31.8 ± 3.8	.003*
Fat Mass (kg)	11.8 ± 4.6	30.5 ± 6.7	.000**
Lean Mass (kg)	72.0 ± 10.4	65.2 ± 7.4	.131
VO2Max	47.6 ± 9.6	30.7 ± 6.6	.001*
VO2Max(L/min)	4.1 ± 0.74	3.0 ± 0.59	.004*
Max HR	185.9 ± 14.9	172.4 ± 10.8	.045*
Max Watt	277.8 ± 47.5	216.7 ± 17.7	.005*
RPE	18.4 ± 1.7	18.2 ± 2.4	.827
IT Max HR	173.1 ± 9.7	167.3 ± 5.9	.152
IT Max Watt	277.8 ± 44.1	225.0 ± 17.7	.007*
IT Stage	12.7 ± 1.6	11.44 ± 1.0	.072
Optimal EB (hr ± 400kcal)	13.6 ± 6.1	12.89 ± 4.3	.79
EB hr > 400 (kcal) Above EB	.11 ± .33	2.33 ± 4.0	.14
EB hr < -400(kcal) Below EB	10.33 ± 6.2	8.78 ± 5.7	.57

Values are represented as mean ± SD

*P < 0.05, **P < 0.01 indicate significant difference between groups

BMI - Body Mass Index (Ht-cm2/Wt-kg), VO2Max – Maximal Oxygen Consumption, Maximal HR – Max Heart Rate, RPE – Rate of Perceived Exertion, IT Maximal HR – Interval Training Max Heart Rate, IT Max Watt – Interval Training Maximal Wattage, IT Stage – Interval stages completed

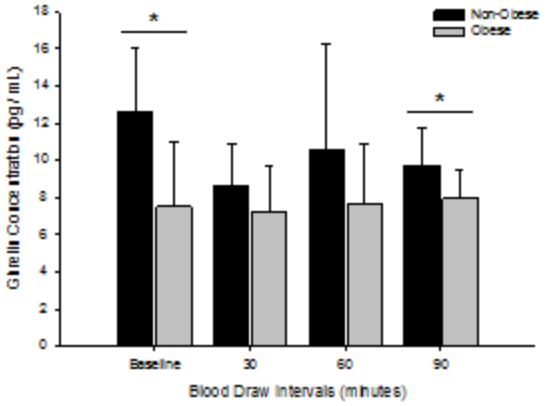


Figure 3. Plasma ghrelin concentration, control day. Independent sample t-test comparison of non-obese and obese. Values reported as means +/- SD (n = 5-6). Significance accepted at $p \leq 0.05$*

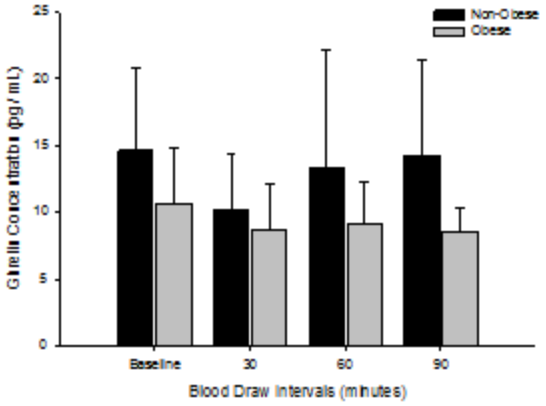


Figure 4. Plasma ghrelin concentration on high intensity, exercise day. Independent sample t-test comparison of non-obese and obese. Values reported as means +/- SD (n = 6-9). Significance accepted at $p \leq 0.05$

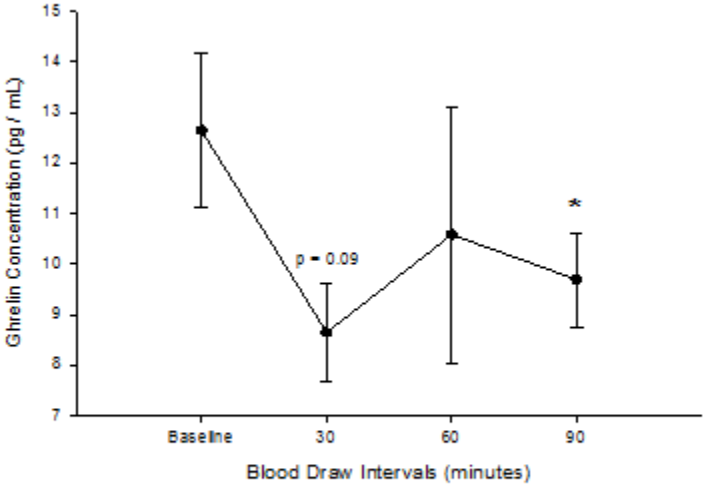


Figure 5. Plasma ghrelin concentration in non-obese subjects, control day (within group). Values reports as means +/- SEM (n = 5). Significance accepted at $p \leq 0.05$.*, compared to baseline. #, compared to 30 minutes.

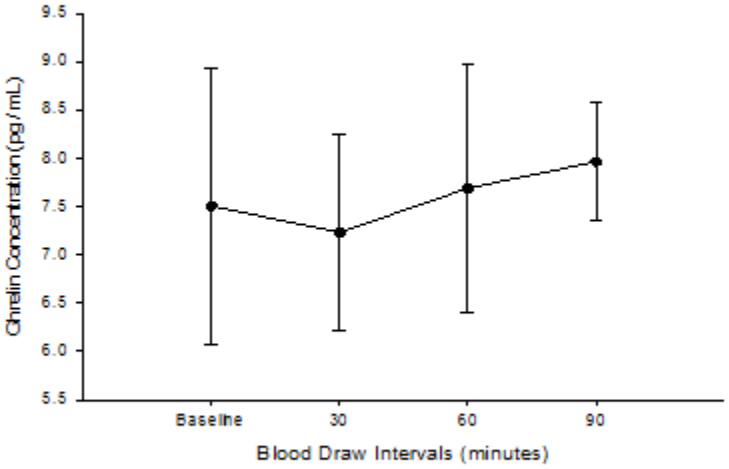


Figure 6. Plasma ghrelin concentration obese subjects, control day (within group). Values reports as means +/- SEM (n = 6). Significance accepted at $p \leq 0.05$.

found between plasma-acylated ghrelin and any of the energy balance variables in the non-obese group.

Discussion

The purpose of this study was to investigate the relationship between an acute bout of short-term high intensity interval training on plasma ghrelin concentrations in a population of obese and non-obese males. The primary findings are that during the control trial significant differences were found between the two groups at baseline and 90 minutes. Furthermore, while high intensity interval exercise did not have the impact of reducing acylated ghrelin in the obese group, it was effective in reducing this hormone in the non-obese group. Although acylated ghrelin levels decreased significantly post-exercise, it rebounded back to near baseline levels 60 minutes post-exercise. Despite not being significantly reduced in the obese group, acylated ghrelin levels exhibited a trend towards decline during the post exercise assessments in this population and continued this downward trend throughout the period of analysis.

The finding that baseline ghrelin levels were higher in the non-obese groups is consistent with research that suggests that acylated ghrelin levels are depressed in obese individuals when compared to non-obese subjects (1). However, such changes in ghrelin do not appear to reduce appetite or result in reduction in weight in this population (6). Our findings that plasma acylated ghrelin was decreased post exercise in the non-obese group is consistent with the findings of other research completed on this topic (2, 4, 7, 13, 16, 19, 32, 33). Burns et al. (5) assessed the effects of treadmill exercise on total ghrelin and found no significant change, however these findings cannot be compared to the findings of the current research because only acylated ghrelin was measured in this study. Other research has found that while exercise was effective in reducing acylated ghrelin, total ghrelin levels remained the same (19, 31).

The results of this experiment determined that exercise was ineffective in reducing acylated ghrelin levels in the obese group. While these findings are consistent with other research completed on this topic (13, 31), it must be understood that the difference in the current research is that only males were assessed, in the previous two studies both male and female were used (13) or a female only (31) population. Previous research has demonstrated that there are sex related differences in acylated ghrelin and energy intake in response to exercise (11). In conflict with the findings of our data, other research studies found that exercise was effective in reducing AG in obese populations (19, 28).

However, in contrast to our experimental protocol, these research studies used an exhaustive form of exercise that could not be used in a clinical setting to facilitate weight loss in a deconditioned, obese population. Furthermore, one of these studies (19) did not specify an average time of exercise nor did they affirm whether or not the population was all male or mixed. These omissions make it impossible to determine whether or not the findings of these two experiments are comparable.

The finding that plasma ghrelin levels were significantly reduced from baseline compared to 90 minutes was unanticipated due to the fact that the subjects remained in a fasted state throughout the entire evaluation period. Although subjects were permitted to drink water ad libitum, research studies using animal (21) and human (26) models have shown no significant effect of water on plasma ghrelin concentrations. Because no other hormones were assessed it is impossible to make a causative assumption about why the anomaly occurred.

Our findings revealed a correlation between hunger and AG 30 minutes post-exercise in the non-obese group, however no correlation between plasma acylated ghrelin concentrations and hunger were found at any other time point after exercise or during control.

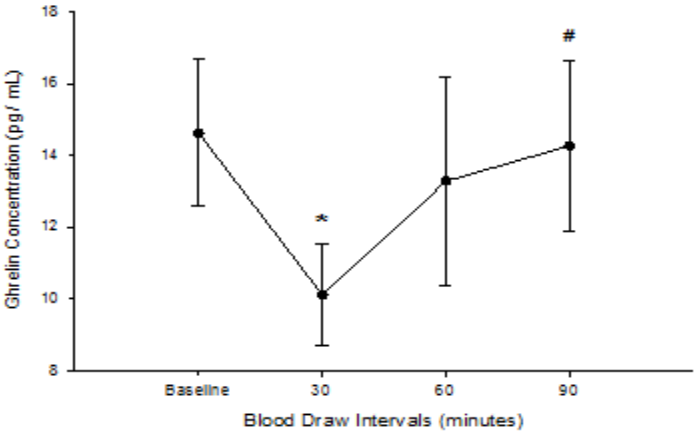


Figure 7. Plasma ghrelin concentration in non-obese subjects, exercise day (within group). Values reports as means +/- SEM (n = 9). Significance accepted at $p \leq 0.05$. *, compared to baseline. #, compared to 30 minutes.

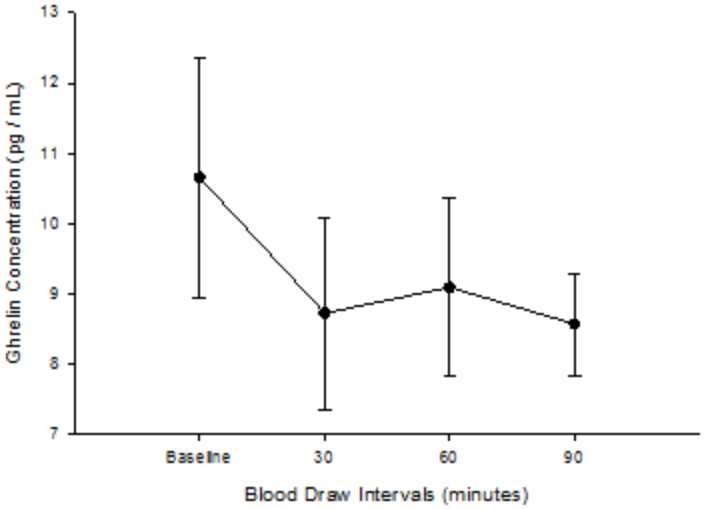


Figure 8. Plasma ghrelin concentration obese subjects, exercise day (within group). Values reports as means +/- SEM (n = 6). Significance accepted at $p \leq 0.05$.

No correlation was found at any time point, during either protocol in the obese group. This was a surprise due to the overwhelming evidence in the literature that support the link between the two (3, 4, 7, 16, 31, 32, 33). However, it must also be understood that ghrelin is only one component of the appetite-regulating neuroendocrine system and that appetite is most likely impacted by other hormonal and physiological elements (17). Still, these findings add to the discussion of whether or not acylated ghrelin can be used as a physiological element of appetite. An answer may come through the utilization of specific ghrelin antagonists in clinical investigations.

A positive correlation was observed between hours spent in optimal energy balance and plasma acylated ghrelin in the obese population. This is a novel finding due to the fact that to the authors' knowledge there is no known published research that has identified a link between hours spent in optimal energy balance and acylated ghrelin in obese individuals. However, research has determined that no differences existed in post-exercise AG levels when exercise resulted in an energy deficit compared to when energy balance was maintained (11). This finding may be evidence that in addition to weight loss, the dysregulation of ghrelin observed in this population may be counterbalanced by adequately matching energy expenditure with energy intake.

One limitation of this study was the sample size. Several control plasma samples where lost due to hemolysis of the samples during both protocols. Although this may have affected the samples taking on the day of control it did not have an impact on the non-obese exercise trial. Most samples taken during the exercise protocol were usable samples. No non-obese subjects had to be removed from the exercise data. In addition, the hunger scale used in this experiment was a limitation. The scale did not provide a robust enough range to allow participants to accurately detail their hunger perceptions. Due to this fact the mean range for hunger ratings was 2, meaning all ratings were between 3 and 5. Therefore, although significance was found at

one time point the trend for the two variables was not similar. The average rating of hunger found at baseline was similar to all other time points, consequently the significance found between these two variables could be attributed to chance and not to an actual relationship.

Conclusions

In conclusion, this is the first study to investigate the effects of a safe and appropriate form of exercise for use in a sedentary, obese population for the purposes of weight loss and plasma acylated ghrelin measurement and to compare the effects of energy balance on this gastric hormone in an obese and non-obese male population. Exercise had the effect of reducing acylated ghrelin levels in the non-obese subjects; however it did not have this impact on the obese group. Furthermore, baseline ghrelin levels were lower in the obese group when compared to the non-obese group. Lastly, hours spent in optimal energy balance were positively correlated to plasma acylated ghrelin in the obese population and no correlation was found in the non-obese population. Although the form of exercise used in this experiment was not effective in reducing ghrelin levels or the perception of hunger, it also did not result in an increase in either parameter. This outcome is a positive one for all clinicians that are responsible for designing weight loss plans for overweight and obese individual. Future studies should compare the effects of high intensity interval exercise on insulin, leptin, and peptide YY on this population and go further to compare these changes in a population of obese and non-obese females.

References

1. Asakawa A, Inui A, Kaga O, et al. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology*. 2001;120(2):337-45.
2. Bilski J, Teleglów A, Zahradnik-Bilska J, Dembiński A, Warzecha Z. Effects of exercise on appetite and food intake regulation. *Medicina Sportiva*. 2009;13(2):82-94.
3. Broom DR, Stensel DJ, Bishop NC, Burns SF, Miyashita M. Exercise-induced suppression of acylated ghrelin in humans. *Journal of applied physiology*. 2007;102(6):2165-71.
4. Broom DR, Batterham RL, King JA, Stensel DJ. Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *Am J Physiol Regul Integr Comp Physiol*. 2009;296:R29–35
5. Burns SF, Broom DR, Miyashita M, Mundy C, Stensel DJ. A single session of treadmill running has no effect on plasma total ghrelin concentrations. *Journal of sports sciences*. 2007;25(6):635-42.
6. Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med*. 2002;346:1623–30
7. Deighton K, Barry R, Connon CE, Stensel, DJ. Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl Physiol*. 2013;113:1147–56
8. Eisenstein J, Greenberg A. Ghrelin: update 2003. *Nutrition reviews*.2003;61(3):101-4.

9. Foster-Schubert KE, Overduin J, Prudom CE, et al., Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. *The Journal of Clinical Endocrinology & Metabolism*.2008;93(5):1971-79.

10. Gibbons C, Caudwell P, Finlayson G, Webb DL, Hellström PM, Näslund E, Blundell JE. Comparison of postprandial profiles of ghrelin, active GLP-1, and total PYY to meals varying in fat and carbohydrate and their association with hunger and the phases of satiety. *The Journal of Clinical Endocrinology & Metabolism*. 2013;98(5): E847-55.

11. Hagobian TA, Sharoff CG, Stephens BR, Wade GN, Silva JE, Chipkin SR, Braun B Effects of exercise on energy-regulating hormones and appetite in men and women. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 2009;296(2):R233-42.

12. Haskell WL, Lee IM, Pate RR, et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Circulation*. 2007;116(9):1081-93.

13. Heden TD, Liu Y, Park Y, Dellsperger KC, Kanaley JA. Acute aerobic exercise differentially alters acylated ghrelin and perceived fullness in normal-weight and obese individuals. *Journal of Applied Physiology*. 2013;115(5):680-87.

14 Heppner KM, Piechowski CL, Müller A, et al. Both acyl and des-acyl ghrelin regulate adiposity and glucose metabolism via central nervous system ghrelin receptors. *Diabetes*. 2014;63(1):122-31.

15. Hosoda H, Kojima M, Mizushima T, Shimizu S, Kangawa K. Structural divergence of human ghrelin Identification of multiple ghrelin-derived molecules produced by post-translational processing. *Journal of Biological Chemistry*. 2003;278(1):64-70.

16. King JA, Wasse LK, Ewens J, et al. Differential acylated ghrelin, peptide YY3-36, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *J Clin Endocrinol Metab*. 2011;96:1114–21

17. King NA, Hopkins M, Caudwell P, Stubbs RJ, Blundell JE. Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise induced weight loss. *Int J Obes*. 2008;32:177–84

18. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999;402(6762):656-60.

19. Marzullo P, Salvadori A, Brunani A, et al. Acylated ghrelin decreases during acute exercise in the lean and obese state. *Clinical endocrinology*. 2008;69(6):970-71.

20. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clinical Chemistry*.2004;50(9):1511-25.

21. Nakahara K1, Okame R, Katayama T, Miyazato M, Kangawa K, Murakami N. Nutritional and environmental factors affecting plasma ghrelin and leptin levels in rats. *J Endocrinol.* 2010;207(1):95-103.

22. Organization, W.H., Obesity and Overweight. *World Organization*, 2015. http://www.who.int/mediacentre/factsheets/fs311/en/(MediaCentre (Fact Sheet)): p. N311.

23. Pescatello LS, Arena R, Riebe D, Thompson PD. ACSM's Guidelines for Exercise Testing and Prescription. *J Can Chiropr Assoc*. 2014;58(3)

24. Sangiao-Alvarellos S. Cordido F. Effect of ghrelin on glucose-insulin homeostasis: therapeutic implications. *International journal of peptides*. 2010;2010.

25. Schubert MM, Sabapathy S, Leveritt M, Desbrow B. Acute exercise and hormones related to appetite regulation: a meta-analysis. *Sports Medicine*. 2014;44(3):387-403.

26. Sheth MK, Gupta N. Metabolic effect of FOS (fructooligosaccharide) in terms of gut incretin (glp-1) gut microflora and weight reduction in obese adults. *International Journal of Applied Biology and Pharmaceutical Technology*. 2014;5(3):256-64.

27. Shiiya T, Nakazato M, Mizuta M, et al. Plasma Ghrelin Levels in Lean and Obese Humans and the Effect of Glucose on Ghrelin Secretion. *The Journal of Clinical Endocrinology & Metabolism. 2002*;87(1):240–44

28. Tiryaki-Sonmez G, Ozen S, Bugdayci G, et al. Effect Of Exercise On Appetite-Regulating Hormones In Overweight Women. *Biology of sport*. 2013;30(2):75-80.

29. Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML, Circulating ghrelin levels are decreased in human obesity. *Diabetes*. 2001;50:707–09

30. Ukkola O, Pöykkö S. Ghrelin, growth and obesity. *Annals of medicine*. 2002;34(2):102-08.

31. Unick JL, Otto AD, Goodpaster BH, Helsel DL, Pellegrini CA, Jakicic JM. Acute effect of walking on energy intake in obese women. *Appetite*. 2010;55(3):413-19.

32. Vatansever-Ozen S, Tiryaki-Sonmez G, Bugdayci G, Ozen G. The effects of exercise on food intake and hunger: Relationship with acylated ghrelin and leptin. *Journal of sports science & medicine*. 2011;10(2):283.

33. Wasse LK, Sunderland C, King JA, Miyashita M, Stensel DJ. The influence of vigorous running and cycling exercise on hunger perceptions and plasma acylated ghrelin concentrations in lean young men. *Applied Physiology, Nutrition, and Metabolism*. 2012;38(999):1-6.

34. Zigman JM, Elmquist JK. Minireview: from anorexia to obesity—the yin and yang of body weight control. *Endocrinology*. 2003;144(9):3749-56.

APPENDIX A

CSEP approved Sept 12 2011 version

PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

SECTION 1 - GENERAL HEALTH

	Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?	☐	☐
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	☐	☐
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	☐	☐
5.	Are you currently taking prescribed medications for a chronic medical condition?	☐	☐
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	☐	☐
7.	Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.

Go to Section 3 to sign the form. You do not need to complete Section 2.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow the Canadian Physical Activity Guidelines for your age (www.csep.ca/guidelines).
- You may take part in a health and fitness appraisal.
- If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist® (CSEP-CEP) or CSEP Certified Personal Trainer® (CSEP-CPT).
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the questions above, please GO TO SECTION 2.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity programme.

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?		☐ If yes, answer questions 1a-1c	☐ If no, go to question 2
	1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/ or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
	1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	☐	☐
2.	Do you have Cancer of any kind?		☐ If yes, answer questions 2a-2b	☐ If no, go to question 3
	2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	☐	☐
	2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm		☐ If yes, answer questions 3a-3e	☐ If no, go to question 4
	3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	☐	☐
	3c.	Do you have chronic heart failure?	☐	☐
	3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	☐	☐
	3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes		☐ If yes, answer questions 4a-4c	☐ If no, go to question 5
	4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	☐	☐
	4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	☐	☐
	4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	☐	☐
5.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)		☐ If yes, answer questions 5a-5b	☐ If no, go to question 6
	5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	5b.	Do you also have back problems affecting nerves or muscles?	☐	☐

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure		☐ If yes, answer questions 6a-6d	☐ If no, go to question 7
	6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	6b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
	6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	☐	☐
	6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	☐	☐
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia		☐ If yes, answer questions 7a-7c	☐ If no, go to question 8
	7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	☐	☐
	7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	☐	☐
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event		☐ If yes, answer questions 8a-c	☐ If no, go to question 9
	8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	8b.	Do you have any impairment in walking or mobility?	☐	☐
	8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?		☐ If yes, answer questions 9a-c	☐ If no, read the advice on page 4
	9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	☐	☐
	9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	☐	☐
	9c.	Do you currently live with two chronic conditions?	☐	☐

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

- You should seek further information from a licensed health care professional before becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise profesional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes - please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

SECTION 3 - DECLARATION

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.
- Please read and sign the declaration below:

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact:
Canadian Society for Exercise Physiology
www.csep.ca

KEY REFERENCES
1. Jamnik VJ, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the eectiveness of clearance for physical activity participation; background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or BC Ministry of Health Services.

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

CSEP approved Sept 12 2011 version

APPENDIX B

Dietary Analysis Data Entry Form
NutriTiming®

Name/Code:_____ Age: _____Years DOB____/____/____ Gender: M / F
MM DD YYYY

Height: _____Feet _____Inches Weight: _____Pounds Date Analyzed___/___/_____
MM DD YYYY

Time of Last Meal Before Day of Analysis:_____

Instructions: Completing this form will help us understand whether the amount of energy (calories) you consume comes close to matching the energy (calories) you expend. This form provides a way of entering your energy expended by using an 'Activity Factor', and your energy consumed by using a description of the foods and drinks you ate. The information is entered by hourly units, so you don't have to remember precisely the time you had an activity or ate some food. Rather, you are asked to enter when you had an activity, its intensity by using the activity factor scale, and how long you did it (example: I had a slow jog between 10 and 11 in the morning that lasted for 30 minutes). Use the Activity Factor Scale Descriptions to help you figure out the best factor to enter when describing an activity. When entering food, describe the food and the way it was prepared fully (example: chicken breast with no skin that was baked; or fried, battered chicken breast, etc), and the amount you consumed (example: 1 apple; 1 ½ cups; 15 red grapes; 1 large banana, etc.). A factor of 1.5 is considered normal daytime activity, and we will assume a factor of 1.5 unless you indicate otherwise. A factor of 1 is equal to sleep, and a factor greater than 1.5 suggests you are doing something more vigorous than normal daytime activity. Please enter a full 24 hours of all your activities and all the foods/drinks you consume. Use the example below to help you understand how to enter the information.

Activity Factor Scale

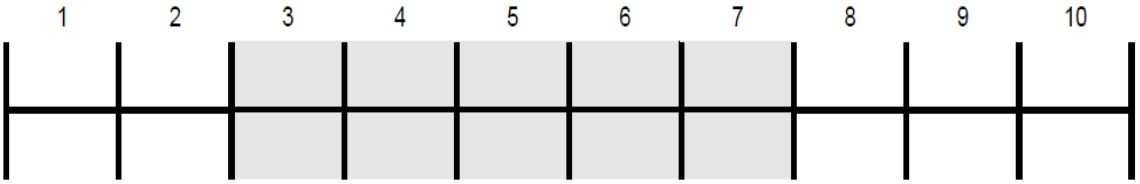
Factor	Description
1	**Resting, Reclining:** Sleeping, reclining, relaxing
1.5	**Rest +:** Normal, average sitting, standing daytime activity
2.0	**Very Light:** More movement, mainly with upper body. Equivalent to tying shoes, typing, brushing teeth
2.5	**Very Light +:** Working harder than 2.0
3.0	**Light:** Movement with upper and lower body. Equivalent to household chores
3.5	**Light +:** Working harder than 3.0; Heart rate faster, but can do this all day without difficulty
4.0	**Moderate:** Walking briskly, etc. Heart rate faster, sweating lightly, etc but comfortable
4.5	**Moderate +:** Working harder than 4.0. Heart rate noticeably faster, breathing faster
5.0	**Vigorous:** Breathing clearly faster and deeper, heart rate faster, must take occasional deep breath during sentence to carry on conversation
5.5	**Vigorous +:** Working harder than 5.0. Breathing noticeably faster and deeper, and must breath deeply more often to carry on conversation
6.0	**Heavy:** You can still talk, but breathing is so hard and deep you would prefer not to. Sweating profusely. Heart rate very high
6.5	**Heavy +:** Working harder than 6.0. You can barely talk but would prefer not to. This is about as hard as you can go, but not for long
7.0	**Exhaustive:** Can't continue this intensity long, as you are on the verge of collapse and are gasping for air. Heart rate is pounding

Begin Hour	End Hour	Activity Factor	Activity Description	Food/Drink Description	Food/Drink Amount
****Begin Example****					
12am	7am	1.0	Sleep		
7am	8am	1.5	Nothing Special	Whole Wheat Waffles (Frozen-Kellogg)	3
				Maple Syrup	2 Tablespoons
				1 % Milk	1 Cup
				Orange Juice (from concentrate)	1.5 Cups
				Coffee	2 Cups
				1 % Milk for Coffee	2 Tablespoons
10am	11am	5.0	Jog 30 minutes	Gatorade	16 Ounces
12noon	1pm	1.5	Nothing Special	Medium size beef sandwich with white	1 Sandwich
				bread, mayonnaise, lettuce, and tomato.	
				Coffee	2 Cups
				Artificial Coffee Creamer	2 Packets
				Apple Pie	1 Slice (small)
5pm	6pm	4.0	Walk 1 hour	Water	16 ounces
7pm	8pm	1.5	Nothing Special	Lasagna with ground beef and cheese	Large Plate
				Lettuce Salad with Tomatoes and Cucumbers	Medium Size Salad
				Blue Cheese Salad Dressing	1 Tablespoon
				Red Wine	1 Medium Glass
10pm	11pm	1.5	Nothing Special	Popcorn (air popped; no butter)	100 Calorie Pack
End Example					

Page _____

APPENDIX C

Hunger/Satiety Scale



1 = Famished, starving

2 = Headache, weak, cranky, low energy

3 = Want to eat now, stomach growls and feels empty

4 = Hungry - but could wait to eat, starting to feel empty but not there yet

5 = Not hungry, not full

6 = Feeling satisfied, stomach feels full and comfortable

7 = Feeling full, definitely don't need more food

8 = Uncomfortably full

9 = Stuffed, very uncomfortable

10 = Bursting, painfully full

Rate how your stomach feels before, during and after each meal or snack. Be sure to put a number to your hunger and fullness each time you eat to help you develop an understanding of eating based on your internal physical cues.

Developed by Lisa Burgoon MS, RD, LD, Sports Nutritionist, SportWell Center,
University of Illinois at Urbana - Champaign, 1998.

APPENDIX D

Table 3. Control Day Ghrelin Levels

Descriptive Statistics

Group		Mean	Std. Deviation	N
Non-Obese	ConBL	12.63748	3.412720	5
	Con30	8.64730	2.185062	5
	Con60	10.57973	5.662090	5
	Con90	9.69067	2.076221	5
Obese	ConBL	7.50721	3.497867	6
	Con30	7.23335	2.488228	6
	Con60	7.69245	3.152897	6
	Con90	7.96552	1.514791	6

Values are represented as mean ± SD

APPENDIX E

Table 4. Exercise Day Ghrelin Levels

Descriptive Statistics

Group		Mean	Std. Deviation	N
Non-Obese	ExBL	14.63738	6.127499	9
	Ex30	10.11546	4.211545	9
	Ex60	13.28626	8.775724	9
	Ex90	14.27690	7.111714	9
Obese	ExBL	10.65790	4.182061	6
	Ex30	8.73105	3.339732	6
	Ex60	9.09747	3.109702	6
	Ex90	8.57359	1.768613	6

Values are represented as mean ± SD