THE EFFECT OF AN ACUTE BOUT OF HIGH-INTENSITY INTERVAL EXERCISE COMPARED TO MODERATE-INTENSITY OR NO EXERCISE ON GROWTH HORMONE SECRETION IN OVERWEIGHT, SEDENTARY, YOUNG WOMEN

A DISSERTATION

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BY

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To the Dean of the Graduate School:

I am submitting herewith a dissertation written by Sarah Deemer entitled "The Effect of an Acute Bout of High-Intensity Interval Exercise Compared to Moderate-Intensity or No Exercise on Growth Hormone Secretion in Overweight, Sedentary, Young Women." I have examined this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Kinesiology.

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ABSTRACT

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THE EFFECT OF AN ACUTE BOUT OF HIGH-INTENSITY INTERVAL EXERCISE COMPARED TO MODERATE-INTENSITY OR NO EXERCISE ON GROWTH HORMONE SECRETION IN OVERWEIGHT, SEDENTARY, YOUNG WOMEN

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There is a strong positive relationship between exercise intensity and pulsatile GH release. It has yet to be determined if high-intensity interval exercise (HIE) can influence pulsatile GH secretion, especially overnight, which accounts for the majority of daily GH release. The purpose of this study was to determine if HIE significantly increased GH secretion compared to continuous moderate-intensity exercise (MOD), or no exercise (CON) in young women. Five young, sedentary women (mean ± SD age: 22.6 ± 1.3 years; BMI: 27.4 ± 3.1 kg/m^2; body fat: 39.2 ± 1.7%; VO_{2max}: 29.4 ± 5.7 ml/kg/min) were studied on three different occasions during the follicular phase of their menstrual cycle (CON: no exercise; MOD: 30-min of continuous cycling at 50% of peak power determined from the VO_{2max} test; and HIE: 4 30-s "all-out" sprints with 4.5 min of recovery between each sprint). Each trial was randomly assigned and separated by a minimum of one month. For each visit, participants reported to the lab at 1700 hr, exercised from 1730 – 1800 hr, and remained in the lab until 0700 hr the following morning. The overnight GH secretory profile of each trial was determined from 10-min sampling of venous blood from 1730 – 0600 hr (12.5 h) using deconvolution analysis.

Deconvolution GH parameters were log transformed prior to statistical analyses. Calculated GH AUC (0 – 120 min) was significantly greater in HIE (1018.2 ± 576.1 ng/mL/120 min) than CON (181.7 ± 138.9 ng/mL/120 min, $p = .04$), but not MOD (544.7 ± 160.7 ng/mL/120 min). Total GH secretory rate (ng/mL/12.5 h) was significantly different between CON (1040.3 ± 242.0) and HIE (1831.2 ± 873.8, $p = .05$), but MOD (1429.2 ± 206.0) was not different from CON ($p = .08$). For these untrained, overweight, sedentary young women, a single bout of exercise was insufficient to significantly affect overnight pulsatile GH secretion. Aerobic fitness, prior training, as well as several metabolic factors associated with obesity (e.g., increased insulin and circulating free fatty acids), can also influence GH secretion and should be taken into account as potential mediators of the GH response to exercise.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Overweight and obesity are world-wide problems that are associated with the development of insulin resistance, Type 2 diabetes, cardiovascular disease, and some types of cancers. More than two-thirds of U.S. adults (68.5%) were classified as overweight (BMI = 25.0 – 29.9 kg/m^2) or obese (BMI $\geq$ 30 kg/m^2), with 34.9% of all adults being classified as obese (Ogden, Carroll, Kit, & Flegal, 2014). Additionally, more than 58% of women between the ages of 20 – 39 years of age are considered overweight or obese and women in this age range have more than double the prevalence (7.7%) of grade III obesity (BMI $\geq$ 40 kg/m^2, extreme obesity) compared to men (3.5%, Ogden et al., 2014). These trends of overweight and obesity and resultant health outcomes can most likely be attributed to the emergence of what has been termed an "obesogenic" environment: large portion sizes; wide-spread availability of unhealthy, fatty foods; and physical inactivity (Lake & Townshend, 2006).

Regular physical activity is an excellent modality touted to help promote weight loss and prevent weight gain or weight re-gain after weight loss (Donnelly et al., 2009). Traditional endurance training (~45%-65% VO_{2max} for 150-300 min/week) is the current recommendation by the American College of Sports Medicine for weight loss (American College of Sports Medicine, 2014a; Donnelly et al., 2009) and thus most weight loss programs center around 30-60 min of steady-state exercise at moderate intensity on most

days of the week. However, given many people cite "lack of time" as being the main reason for not exercising (Leslie et al., 1999; Schutzer & Graves, 2004; Stutts, 2002; Trost, Owen, Bauman, Sallis, & Brown, 2002), high intensity interval exercise (HIE) may be a time-efficient and effective alternative exercise protocol for potentially reducing body mass and percent fat in overweight/obese individuals (Gibala & McGee, 2008; Gillen & Gibala, 2014). There is an approximately10% increase in total daily energy expenditure following a single HIE training session, suggesting that HIE may be an appropriate modality to help prevent weight gain (Sevits et al., 2013).

High intensity interval training (HIIT) is generally defined as a bout of exercise performed at an "all-out" (> 90% VO_{2max}) effort, followed by a recovery phase of rest or low-intensity exercise (Gibala & McGee, 2008). A single training session can include anywhere from 4 – 60 bouts of intense exercise depending upon the protocol selected. The most widely researched protocol regarding adaptations to HIIT utilizes the Wingate test: a 30-s supra-maximal cycling test (Inbar, Bar-Or, & Skinner, 1996) followed by a 4-min low-intensity recovery session, repeated 4 – 6 times. This equals a total of only 2 – 3 min of intense exercise per session with 3 training sessions completed per week (Gibala & McGee, 2008). Due to the difficult nature of the Wingate test, other researchers have used protocols that are of lower-intensity and longer duration and found similar beneficial adaptations in aerobic fitness and skeletal muscle oxidative capacity as traditional Wingate training (Boutcher, 2011).

While aerobic and skeletal muscle adaptations following HIE can be seen in as little as two weeks, longer interventions are necessary to detect actual changes in fat and

body mass. Tremblay et al. (1994) compared 20 weeks of traditional endurance training (ET) to 15 weeks of HIIT in nonobese, healthy men and women (Tremblay, Simoneau, & Bouchard, 1994). Despite total exercise energy expenditure for the intervention in the HIIT group being half that of the ET group (13,838 kcal vs 28,776 kcal, respectively), the HIIT group had a nine-fold greater loss in subcutaneous fat compared to ET (Tremblay et al., 1994). Trapp et al. (2008) documented a significant decrease in total body mass and fat mass with 15 weeks of HIIT while no change was seen within the same time frame in the moderate-intensity endurance trained or control (no exercise) groups (Trapp, Chisholm, Freund, & Boutcher, 2008). Additionally, HIIT contributed to a significant loss of abdominal fat and decreased fasting insulin concentrations (Trapp et al., 2008), demonstrating the potential for augmented health outcomes with HIIT compared to moderate-intensity steady-state training. Similarly, a 12-week program with three, 20-min HIIT sessions (alternating 8 s sprints with 12 s recovery) per week resulted in significant decreases in total body mass (2%), fat mass (6.7%), percent abdominal fat (6.6%), and percent trunk fat (8.4%) in young, overweight males (Heydari, Freund, & Boutcher, 2012). Furthermore, although there was no change in resting metabolic rate following the intervention, fat oxidation was increased by 13%, reflected by a significantly reduced RQ (Heydari et al., 2012). In a shorter duration study, 6-weeks of HIIT in a fed or fasted state resulted in decreased total body and abdominal fat as well as increased lean body mass in overweight women (Gillen, Percival, Ludzki, Tarnopolsky, & Gibala, 2013). Thus, it appears that HIIT is an effective modality for fat loss involving significantly less time

commitment from the participant while achieving similar (and sometimes better) health outcomes as traditional aerobic training programs.

The mechanisms responsible for changes in body mass and fat mass with HIE are undetermined, but may include changes in adipose tissue lipolysis through catecholamine and lipolytic hormone responses. During high-intensity exercise there are significant increases in epinephrine and norepinephrine concentrations (Boutcher, 2011; Brooks, Fahey, & Baldwin, 2005d) that are in contrast to the minor increases seen during moderate steady-state exercise (Zouhal, Jacob, Delamarche, & Gratas-Delamarche, 2008). Catecholamines, in particular epinephrine, are known to stimulate lipolysis and mobilize fat stores (Mora-Rodriguez & Coyle, 2000); and, a greater proportion of β-receptors are found in visceral adipose tissue as compared to subcutaneous adipose tissue in nonobese individuals (Rebuffé-Scrive, Andersson, Olbe, & Björntorp, 1989). Catecholamines stimulate adipose tissue triglyceride (TG) hydrolysis by activating hormone sensitive lipase (HSL), and once mobilized, circulating non-esterified fatty acids (NEFA) concentration are elevated (Lafontan et al., 1997). Plasma NEFAs provide a major source of energy under situations of stress, fasting, starvation, and exercise. Thus, the augmented catecholamine response driven by HIE may contribute to a greater loss in both total fat, and possibly visceral fat, thereby resulting in weight loss and potentially decreasing an individual's disease-risk.

The "crossover concept", a term coined by George Brooks depicts a shift from primarily fat oxidation at low-intensity exercise to carbohydrate oxidation at higher intensity exercise (Brooks, 1997; Brooks & Mercier, 1994). This shift is primarily

explained by a greater recruitment of fast-twitch muscle fibers at high-intensity exercise, increased sympathetic nervous system stimulation, as well as increased lactate production (Brooks, Fahey, & Baldwin, 2005d). Following high-intensity exercise, there is an increase in fat oxidation that may be attributed not only to increased circulating catecholamines, but also to elevated circulating GH, which displays a positive linear dose-response relationship with exercise intensity (Pritzlaff, Wideman, & Weltman, 1999). Increased fat oxidation following recovery from high intensity steady-state exercise was directly related to growth hormone release and to a lesser extent, circulating epinephrine (Pritzlaff et al., 2000).

Growth hormone has a profound influence on body composition and lipid metabolism (Johannsson, 1999; Rosen, 1999). Growth hormone responses to traditional endurance training or strength training have been widely documented, yet the effect of HIIT on growth hormone concentration has not been extensively studied. For the purposes of this paper, HIE will be defined as a bout of exercise performed at an intensity above VO_{2max} and for less than 60 s. A single bout of 30 s of maximal exercise on a treadmill resulted in elevated growth hormone concentration 10 times above baseline at 1 hr post-exercise (Nevill et al., 1996). Similarly, seven 1-min bouts of intermittent cycling at an absolute intensity of 285 W in young men significantly elevated GH concentrations above those observed during continuous cycling for 20-min at 100 W (Vanhelder, Goode, & Radomski, 1984a). Stokes et al. (2002) had healthy male volunteers complete either a 6-s or 30-s all-out sprint on a cycle ergometer and measured GH concentrations up to 3 hr post-exercise. The duration of the bout of exercise has a profound influence on the

magnitude of the GH response, such that GH was elevated approximately 217% above baseline following a 6-s sprint, while there was a 530% increase in GH after the 30-s sprint (Stokes, Nevill, Hall, & Lakomy, 2002b). Furthermore, the mean integrated AUC of GH values was significantly increased over 3 hours from the 30-s bout compared to the 6-s bout (Stokes, Nevill, Hall, & Lakomy, 2002b). Growth hormone concentrations had returned to baseline values within 60-min post-exercise following the 6-s sprint while it took almost 120 min following the 30-s sprint (Stokes, Nevill, Hall, & Lakomy, 2002b). However, the above studies only measured GH concentrations up to a few hours after exercise; it is unclear what the effect a bout of HIIT has on 12 hr GH secretion, particularly overnight, when GH pulses are greatest.

Problem Statement

Obesity prevalence remains high in the United States (Ogden et al., 2014) contributing to a decrease in worker productivity and increasing medical expenditures due to development of obesity-related chronic diseases such as type 2 diabetes and cardiovascular disease (Trogdon, Finkelstein, Hylands, Dellea, & Kamal-Bahl, 2008). Furthermore, almost 60% of women between the ages of 20 – 39 y are considered overweight or obese (Ogden et al., 2014), potentially leading to earlier development of these obesity-related diseases and incurred costs. It thus becomes paramount to examine methods which can lessen this burden and improve health and quality of life in these women.

Exercise is one of the most effective modalities for weight loss, yet many people cite “lack of time” and “lack of enjoyment” as a reason for not exercising (Leslie et al.,

1999; Stutts, 2002; Trost et al., 2002). One of the most popular trends in recent years for fitness is high-intensity interval training (HIIT) (Vanhelder, Casey, & Radomski, 1987) which is short bursts of high-intensity exercise followed by a similar period of recovery, completed in less than 30 minutes. Participants in HIIT programs seem to enjoy this type of exercise (Bartlett et al., 2011; Vanhelder et al., 1987), however, its applicability and enjoyment across a broad range of populations has yet to be determined. Furthermore, HIIT has repeatedly demonstrated to be an effective exercise modality for fat loss (Gillen et al., 2013; Heydari et al., 2012; Trapp et al., 2008; Tremblay et al., 1994). Yet the mechanisms explaining the accelerated fat loss that occurs with HIIT have yet to be determined.

The relationship between GH and exercise in humans has been widely studied, and a simple search of the literature revealed more than 2,000 peer-reviewed papers on the topic in PubMed. One of the major actions of GH is to stimulate free fatty acid (FFA) release from adipose tissue which provides an increase in substrate availability (fat) for metabolism. Patients with documented GH deficiency have increased body fat with a greater proportion of fat mass distributed in the visceral area (Rosen, 1999). Treatment with GH therapy typically results in decreased fat mass within a few months that is maintained throughout the duration of treatment (Rosen, 1999). Exercise is also a potent stimulator of GH, however, much of the existing literature regarding growth hormone secretion following exercise has been done in young, healthy males. Given the potent lipolytic effect of growth hormone, and knowing that the magnitude of exercise-induced growth hormone release is intensity-dependent, the effect of HIIT on growth hormone

secretion may have significant implications in treatment of overweight and obesity in young women.

Hypotheses

This study will examine the effect of an acute bout of high intensity interval exercise (HIE) compared to moderate-intensity exercise (MOD) or no exercise (control, CON) on 12-h growth hormone secretion and resting fat oxidation in sedentary young women. The hypotheses tested for this study will be:

1. High intensity interval exercise will result in greater total pulsatile GH secretion compared to moderate-intensity exercise or no exercise in young women.
2. High intensity interval exercise will result in a lower RER at rest 12-h post-exercise compared to moderate-intensity exercise or no exercise in young women.

Definitions

Aerobic Exercise: planned physical activity that is dynamic and rhythmic in nature and utilizes oxygen for the production of ATP from carbohydrates and fats (McArdle, Katch, & Katch, 2014)

Body Mass Index: used to measure an individual's disease risk and body size 'normalcy'; derived from a person's height (in meters) and weight (in kilograms) (McArdle et al., 2014)

$$\text{BMI} = \frac{\text{kg}}{\text{m}^2}$$

Catecholamines: organic compounds, including epinephrine, norepinephrine, and dopamine (Powers & Howley, 2012)

Deconvolution Analysis: determination of underlying hormone secretion or elimination rates (or both) from a hormone-concentration profile (Veldhuis, Keenan, & Pincus, 2008)

Epinephrine: a hormone synthesized by the adrenal medulla (Powers & Howley, 2012)

Estradiol (E_2): primary female sex steroid hormone in the family of estrogens produced in the ovaries (Deschenes & Dohi, 2005)

Follicle-Stimulating Hormone: hormone that is secreted by the anterior pituitary and is essential for follicular growth; induces estrogen secretion from the ovaries (Vanheest, Mahoney, & Rodgers, 2005)

Follicular Phase: the phase of the menstrual cycle associated with maturing follicles and elevated follicle-stimulating hormone (FSH); ends with ovulation, usually around day 10 – 18 (Vanheest et al., 2005)

Excess Post-Exercise Oxygen Consumption (EPOC): related to the replacement of creatine phosphate, lactic acid clearance and re-synthesis to glucose, and elevated body temperature, catecholamines, heart rate, and breathing (Powers & Howley, 2012)

Free Fatty Acids (FFA): a type of fat that combines with glycerol to form triglycerides; used as an energy source under aerobic conditions (Powers & Howley, 2012)

Ghrelin: a growth hormone releasing peptide secreted from the stomach, anterior pituitary, and hypothalamus that amplifies growth hormone secretion (Kraemer & Rogol, 2005)

Growth Hormone (GH): a hormone synthesized and secreted by the anterior pituitary that stimulates growth of the skeleton and soft tissues during puberty. It is also involved in the mobilization of fatty acids from adipose tissue for energy (Powers & Howley, 2012)

High Intensity Interval Training (HIIT): a mode of exercise that alternates short bursts of very high intensity exercise with periods of rest or very low intensity exercise

Hormone Sensitive Lipase (HSL): an enzyme responsible for hydrolyzing free fatty acids from glycerol; activated in response to catecholamines and inhibited in response to insulin (Brooks, Fahey, & Baldwin, 2005b)

Indirect Calorimetry: estimation of heat or energy production on the basis of oxygen consumption, carbon dioxide production, and nitrogen excretion (Powers & Howley, 2012)

Lactate/Lactic Acid: an end product of glucose metabolism in the glycolytic pathway; formed under conditions of anaerobic metabolism and in muscle fibers with few mitochondria (Powers & Howley, 2012)

Maximal Oxygen Consumption $(\dot{V}O_{2max})$: greatest rate of oxygen uptake by the body measured during severe dynamic exercise; usually on a cycle ergometer or treadmill; dependent on maximum cardiac output and the maximal arteriovenous oxygen difference (Powers & Howley, 2012)

Moderate Intensity Steady-State Exercise: aerobic exercise completed at 40 – 60% of heart rate reserve for a minimum of 30 minutes (American College of Sports Medicine, 2014a)

Negative Feedback: describes the response from a control system that reduces the size of the stimulus (Powers & Howley, 2012); considered the most prevalent form in regulation of endocrine gland secretions (Deschenes & Dohi, 2005)

Obesity: defined by a BMI greater than 30 kg/m^2 (American College of Sports Medicine, 2014a; McArdle et al., 2014)

Overweight: defined by a BMI between 25.0 and 29.9 kg/m^2 (American College of Sports Medicine, 2014a; McArdle et al., 2014)

Oxygen Deficit: refers to the lag in oxygen uptake at the beginning of exercise (Powers & Howley, 2012)

Pituitary Gland: a gland at the base of the hypothalamus of the brain having an anterior portion that produces and secretes numerous hormones that regulate other endocrine glands and a posterior portion that secretes hormones that are produced in the hypothalamus (Powers & Howley, 2012)

Respiratory Exchange Ratio: a ratio of the carbon dioxide produced (VCO_2) to the amount of oxygen consumed (VO_2) that can be used to determine substrate oxidation during steady state.

$$\mathrm{RER} = \frac{\mathrm{VCO_2}}{\mathrm{VO_2}}$$

Resting Metabolic Rate (RMR): metabolic rate measured in the supine position following a period of fasting (4 – 12 hours) and rest (4 – 8 hours) (Powers & Howley, 2012)

Somatostatin: hormone produced in the hypothalamus that inhibits growth hormone release from the anterior pituitary gland; secreted from cells in the islet of Langerhans and causes a decrease in intestinal activity (Powers & Howley, 2012)

Sympathetic Nervous System (SNS): portion of the autonomic nervous system that releases norepinephrine from its postganglionic nerve endings; epinephrine is released from the adrenal medulla (Powers & Howley, 2012)

Urea Nitrogen: a waste product of protein breakdown in the body; typically measured in blood or urine (Brooks, Fahey, & Baldwin, 2005b)

Wingate Test: a 30-second cycling anaerobic power test used to evaluate the maximal rate at which glycolysis can deliver ATP (Powers & Howley, 2012)

Assumptions

The assumptions of this study will be:

1. Women will be non-smoking, sedentary, not taking any medication for metabolic or cardiovascular disease, and not taking birth control other than oral contraceptives.
2. The participants will honestly report their menstrual cycle.
3. Participants will not change weight or actively diet or exercise during their participation in this study.
4. Participants will be fasted at least 3 hours prior to arrival at the lab for testing.
5. Participants will not exercise for 48 hours prior to their scheduled test visit in the exercise physiology lab.

Limitations

The limitations of this study will be:

1. This study will be looking at pre-menopausal, sedentary women only.
2. Results can only be applied following a single bout of exercise, and may be different or altered as a result of a long-term high-intensity interval training program

Significance of Study

Few studies have addressed the effect of HIIT on fat metabolism and potential mechanisms underlying fat loss with HIIT. Furthermore, there is a paucity of literature regarding GH secretion profiles, especially following exercise, in young, overweight and obese women; a population at risk for attenuated GH secretion, as well as developing obesity-related diseases. This study will address a unique area within the body of literature of high-intensity interval training and fat metabolism. The need for approaches to promote weight loss has become ever more pressing with the increased prevalence of obesity and metabolic disorders. Given the potent lipolytic effect of growth hormone, the effect of training intensity on pulsatile GH secretion may have significant clinical implications in the treatment of the obesity epidemic.

CHAPTER II

REVIEW OF LITERATURE

Regulation of Fat Metabolism

Lipids (fats) provide a major source of energy to the body when carbohydrate (CHO) availability is limited, such as during an overnight fast or prolonged exercise when CHO supplementation is not available. Fat is a very efficient storage form of energy, yielding approximately 9.5 $kcal \cdot g^{-1}$ compared to the 4.2 $kcal \cdot g^{-1}$ available from CHO (Brooks, Fahey, & Baldwin, 2005a). The majority of lipid found in the body is stored in the form of a triglyceride: a glycerol molecule with three fatty acid chains attached (Brooks, Fahey, & Baldwin, 2005c).

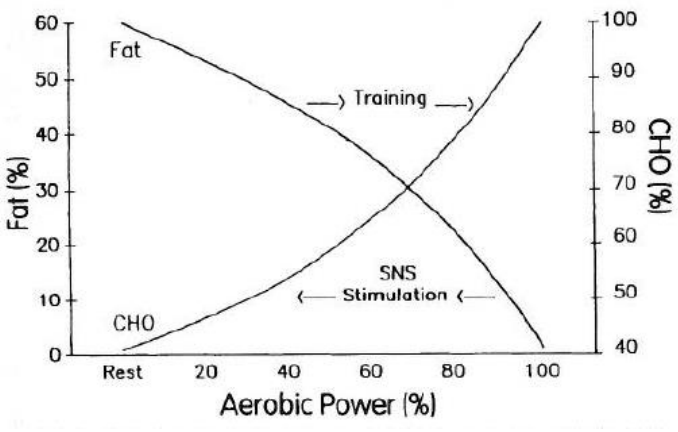

Figure 1. The Crossover Concept - carbohydrate (CHO) utilization is increased and lipid oxidation (Fat) is decreased as a function of exercise intensity. Copied from Brooks & Mercier (1994) *J Appl Physiol*, *76*(6): p. 2254.

During exercise, energy to do work is derived from four main sources: muscle glycogen, blood glucose, free fatty acids (FFA), and intramuscular triglycerides (IMTGs). It has generally been believed, and demonstrated, that exercise intensity is the primary determinant of substrate utilization. Demonstrated by the Crossover Concept (Figure 1), as exercise intensity increases, there is increased utilization of carbohydrate, with high power activities deriving all energy from carbohydrate stores (Brooks & Mercier, 1994). With exercise training, the body can increase its ability to use lipid for higher intensity work, thus shifting the crossover point to the right. These changes occur due to a decrease in sympathetic nervous system stimulation as well as other biochemical and hormonal adaptations (Brooks & Mercier, 1994; Holloszy & Coyle, 1984).

During exercise, fat is mobilized from adipose tissue stores at a relatively slow rate, a process stimulated by hormone-sensitive lipase (HSL) which breaks down lipid and TG molecules into FFA and glycerol (Brooks, Fahey, & Baldwin, 2005c). The primary factor thought to be responsible for the stimulation of lipolysis is the release of epinephrine, which activates beta-receptors on adipocytes (Mora-Rodriguez & Coyle, 2000). During low-intensity exercise, the rate of appearance of FFA in the bloodstream increases by up to five fold (Klein, Coyle, & Wolfe, 1994; Romijn et al., 1993) where they can then be transported into the mitochondria for oxidation or re-esterified. As the intensity of exercise increases, the rate of appearance of FFA in the bloodstream declines due to vasoconstriction of blood supply to adipocytes and therefore decreased albumin is available for FFA transport (Bulow, Madsen, Astrup, & Christensen, 1985). The decline in adipocyte blood flow during high-intensity exercise can most likely be attributed to the

necessity of oxygen delivery to the working muscle. Oxygen availability to the muscle is more important than FFA availability, since muscle glycogen breakdown and blood glucose can provide adequate energy substrate for a short period of time (Romijn et al., 1993).

High-Intensity Interval Exercise (HIIE)

According to recent surveys by the American College of Sports Medicine (ACSM) on Worldwide Fitness Trends, high-intensity interval training (HIIT) was at the no. 1 spot in 2014 and no. 2 spot in 2015, demonstrating that this type of exercise has become extremely popular in gyms and fitness centers worldwide (W. R. Thompson, 2014). High-intensity interval exercise (HIE) is typically characterized by short bursts (< 60 seconds) of intense exercise followed by short periods of rest or recovery (1 – 5 minutes). A HIIT session is considered "low-volume," which means that in a typical 30-min training session, approximately 10 minutes or less of that time is devoted to intense exercise, with the majority of the session composed of low-intensity exercise (e.g., warm-up, recovery in-between intervals, and cool-down) (Gillen & Gibala, 2014). The ACSM recommends that individuals interested in engaging in vigorous intensity exercise to do so at least 3 $d \cdot w^{-1}$, at 60 – 90% heart rate reserve, for 20 – 60 minutes (American College of Sports Medicine, 2014b). One of the more common research models for examining the effects of HIE uses the Wingate Test (Inbar et al., 1996), a 30-second "all-out" sprint typically done on an specialized cycle ergometer at a resistance equal to 7.5% or greater of the individual's body weight. A typical HIIT session of this type would consist of 4 – 6 sprints interspersed with 4 – 5 minutes of recovery cycling.

Regular endurance training will produce skeletal muscle adaptations that lead to improved exercise capacity as well as health outcomes (e.g., improved insulin sensitivity, decreased blood pressure). These adaptations occur in large part by increasing the body's ability to transport and utilize oxygen as well as altering substrate utilization such that lipid metabolism can occur at higher exercise intensities. In contrast, HIE would be expected to have less of an effect on oxidative metabolism and endurance capacity given its anaerobic nature. However, it has been repeatedly demonstrated that in as little as two weeks of HIIT, activity of mitochondrial enzymes is increased and improvements in aerobic fitness (VO_{2max}) and performance can be seen (Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005; Gibala & McGee, 2008; Gillen & Gibala, 2014). Six sessions of sprint interval training over a two week time period almost doubled time to exhaustion (26 min to 51 min) when cycling at 80% of the pre-training VO_{2peak} (Burgomaster et al., 2005). This improvement was accompanied by a 38% increase in citrate synthase activity and a 26% increase in resting muscle glycogen concentration (Burgomaster et al., 2005). When compared to continuous cycling at 65% VO_{2peak}, two weeks of HIIT produced similar adaptations in skeletal muscle oxidative capacity (measured by cytochrome C oxidase mRNA) and improved time trial performance despite there being a 90% reduction in training volume in the HIIT group (Gibala et al., 2006). Thus, HIE appears to be a time efficient alternative to traditional endurance training to improve endurance performance and skeletal muscle oxidative capacity.

Part of the popularity and appeal of HIE to the general population is that participants see greater and faster changes in body composition with HIIT compared to lower-intensity steady-state exercise (Boutcher, 2011). Talanian et al. (2007) used 4-min intervals at 90% VO_{2max} and saw a marked increase in whole body fat oxidation after 2-weeks of HIIE. Furthermore, enzymes associated with fat oxidation in the mitochondria (FABP, β-HAD, and citrate synthase) were increased after HIIT (Talanian, Galloway, Heigenhauser, Bonen, & Spriet, 2007). Twenty-minutes of HIE for 12 weeks resulted in significant total, abdominal, trunk, and visceral fat in overweight young males, while simultaneously increasing their fat-free mass and VO_{2max} (Heydari et al., 2012).

Specifically, HIE may contribute to a greater loss of abdominal visceral fat due to the following reasons. First, HIE will induce secretion of the lipolytic hormones, growth hormone and epinephrine (Pritzlaff et al., 1999; 2000) which could increase post-exercise fat oxidation. When matched for work output, a single bout of HIE resulted in greater growth hormone and epinephrine concentrations up to two hours post-exercise compared to moderate-intensity exercise (Peake et al., 2014). However, concentrations of non-esterified fatty acids following exercise were not different between the exercise trials (Peake et al., 2014). The lack of difference in mobilized fatty acids between exercise intensities may be a result of the intensity of HIE, this study employed 4-min intervals at 80% of measured VO_{2max}. Secondly, there is evidence to suggest that HIE may blunt appetite following an exercise bout, resulting in an overall daily negative energy balance (Thivel et al., 2012; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009; Williams et al.,

2013). However, as this response is outside the main scope of this study it will not be reviewed here.

Growth Hormone

Growth hormone (GH) is a 22-kDa protein containing 191 amino acids (Møller & Jørgensen, 2009) and is secreted from the anterior pituitary in a pulsatile fashion, playing a significant role in growth and metabolism in both humans and animals (Jansson & Dickson, 1999). Binding of GH to its receptor has acute effects on lipid metabolism, resulting in increased lipolysis and circulating free fatty acids in the bloodstream during conditions of stress (Møller & Jørgensen, 2009). The specific mechanisms of secretion and regulation of GH will be discussed in more detail below.

Mechanism of Growth Hormone Secretion

The effect of a hormone on a target tissue is proportional to its concentration within the bloodstream. While there are several other related factors, the principal determinant of a hormone's concentration within the body is: 1) the rate of secretion of the hormone into circulation, and 2) the rate of inactivation and clearance of the hormone from the bloodstream (Deschenes & Dohi, 2005). Several hormones display a circadian rhythm, or a pattern of peaks and valleys (*i.e.*, nadir) throughout a 24-hour period. In addition, hormone secretion can also display a pulsatile pattern, often referred to as an 'ultradian' rhythm that is superimposed on the circadian rhythm (Deschenes & Dohi, 2005). A growth hormone pulse is secreted approximately every 35 – 60 minutes in young healthy males (Holl, Hartman, Veldhuis, Taylor, & Thorner, 1991).

The pituitary gland is considered to be the master endocrine gland, and is divided into anterior and posterior sections. The posterior pituitary gland is responsible for oxytocin and vasopressin (anti-diuretic hormone) secretion; while the anterior pituitary is mainly under the control of the hypothalamus and is a collection of various endocrine cell types that make up the hypothalamic-pituitary-adrenal axis (Carmean, Cohen, & Brady, 2014). The major regulators of growth hormone secretion are: growth hormone releasing hormone (GHRH, stimulatory), somatostatin (SRIF, inhibitory), and ghrelin (stimulatory) (Figure 2) (Anderson & Scanes, 2012).

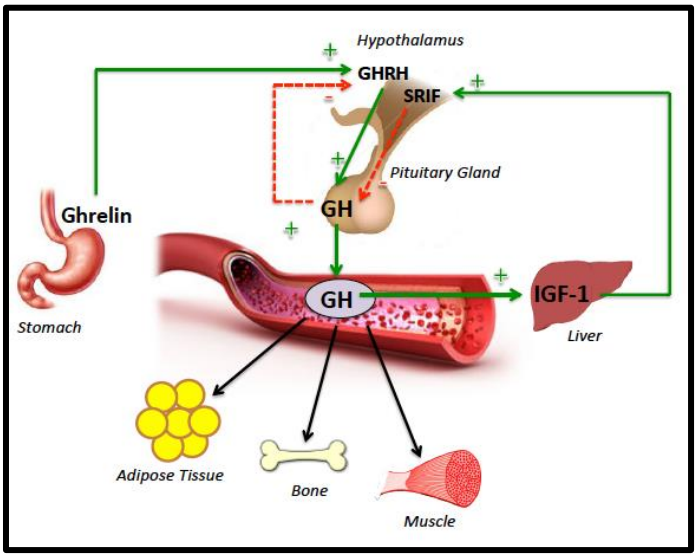

Figure 2. Growth hormone has a multitude of effects within the body, influencing growth and metabolism in adipose tissue, bone, and skeletal muscle. Somatostatin (SRIF), Insulin-like growth factor-1 (IGF-1).

Growth hormone is produced by somatotrophs, which are found in the anterior pituitary. Somatotrophs are slow secreting cells which, when stimulated, have the ability to transiently fuse to the cell membrane (Anderson & Scanes, 2012). The signaling cascade begins when GHRH is released from the neurosecretory terminals found in the

median eminence of the hypothalamus (Frohman & Kineman, 2010). Following binding to a G_s-protein-coupled receptor (GPCR), GHRH stimulates the following (Figure 3) (Frohman & Kineman, 2010; Lobie, 1999):

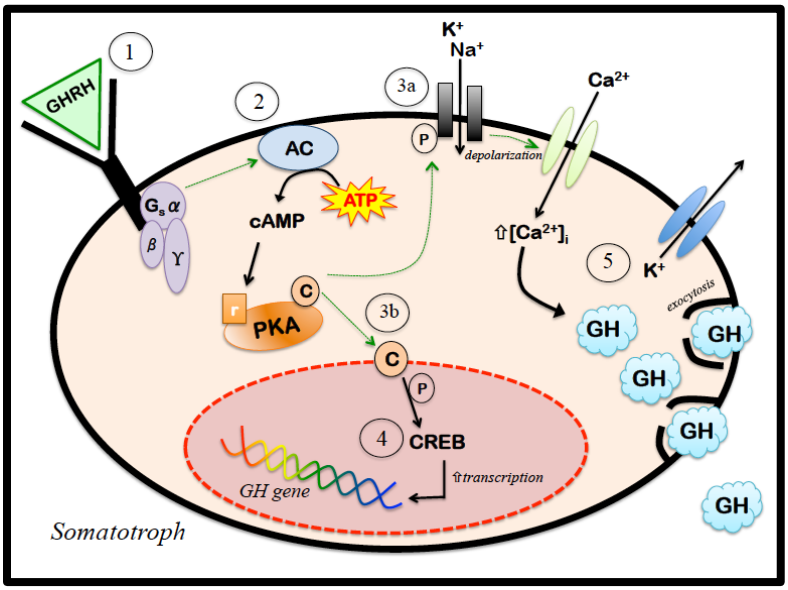

Figure 3. GH signaling cascade. Abbreviations: growth hormone releasing hormone (GHRH), adenylate cyclase (AC), protein kinase A (PKA), cAMP-response-element-binding protein (CREB), growth hormone (GH), catalytic subunit (c), regulatory subunit (r), phosphorylated (p).

1. There is a conformational change in the GPCR causing the α-subunit to dissociate from the β- and ϒ- subunits and become active.
2. Activation of G_s-α stimulates adenylate cyclase (AC) to drive the conversion of ATP to cAMP, thereby raising cAMP levels within the cell.
3. Increased cAMP bind to the regulatory subunits (r) on the enzyme protein kinase A (PKA) resulting in a dissociation of the catalytic subunits (c). It has been demonstrated that PKA activity is *critical* to GH signaling, as blockade of PKA activity inhibits GHRH-induced GH release (A. O. L. Wong, Moor, Hawkins, Narayanan, & Kraicer, 1995).
    a. Activation of the catalytic subunit of PKA phosphorylates monovalent ion channels (Na^+) leading to depolarization of the plasma membrane and opening of L- and T-type voltage-gated Ca^{2+} channels.
    b. The catalytic subunit from PKA can also translocate to the nucleus and phosphorylate the nuclear cAMP-response-element-binding (CREB) protein.
4. CREB binds to cAMP-response-elements (CREs) located within the promoter regions on the GH gene, increasing transcription.
5. An increase in intracellular calcium (Ca^{2+}) leads to GH release through exocytosis.

Mechanistically, it is thought that the intracellular GH secretory granules fuse (or dock) with 'pits' (*i.e.*, porosomes) located on the cell membrane in response to rising intracellular Ca^{2+} concentrations (Anderson & Scanes, 2012). This results in SNAP/SNARE-mediated fusion of the secretory vesicles (somatotrophs) with the porosomes and subsequent exocytosis of GH into the circulation (Anderson & Scanes, 2012).

Additionally, some neurotransmitters and hormones (e.g., glucocorticoids, catecholamines) can act directly on the anterior pituitary, affecting GH release (Giustina & Veldhuis, 1998). The intestinal-secreted hormone, ghrelin, has been found to augment GH secretion by hypothalamic stimulation of GHRH (Anderson & Scanes, 2012) as well as pituitary GH release (Melmed & Kleinberg, 2003). Somatostatin, which is released from the hypothalamus, is the major counter-regulatory hormone that inhibits GH release (Anderson & Scanes, 2012; Giustina & Veldhuis, 1998; Jansson & Dickson, 1999). Somatostatin appears to exert its inhibitory effects on GH release by elevating intracellular $[K^+]$ and suppressing Ca^{2+} influx (Anderson & Scanes, 2012; Carmean et al., 2014). Another major inhibitor of GH secretion is insulin-like growth factor-1 (IGF-1), which is produced in the liver (Anderson & Scanes, 2012; Jansson & Dickson, 1999); it appears that IGF-1 stimulates SRIF, thereby indirectly inhibiting GH release (Clemmons, 1999; Jansson & Dickson, 1999).

Growth hormone secretion per unit surface area ranges from less than 60 $\mu g/m^2$ in elderly individuals to more than 1200 $\mu g/m^2$ in adolescents entering puberty (Giustina & Veldhuis, 1998). As mentioned above, GH secretion is pulsatile, and the frequency of GH

pulses is about 14–18 per day, independent of gender, hormonal milieu, fitness level, or age (Veldhuis & Bowers, 2003). A given pulse will range in size, with larger pulses (more mass) secreted during sleep compared to waking hours (Veldhuis & Bowers, 2003). On average, the plasma half-life of GH is 14–18 minutes, with the extreme range being 4–25 min (Veldhuis & Bowers, 2003). Several variables are known to influence the magnitude of GH secretion, including: sleep time, menstrual cycle, age, gender, body composition, and exercise.

Regulation of Growth Hormone Secretion: Sleep

There are several factors that regulate GH secretion. The majority of daily GH secretion (~70%) occurs during the first wave of deep sleep (e.g., stages 3 and 4 of non-REM sleep) (Van Cauter, Plat, & Copinschi, 1998). Twenty-four hour GH secretory profiles decline by almost 75% from young-life (16–25 years old) to midlife (36–50 years old) independent of changes in BMI (Van Cauter, 2000). It has also been demonstrated that sleep time declines by about 27 min per decade following mid-life (50 years old) (Van Cauter, 2000); and there may be an important link between sleep time and GH secretion. Individuals with increased amounts of deep sleep had higher GH concentrations, in both young and middle-aged men (Van Cauter, 2000).

Fewer studies have examined the association between sleep and GH secretion in women, and thus a clear relationship between these two variables has not been defined. One study which examined the association between GH and deep sleep in pre-menopausal (~48 years old) and post-menopausal (~63 year old) women, concluded that sleep stages did not differ between the two groups, even when hormone replacement

therapy (HRT) was provided to the post-menopausal women (Kalleinen et al., 2012). Although GH secretion and number of peaks did not differ between pre- and post-menopausal women, the first GH surge occurred, on average, about 45 minutes later in post-menopausal women compared to pre-menopausal women (Kalleinen et al., 2012). Clearly, further research needs to be done to assess the relationship between sleep and subsequent GH secretion in women, and the influence that menopause and hormonal changes may have on this response.

Regulation of Growth Hormone Secretion: Menstrual Cycle

The hormonal fluctuations that occur during the menstrual cycle in a normal, healthy woman are described (Bulun & Adashi, 2003). Follicle-stimulating hormone (FSH) concentration is elevated immediately prior to and during menses. As the endometrium thickens, there is a surge in estradiol (E_2) that triggers ovulation. Immediately following the E_2 surge, luteinizing hormone (LH) concentration increases, causing release of the egg and formation of the corpus luteum. After ovulation, progesterone increases, along with a second rise in E_2, characterizing the luteal phase. During day 14–28, FSH and LH levels continue to drop. If pregnancy does not occur, the corpus luteum will begin to degrade in conjunction with falling concentrations of progesterone and E_2 which signals the end of the luteal phase; this releases the inhibition of these hormones on FSH, thereby initiating a new cycle, and the beginning of the new follicular phase (Bulun & Adashi, 2003).

Growth hormone concentration doubles during the late follicular phase (2–5 days prior to the LH surge), in conjunction with rising estradiol concentration (Faria et al.,

1992; Ovesen et al., 1998; Yen, Vela, Rankin, & Littell, 1970). Pre-menopausal women have higher GH secretion rates compared to post-menopausal women, and this has been attributed to estradiol (E_2) concentration (Veldhuis, 1995; Veldhuis & Bowers, 2003). Post-menopausal women treated with oral E_2 had an approximate 45% increase in 24-h mean GH concentration after 15 days (Dawson-Hughes, Stern, Goldman, & Reichlin, 1986), and 3-weeks of oral E_2 increased serum GH in post-menopausal women (Duursma, Bijlsma, Van Paassen, van Buul-Offers, & Skottner-Lundin, 1984). Pubertal girls were found to have a positive correlation between GH and E_2 concentrations ($\tau = 0.59 – 0.71$) (Wennink et al., 1991); and women with ovariectomies had reductions in GHRH (growth-hormone releasing hormone), the pre-cursor to GH secretion (De Leo, Lanzetta, D'Antona, & Danero, 1993). It is unknown if estrogen's effect on GH concentration is a primary effect (direct action on the hypothalamus and/or the anterior pituitary) or a secondary effect by inhibiting IGF-1 production (Ovesen et al., 1998). In 1976, Wiedemann et al. demonstrated that estrogen increased GH secretion by decreasing IGF-1, which negatively feedbacks to inhibit GH secretion (Wiedemann, Schwartz, & Frantz, 1976). However, this effect appears to be dose dependent, as low doses of estrogen, or estrogen delivered transdermally appear to have a positive (stimulatory) effect on IGF-1 (Veldhuis, 1995).

Regulation of Growth Hormone Secretion: Age

Age appears to have a negative influence on the half-life of GH. Circulating GH concentrations begin to decline after puberty and continue to decline with senescence. Growth hormone secretory bursts in young healthy men (~25 year old) were significantly

more prominent compared to middle-aged (~47 year old) and older (~66 year old) men. Additionally, the time between each GH burst was significantly increased with age from approximately 83 minutes in young men to 143 minutes in the old men (65+ years) (Iranmanesh, Lizarralde, & Veldhuis, 1991). Furthermore, the half-life of GH was found to decrease in healthy men, from approximately 23 minutes in young men to ~15 minutes in old men (65+ years) (Iranmanesh et al., 1991). Regression analysis estimated, from these men, that GH secretion rate falls by about 14% per decade of life after age 25 (Iranmanesh et al., 1991). However, Vestergaard et al. (2014) found age to not be a significant stimulant of GH signaling, and suggested that the decline in GH associated with aging is potentially mediated by an extrinsic property (e.g., body composition, physical activity, or dietary habits) (Vestergaard et al., 2014).

Regulation of Growth Hormone Secretion: Gender

Premenopausal women have higher GH concentrations than similarly aged males (Ovesen et al., 1998; Pincus et al., 1996). Estradiol has been found to be a positive correlate of GH release in a cross-sectional study comprised of both men and women (Ho et al., 1987). However, estradiol negatively influences daytime basal GH secretion in men, while having a positive effect on pulsatile secretion (Veldhuis et al., 1995). Testosterone has been shown to positively control both basal and pulsatile GH secretion (Iranmanesh et al., 1991; Ulloa-Aguirre et al., 1990; Veldhuis et al., 1995), and higher serum testosterone concentrations are associated with greater time-coordinated consistency of GH secretion (Veldhuis et al., 1995). Thus, it has been hypothesized that testosterone's agonist effect on the GH axis may be mediated by the aromatization of

androgens to estrogen in men (Veldhuis et al., 1995), which may explain the decline in GH secretion with age and body fat (aromatase is stored in fat cells) in men.

Women, regardless of age, have greater GH secretion irregularity (also known as approximate entropy, ApEn) than males of comparable ages (Pincus et al., 1996; Veldhuis & Bowers, 2003). Young women (23-25 years) in the early follicular phase of their menstrual cycle had significantly greater ApEn values and mean circulating GH concentration compared to similarly aged males (22-28 years) (Pincus et al., 1996). Women also had greater ApEn values than similarly aged men when previously collected GH data (van den Berg, Frölich, Veldhuis, & Roelfsema, 1994) was re-analyzed by Pincus et al. (1996); and in normal rats an identical response was measured (Pincus et al., 1996). During both dark and light cycles in the rats, female rats had higher GH concentration and greater ApEn than males. In fact, this response showed 100% specificity and sensitivity as during the dark period all female rats had greater ApEn than male rats, and only one female rat's ApEn value dropped into the male ApEn range during the light period (Pincus et al., 1996). Thus it appears this response is highly conserved, at least in mammals who are normal fed and normal weight. The difference in GH release pattern between men and women appears to relate to sex steroid hormones.

In fasted adults, there is no gender difference in ApEn, which may be attributed to a withdrawal of SRIF while GHRH secretion remains preserved and possibly amplified. Thus, the difference in GH secretion between genders may be dependent on SRIF control and how estrogen (or testosterone) subsequently regulates its release (Pincus et al., 1996). Both hypothalamic mRNA and peptide concentrations of SRIF are higher in male

compared to female mice (Srikant, 2004), suggesting that hypothalamic signaling of SRIF to somatotrophs plays an important role in the sexual diergic pattern of GH secretion. In a rodent model, testosterone significantly up-regulated SRIF mRNA (Chowen-Breed, Steiner, & Clifton, 1989), and castration of male rats with 17-β-estradiol "feminized" the GH secretory profile, while treating female rats with testosterone "masculinized" GH secretion (Painson, Thorner, Krieg, & Tannenbaum, 1992; Painson, Veldhuis, & Tannenbaum, 2000). Thus it is highly probable that SRIF is a primary factor in gender differences of GH secretion.

To examine the effect of age and gender on intracellular GH signaling *in vivo*, young (~ 25 years, n = 10) and old (~ 65 years, n = 10) males and females were given a 0.5 mg intravenous bolus of GH followed by both muscle and adipose tissue biopsies at 30 and 120 min post-bolus. Females, regardless of age, had significantly greater transcription of GH-dependent genes following the GH bolus, as a result of induction of pSTAT5B (phosphorylated Signal Transducer And Activator Of Transcription factor 5B) (Vestergaard et al., 2014). Interestingly, women in this study had higher concentrations of cytokine-inducible SH2 protein (*CISH*) which acts as a feedback inhibitor of GH (Vestergaard et al., 2014). The authors suggest that the elevated *CISH* levels in the female participants may be induced by the continuous elevation of endogenous GH levels and therefore a result of 'chronic' GH exposure (Vestergaard et al., 2014).

Regulation of Growth Hormone Secretion: Body Composition

Additionally, obesity is a negative determinant on GH secretion. In a small group of older men, body composition measured by hydrodensitometry was negatively

correlated with both GH secretion rate and secretory burst amplitude (unpublished observations) (Iranmanesh et al., 1991). In the same group of men from above, it was estimated that for a 20 year old man, each unit increase (1 kg/m^2) in BMI would decrease GH secretion by 5.6% (Iranmanesh et al., 1991). This can be translated into as much as a 50% decrease in GH secretion if BMI were to increase from 21 kg/m^2 to 28 kg/m^2 (Iranmanesh et al., 1991). It was subsequently identified, using an ultrasensitive GH assay, that the primary neuroendocrine mechanism linking the relationship between increasing body fat and decreasing GH concentration is attributed to a decrease in the mass of an individual GH secretory burst (Veldhuis et al., 1995). Further, obese men had greater secretion irregularity (ApEn) suggesting that obesity may contribute to a loss of somatostatinergic tone and/or decreased activity of hypothalamic GHRH (Veldhuis et al., 1995).

Visceral obesity is found to have similar effects as estrogen – reducing GH half-life by increasing the rate of removal from the plasma (Langendonk et al., 2001; Pijl et al., 2001; Schaefer et al., 1996; Shah, Aloi, Evans, & Veldhuis, 1999; Vahl et al., 1997a). Although not statistically significant, young, pre-menopausal women with upper body obesity (defined as a waist:hip ratio >0.89) had reduced GH secretion rate estimated by deconvolution analysis compared to women with lower body obesity (waist:hip ratio < 0.81), however, upper body obesity had significantly reduced GH secretion compared to normal weight controls (Langendonk et al., 2001). In fact, the peak stimulated GH concentration is reduced by 1 μg/L for each 1 cm increase in waist circumference (Grinspoon, 2009). Possible mechanisms for decreased GH secretion with increasing

adiposity are: a) increased somatostatin; b) diminished amount and/or decreased efficacy of GHRH or other GH secretagogues; c) attenuated pituitary responsiveness (Iranmanesh et al., 1991). Weight loss can partially restore impaired GH secretion from obesity (Rasmussen et al., 1995), however this was determined from an average 30 kg weight loss in 9 obese individuals, which is an atypical response to diet-induced weight loss. Additionally, treatment with GH-releasing hormone (GHRH) provides a means to reverse reduced basal and pulsatile GH secretion, increasing endogenous basal and pulsatile GH secretion without altering the pulse frequency (Stanley & Grinspoon, 2015).

Regulation of Growth Hormone Secretion: Response to Exercise

Exercise is one of the most potent 'controllable' mediators of growth hormone secretion, and although the exact mechanism regarding how this occurs is still unknown, it is most likely a combination of: 1) neural stimulation, 2) feedback from release of IGF-1 and IGF-2, 3) catecholamine stimulation, 4) nitric oxide release, and 5) lactate accumulation and changes in acid-base balance (Godfrey, Madgwick, & Whyte, 2003).

When discussing the response of GH to a given exercise stimulus, it is important to also address the type of exercise used to cause the response. The magnitude of the GH response to an exercise bout is dependent upon the type of exercise as well as the amount of muscle mass recruited to complete the exercise (Kraemer & Ratamess, 2005). Furthermore, the muscle action (concentric *vs.* eccentric) (R. J. Durand et al., 2003), intensity (Ahtiainen, Pakarinen, Kraemer, & Häkkinen, 2003; Vanhelder, Radomski, & Goode, 1984b), volume (Gotshalk et al., 1997), rest intervals (Kraemer et al., 1991; 1990), and training status of the individual play an integral role in the GH response

(Kanaley et al., 1997; Kraemer & Ratamess, 2005). There is a plethora of literature devoted to resistance training and GH response; however, it will not be reviewed here as this is outside of the scope of this literature review and study design.

Moderate-intensity aerobic. Exercise that is less than 50% of an individuals' lactate threshold has little to no stimulus on GH secretion (Felsing, Brasel, & Cooper, 1992). Twenty minutes of continuous cycling exercise at 100 W elicited no elevated GH response in young men who regularly participated in a twice weekly exercise program (Vanhelder, Goode, & Radomski, 1984a). Similarly, a minimal GH response was observed in male participants who completed 30-min of treadmill exercise at 25% and 75% of the difference between $\dot{V}O_2$ at rest and $\dot{V}O_2$ at the lactate threshold (0.25LT and 0.75LT, respectively) (Pritzlaff et al., 2000). Using a similar study design, Weltman et al. (2000) found that following the 0.25LT exercise trial, peak GH concentration, measured 24 minutes after the end of exercise, was not significantly different from control GH concentrations (A. Weltman et al., 2000). Conversely, Luger et al. (1992) demonstrated in both highly trained, moderately trained, and untrained males, that exercise at 50% of $\dot{V}O_{2max}$ resulted in a significant increase in GH above baseline (Luger et al., 1992). Interestingly, this increase occurred despite no elevation of lactate at this exercise intensity (Luger et al., 1992). However, most studies have reported that a minimum threshold intensity of exercise (typically greater than LT) is necessary before a significant rise in GH concentration is detected (A. Weltman et al., 1992).

High-intensity aerobic. The actual measurement and identification of a threshold intensity for GH secretion has yet to be determined; however, it is generally accepted that

protocols that elicit high blood lactate values tend to produce the most substantial GH responses. Exercise at 50% of the difference between lactate threshold and $\dot{V}O_{2max}$ has demonstrated a GH response (Felsing et al., 1992). Exercise at intensities between lactate threshold (LT) and $\dot{V}O_{2max}$ (1.25LT and 1.75LT) elicited a large increase in peak GH measured, such that, over the 5 exercise trials (including 0.25LT, 0.75LT, and LT trial), peak GH concentration linearly increased with exercise intensity (Pritzlaff et al., 2000; A. Weltman et al., 2000).

Repeated bouts of aerobic exercise at 70% of $\dot{V}O_{2max}$ significantly increased daytime serum GH concentrations by approximately 150% - 160% without altering overnight GH secretion (Kanaley et al., 1997). Compared to the control day, this resulted in an approximate 60% increase in 24-hour GH concentration, as there was no change in overnight GH secretion (Kanaley et al., 1997). Due to the persistent increase in GH regardless of exercise rest time, Kanaley et al. (1997) suggested that exercise of a sufficient intensity to increase GH concentrations might also override the ability for GH auto-negative feedback. As mentioned previously, growth hormone stimulates the production of IGF-1 and IGF-2 in the liver. IGFs can inhibit GH release through negative feedback by acting directly on the anterior pituitary (direct inhibition of GH release) or by acting on the hypothalamus to reduce secretion of GHRH. It is therefore possible to surmise that the exercise stimulus, when of a sufficient intensity to stimulate GH secretion, is more potent than the inhibitory feedback mechanisms and thereby GH secretion is maintained or increased.

High-intensity (sprint) exercise (HIE). For the purpose of this review, high-intensity exercise (HIE) will be defined as exercise that can be sustained for only a short period of time (< 60 seconds). Seven, 1-min bouts of cycling exercise at 285 W, followed by 2 min of rest resulted in a significant elevation of GH that remained substantially elevated for 40-min after the end of exercise (Vanhelder, Goode, & Radomski, 1984a). Trained male and female sprinters had a significantly greater and prolonged GH response to a single 30-second non-motorized treadmill sprint compared to endurance-trained (ET) athletes, such that the peak GH response was 3 times greater for sprinters compared to ET (Nevill et al., 1996). However, both absolute and relative power output measured during the sprint was higher in sprinters compared to ET (Nevill et al., 1996); which, when considering that the GH response to exercise is intensity-dependent (Pritzlaff et al., 1999), may account for the difference in peak GH concentrations. In order to examine the effect of pedal rate on GH response to sprint exercise, male volunteers completed two 30-sec sprints (separated by 1-hr of passive recovery) against a resistance of 7.5% (fast trial) or 10% (slow trial) of their body mass (Stokes, Nevill, Hall, & Lakomy, 2002a). Blood lactate concentration and pH did not differ between the fast and slow trials or between the first and second sprints, yet, peak GH concentration (during the first sprint) for the fast trial was more than twice that from the slow trial (Stokes, Nevill, Hall, & Lakomy, 2002a). Furthermore, although it was not a significant difference, the GH area under the curve (AUC) tended to be lower following the second sprint, regardless of pedal rate (Stokes, Nevill, Hall, & Lakomy, 2002a). Given the amount of recovery time between the first and second sprints (1 hour), it is possible that negative-feedback mechanisms

resulted in the blunted GH response to the second sprint. Indeed, in a subsequent study, Stokes et al. (2005) demonstrated that a 30-sec sprint at a resistance of 7.5% of body mass resulted in no further GH increase in a second sprint completed 60-min after the first sprint, similar to results from the previous study (Stokes, Nevill, Frystyk, Lakomy, & Hall, 2005; Stokes, Nevill, Hall, & Lakomy, 2002a). However, when 240-min separated the two sprints, GH AUC was similar between sprint 1 and sprint 2, and GH had returned to baseline well before the start of the second sprint (Stokes et al., 2005). This study reiterated the concept of GH autoinhibition, but whether or not autoinhibition occurs during shorter bouts of recovery (e.g., 3-4 minutes between sprints), has yet to be determined.

The duration of sprint exercise also plays an important role in the magnitude of the GH response. Nine men completed either a 6-second or 30-second all-out sprint on a cycle ergometer against a resistance equal to 7.5% of body mass, after which blood samples were measured for 4 hours during rest (Stokes, Nevill, Hall, & Lakomy, 2002b). The highest measured mean GH concentration was ~450% greater following the 30-sec sprint compared to the 6-sec sprint, and GH concentrations remained elevated for almost twice as long during recovery after the 30-sec sprint compared to the 6-sec sprint (Stokes, Nevill, Hall, & Lakomy, 2002b). The highest blood lactate concentration measured during the 30-sec sprint was also approximately three times greater than that measured during the 6-sec sprint (11.8 mmol/L vs. 3.6 mmol/L) (Stokes, Nevill, Hall, & Lakomy, 2002b). As discussed below, the lactate and acid load build up in exercising muscle, may be an important mediator in GH release during exercise.

Proposed Mechanisms that Regulate Growth Hormone Response During Exercise

Because of the disparity within the literature regarding GH and exercise response, the mechanisms that regulate GH secretion are not yet fully understood. However, there are a number of suggested possibilities, which are discussed in detail below.

Neural stimuli. The production and secretion of GH from the anterior pituitary is highly complex and although the 22-kDa GH molecule comprises the majority of GH secreted (~43%), several other isoforms have been discovered that have variable specialized responses (Hymer, Grindeland, Nindl, & Kraemer, 2005). Growth hormone molecular size can vary from as small as a 5-kDa molecule to greater than 100-kDa in size (Gosselink et al., 2000; 1998; Hymer et al., 2005). However, several of these isoforms are unidentifiable through traditional immunoassay mechanisms, but the effect of these isoforms can be quantified by bioassays. The most common bioassay utilized to identify GH effects that are un-measureable in serum is the tibial line bioassay. This procedure is based upon the premise that the width (or growth) of the epiphyseal plate is proportional to the dose of GH injected in hypophysectomized rats (Figure 4) (Greenspan, Li, Simpson, & Evans, 1949). Bioassayable GH (bGH) values are reported in the hundreds to thousands of ng/mL because bGH represents biological activity and not a measured amount of (purified) hormone (Hymer et al., 2005). Values are represented as the measured response (biological potency) compared to a standard 22-kDa GH value (i.e., in rats: 3.0 U/mg; in humans 3.0 IU/mg) (Gosselink et al., 1998; Hymer et al., 2005).

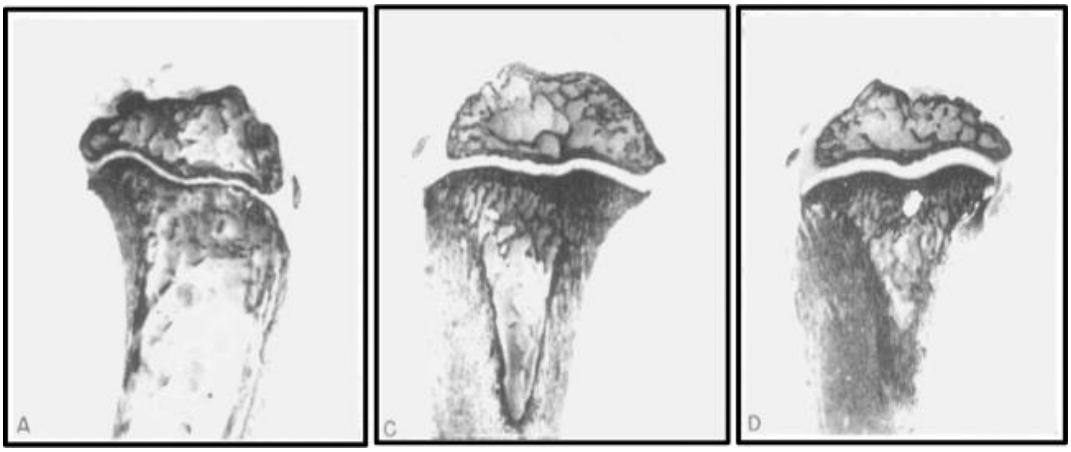

Figure 4. Tibial line bioassay. The effect of growth hormone dose on the epiphyseal plate in hypophysectomized female rats. Intraperitoneal injections of saline (A), 16 μg (C) or 120 μg (D) of GH were given for 4 days, after which the rats were sacrificed. The tibia was dissected and stained, and the width of the epiphyseal cartilage was measured using a low power microscope with micrometer. As is evident in this figure, the growth of the epiphyseal cartilage plate was greater with increased dose of GH (Greenspan et al., 1949).

Severed rat fast-twitch hind-limb nerves were electrically stimulated at the proximal nerve trunk for 15 min at an intensity equivalent to rat treadmill running at 40 m/min (~1.5 mph) (Gosselink et al., 1998). Stimulation of the proximal end of the tibial nerve (fast twitch ankle extensors) and peroneal nerve (fast twitch ankle flexors) increased bGH concentrations in plasma by 254% and 210%, respectively; while decreasing pituitary bGH by 57% and 66%, respectively (Gosselink et al., 1998). Plasma immunoassayable GH (iGH) concentrations remained unchanged (Gosselink et al., 1998). Stimulation of the proximal or distal end of the sural nerve (cutaneous innervation) had no effect on bGH or iGH (Gosselink et al., 1998). Additionally, electrical stimulation of the proximal soleus nerve (slow twitch soleus muscle) decreased plasma bGH by 63% and increased pituitary bGH by 34%, while iGH concentrations remained unchanged (Gosselink et al., 2000). Together, these data present evidence that fast-twitch afferent

input can stimulate bGH release, while slow-twitch innervation appears to inhibit bGH release. Because the stimulation was of severed nerves from the muscle, the results are independent of the influence of muscle metabolites, and thus the response of bGH release from the anterior pituitary is mediated by a neural mechanism. Fifteen minutes of treadmill running at 27 m/min (~1 mph) in rats resulted in a 300% increase in plasma bGH and a 50% decrease in pituitary bGH compared to control rats (no exercise) (Bigbee, Gosselink, Roy, Grindeland, & Edgerton, 2000). Again, plasma concentrations of iGH remained unchanged (Bigbee et al., 2000). Stimulation of the human tibialis anterior by 10-min of vibration (100 Hz) elevated bGH 94%, while there was a 22% decrease in bGH after 10-min of vibration placed on the soleus muscle (McCall, Grindeland, Roy, & Edgerton, 2000). Similar to rat studies, there was no change in iGH (McCall et al., 2000). Thus, it is clear that there is a neural mechanism responsible for GH release. The afferent fibers stimulated in the rat studies above were type I and type II afferent fibers, originating from muscle spindles and/or Golgi tendon organs. Therefore, it could be hypothesized, that increased muscle fiber tension (such as that occurring during higher intensity exercise) may increase muscle spindle activity. This could result in an increased afferent signal to the pituitary, and ultimately enhanced GH secretion.

Catecholamine stimulation. Catecholamines directly stimulate GH secretion from murine pituitary tissue *in vitro* (Giustina & Veldhuis, 1998). Catecholamines are stress hormones that are released to help activate the behavioral and physiological process of overcoming the particular stress (e.g., exercise). In short, stress stimulates afferent nerves that activate catecholamine and other spinal and medullary neurons. This

in turn activates the limbic system, cerebral cortex, and hypothalamus in the brain. There are then two mechanisms by which hormonal release occurs: 1) the hypothalamic pituitary axis (HPA) which releases adrenocorticotropic hormone, which then stimulates cortisol release from the adrenal cortex, and 2) activation of the sympathetic nervous system and adrenal medulla which release norepinephrine (NE) and epinephrine (E) (Tank & Wong, 2015). In general, the HPA axis activation is considered to be a "long-term" stress response, while sympathetic activation mediates the "short-term" and more immediate responses (Tank & Wong, 2015).

Blood concentrations of NE and E ([NE] and [E]) have been shown to increase with progressing exercise intensity (A. Weltman et al., 2000; 1994). Endurance-trained male athletes were asked to cycle to exhaustion while venous samples of GH, lactate [La^-], NE, and E were taken after each 3-min stage (Chwalbinska-Moneta, Krysztofiak, Ziemba, Nazar, & Kaciuba-Uścił ko, 1996). Interestingly, GH, NE, and E changed minimally during low-intensity stages, but began rising abruptly at the workload associated with the lactate threshold (Chwalbinska-Moneta et al., 1996). Treadmill sprinting at 156% of the speed achieved at $\dot{V}O_{2max}$, (anaerobic exercise) until exhaustion (mean time = 1.5 min) resulted in a mean maximum lactate value of 16.3 mmol/L, pH dropped to a minimum value of 7.11, and NE and E increased 15-fold from pre-exercise values (Kindermann et al., 1982). Participants also completed an aerobic exercise trial which was 50 min of continuous running at anaerobic threshold (workload = ~75% $\dot{V}O_{2max}$ and blood lactate = 4.0 mmol/L) (Kindermann et al., 1982). Lactate concentration remained relatively constant during aerobic exercise, while [NE] increased

~7-fold and [E] increased ~3.5-fold. There was only a slight increase in GH during anaerobic exercise which occurred during the post-exercise period, while aerobic exercise resulted in a 14-fold increase in GH (Kindermann et al., 1982). However, it is very possible that the low GH concentrations observed in this study during anaerobic exercise are a result of the short measurement time period (1.5 min of exercise + 6 min of recovery vs. 50 min of exercise + 6 min recovery). Nonetheless, although the relationship between catecholamines and GH is not well defined in this study, it is clear that anaerobic exercise is sufficient stressor that can elicit a substantial sympathetic response.

To test the hypothesis that sympathetic activity is an important mediator of GH secretion during exercise, ten healthy males completed 5 30-min exercise trials and one control (rest) trial (A. Weltman et al., 2000). Participants exercised at the following intensities in random order: 25% and 75% of the difference between $\dot{V}O_2$ at rest and $\dot{V}O_2$ at the lactate threshold (0.25LT and 0.75 LT, respectively), lactate threshold (LT), and 25% and 75% of the difference between $\dot{V}O_2$ at LT and $\dot{V}O_{2max}$ (1.25LT and 1.75LT, respectively). Peak GH, NE, and E concentrations increased with increasing exercise intensity, such that exercise at 1.75LT elicited the maximum GH, NE, and E response (A. Weltman et al., 2000). Furthermore, peak [NE] and [E] were achieved 20-min after the onset of exercise, regardless of intensity, in all participants; and this always preceded the peak GH concentration, which occurred after the cessation of exercise (A. Weltman et al., 2000). At the lowest exercise intensity (0.25LT), there was a significant time delay between peak catecholamine concentrations and peak GH concentration, however, GH response at this intensity did not differ from the control day (rest) (A. Weltman et al.,

2000). Regression analyses revealed that NE was primarily responsible for the intensity-dependent increase in GH, rather than E (A. Weltman et al., 2000). Thus, it appears that central adrenergic activation as a result of exercise may drive GH secretion.

Lactate and acid-base balance. More than fifty years ago an association was made between blood lactate and GH concentrations (Sutton, Young, Lazarus, Hickie, & Maksvytis, 1969), yet results showing a direct cause and effect response between lactate levels and GH concentrations have been disparate. Young men completed two bouts of cycling exercise during which individuals cycled continuously for 40 min (CE), or cycled intermittently for 40-min (IE): 1-min at double the workload of CE, followed by 1-min of rest, resulting in 20-min of exercise (Karagiorgos, Garcia, & Brooks, 1979). Although GH concentrations increased similarly between the two cycling bouts of equal external work (CE and IE), lactate concentrations were significantly increased during the intermittent exercise trial compared to the continuous exercise trial (Karagiorgos et al., 1979). In a different set of studies, men were asked to complete two protocols: one protocol was 20 min of continuous 'aerobic' exercise at 100 W, while the second protocol (of equal energy expenditure and duration) was 'anaerobic' exercise consisting of 1-min sprints at 285 W followed by 2 minutes of rest, completed seven times (Vanhelder, Goode, & Radomski, 1984a). Growth hormone concentrations at the end of anaerobic exercise were considerably greater (at 20-min, 2.65 μg/L) and reached a higher peak concentration within 10 minutes after the end of exercise (30-min, 7.25 μg/L) compared to aerobic exercise (0.8 μg/L at 20-min, 2.5 μg/L at 30-min) (Vanhelder, Goode, & Radomski, 1984a). Furthermore, lactate concentrations were significantly

higher following anaerobic exercise (9.2 mmol/L) compared to aerobic exercise (1.96 mmol/L). And there was a strong correlation between GH and lactate concentrations for both anaerobic (r = 0.87) and aerobic (r = 0.93) exercises (Vanhelder, Goode, & Radomski, 1984a). The discrepancy in results between these two studies (Karagiorgos et al., 1979; Vanhelder, Goode, & Radomski, 1984a) may be attributed to the IE of Karagiorgos et al. (1979), not being a "true" anaerobic exercise bout since participants were asked to maintain a 50 rpm pedal cadence compared to the all-out sprint of VanHelder et al. (1984). Interestingly, low intensity leg exercise (20% of 1-RM) performed with vascular occlusion significantly increased lactate concentrations with concomitant increases in GH and norepinephrine (Takarada et al., 2000). It could be well conceived that this response may be attributed to local hypoxia, and thus is an anaerobic response. It has been subsequently suggested that a mechanism that stimulates anaerobic metabolism, resulting in a dramatic increase in lactate, could also independently provide a stimulus for GH release (Stokes, 2003).

Exercise that stimulates lactate accumulation would also likely be associated with hydrogen ion (H^+) accumulation, suggesting that maybe GH release is controlled by an increase in H^+. An acidic environment has been shown to stimulate sympathetic nerve activity (Victor & Seals, 1989) activating the same afferent fibers (group III and IV) known to upregulate GH secretion (Gosselink et al., 1998). When male participants were provided $NaHCO_3$ or NaCl (placebo) prior to an all-out 90s sprint; there was a dramatic increase in GH seen during the placebo trial compared to the $NaHCO_3$ trial (Gordon, Kraemer, Vos, Lynch, & Knuttgen, 1994). Additionally, when $NaHCO_3$ was given prior

to an incremental exercise test to exhaustion, GH release was found to be blunted during exercise and recovery compared to placebo administration (Elias, Wilson, Naqvi, & Pandian, 1997).

Oxygen demand/availability (EPOC). It has already been discussed that GH secretion is proportional to exercise intensity, so it is reasonable to assume that GH would be proportional to oxygen consumption ($\dot{V}O_2$) given that there is a linear relationship between exercise intensity and $\dot{V}O_2$. Following 5 days of living at ~9000 ft, individuals had a greater GH response to both exercise at altitude and sea level exercise while breathing a hypoxic gas mixture, than when exercising at sea level under normoxic conditions (Raynaud et al., 1981). Incidentally, increased blood lactate concentrations were also noted during both hypoxic trials compared to the normoxic exercise trials (Raynaud et al., 1981). This appears to once again emphasize the role that oxygen availability has on regulating the GH response during exercise. Indeed, it has been suggested that the GH response to exercise may be a function of oxygen demand to oxygen availability (D/A ratio) (Vanhelder et al., 1987). This equation was developed under the assumption that oxygen availability (f) is inversely proportional to the change in lactate concentration [La^-] during exercise ($\frac{1}{f}$) and that oxygen demand would be proportional to the cumulative $\dot{V}O_2$ over the time (t) of the exercise (Vanhelder et al., 1987). Therefore, the D/A ratio is calculated using the following equation:

$$D/A\ ratio = \left[\int_0^x \dot{V}O_2 \cdot dt\right] \cdot f \qquad where, f = \left(\frac{[La^-]\ at\ t = x}{[La^-]\ at\ t = 0}\right)$$

(Vanhelder et al., 1987)

This D/A ratio should reflect the relationship between the aerobic requirement of exercise weighted by the inability of the system to meet the requirement (Vanhelder et al., 1987). Five men were asked to complete 7 sets of 7 squats at 80% of measured 7-RM (Vanhelder et al., 1987). When compiling additional data points from a previous laboratory study (Vanhelder, Goode, & Radomski, 1984a), it was determined that there was a strong, significant correlation (r = 0.93) between the change in GH concentration (calculated as: $\frac{[GH]_{t=x}}{[GH]_{t=0}}$) and the D/A ratio (Vanhelder et al., 1987). Additionally, when the authors took data from previously published research that provided $\dot{V}O_2$, [La^-], and GH data (Karagiorgos et al., 1979; Lassarre, Girard, Durand, & Raynaud, 1974; Raynaud et al., 1981; Vanhelder, Radomski, & Goode, 1984b), the strong relationship between the D/A ratio and GH changes remained (r = 0.78) (Vanhelder et al., 1987). Interestingly, when this equation was applied to the data from Karagiorgos et al (1979), a very strong correlation (r = 0.92) persisted between GH and the D/A ratio during continuous exercise (intermittent exercise $\dot{V}O_2$ data was not published), despite the fact that the original paper reported no relationship between lactate and GH (Karagiorgos et al., 1979; Vanhelder et al., 1987). The data examined from above ranged from 20 – 60 minutes in exercise duration, covered a wide range of individual fitness statuses, and various modes and intensities of exercise; therefore, it may be prudent to suggest that the relationship between GH and exercise is a direct function of the D/A ratio. A high D/A ratio would

subsequently affect muscle metabolites such as [La^-] and H^+, as their production is also dependent on oxygen availability to do work, and thus may explain the noted relationships between these metabolites and GH. It remains to be seen if this relationship between the D/A ratio and GH holds true for short-term exercise (such as that completed during HIE bouts).

Deconvolution Analysis

Pulsatility, generally defined as the recurrence of individual peaks interrupting a constant baseline secretion, is a vital aspect of hormone secretion (Xu et al., 2011). A given pulse is defined by an abrupt increase and ensuing decrease of a measured hormone that exceeds random assay variability by some objective amount (e.g., 2-3 times the assay coefficient of variation) (Xu et al., 2011). Therefore, being able to identify the pulsatility of hormone secretion patterns can provide an understanding of the mechanisms by which an endocrine gland communicates with its target organ and the subsequent response(s). There are several fundamental challenges underlying estimation of hormone pulsatility that must be considered (Figure 5).

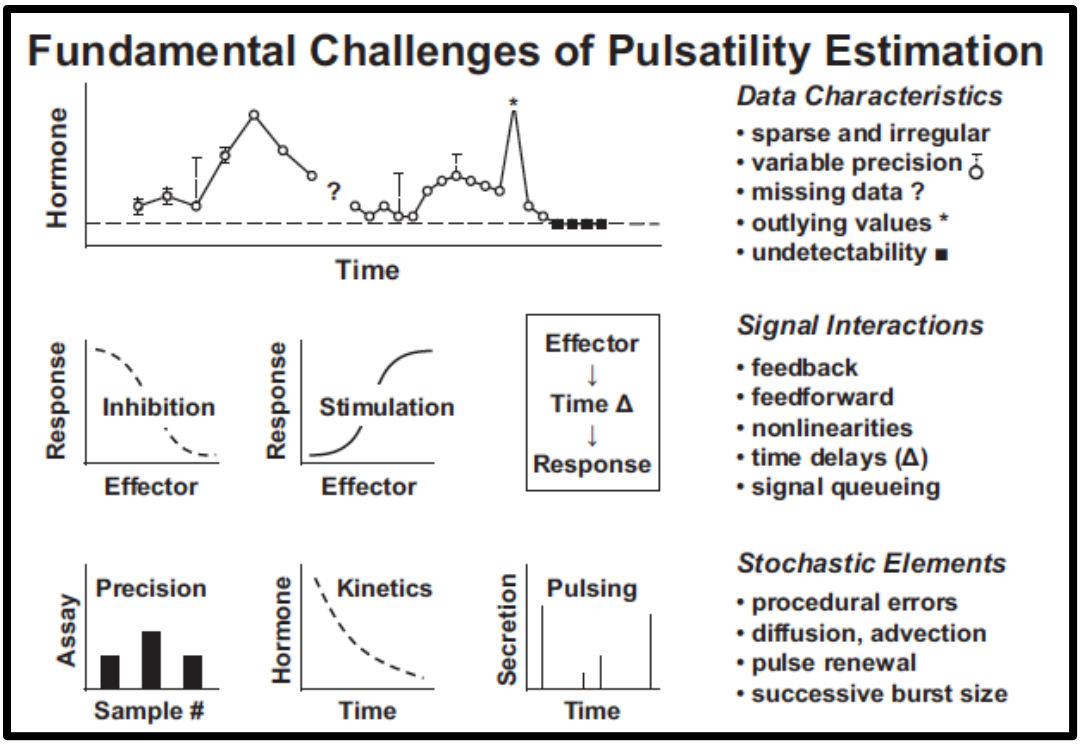

Figure 5. Diagram of the analytical hurdles associated with estimation of hormone pulsatility measurement (copied from Xu et al., 2011).

Such issues that arise and must be considered for valid hormone pulsatility measurements include: 1) the pulsatility pattern (number, size, and shape) of a given hormone, 2) specific hormone secretion and elimination kinetics, 3) the regulation of hormone feed-forward and feed-back signals on secretion control, and 4) biological variation as well as procedural errors introduced through sample collection, analysis, or processing (Veldhuis & Johnson, 1992; Xu et al., 2011).

First, in order to properly assess hormone secretion, it is important to know the half-life of the hormone in question. Too few of samples taken during a time period may 'dampen' the pulsatile response upon analysis, and too many samples can provide no additional benefit to pulse detection and greatly increase cost (Pincus, Hartman,

Roelfsema, Thorner, & Veldhuis, 1999). Second, analysis of hormone pulses can be difficult given that hormone time scales of pulsatility vary greatly and can range from secretion rates every millisecond–second (e.g., $[Ca^{2+}]_i$) to approximately every 3 hours (e.g., luteinizing hormone) (W. S. Evans, 1992; Kwiecien, Tseeb, Kurchikov, Kordon, & Hammond, 1997). The majority of hormones, however, are secreted in an intermediate time frame: every 4 – 30 minutes (e.g., insulin and glucagon) or every 45 – 180 minutes (e.g., anterior pituitary hormones) (Xu et al., 2011).

A measurement of hormone in plasma is a single snapshot that does not provide information regarding: 1) how much hormone was secreted previously and not yet removed; and 2) how much hormone has been removed from the periphery and at what rate. Furthermore, a single hormone measurement is also subject to any unexplained variations to include biological variation or procedural error/variability and the sensitivity, specificity, validity, and reliability of the assay used for the analysis. Deconvolution analysis is the determination of underlying secretion or elimination rates from a hormone-concentration profile (Xu et al., 2011). It is therefore paramount to identify a valid procedure by which the above challenges are addressed in order to accurately assess hormone metabolism.

Deconvolution procedures simultaneously estimate the accumulation (secretion) and dissipation (elimination) to a measured outcome (the hormone concentration). The concentration [*C(t)*] is described by: 1) the elimination of the previously secreted hormone; 2) ongoing secretion *S(t)* into and elimination *E(t)* from the system; and 3) experimental variability (see equation 1 and Figure 6) (Xu et al., 2011).

$$C(t) = E(t) \times C(0) + \int_0^t S(z) \times E(t-z)dz + \varepsilon_i$$

Equation 1. *Model of deconvolution analysis*. The first term on the right side of the equation indicates the amount of hormone remaining at time *t* given a starting concentration, $C(0)$, acted upon by an elimination function, $E(t)$. The middle term is the convolution integral, which denotes that secretion and elimination between time zero and *t* are evaluated by summing the product of their effects over all infinitesimally short intervals, *dz*. Taking the product of the two functions: $S(z)$ and $E(t-z)$, indicates the effects of input and output contribute jointly to describing how much of the secretion at time *z* remains in the concentration at time *t*. The expression $(t-z)$ in the elimination function denotes that removal only proceeds after secretion has occurred at time *t*. The rightmost term, ε_i, signifies unexplained variance in the observed concentrations (copied from Xu et al., 2011).

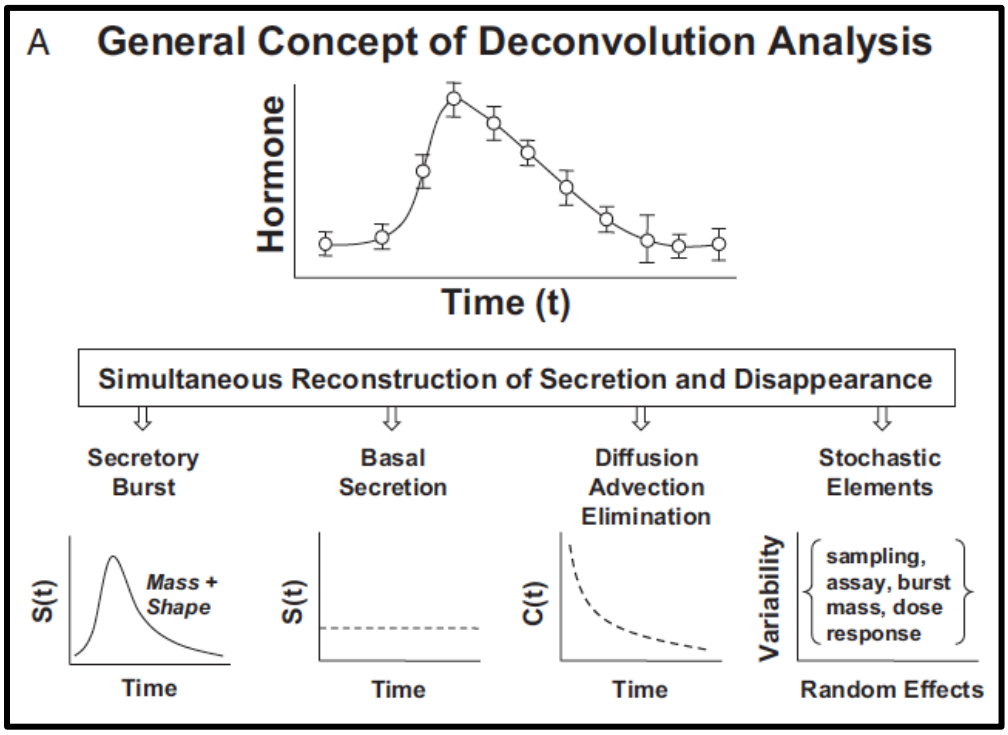

Figure 6. Diagram demonstrating the breakdown of a hormone concentration peak (top) into an underlying secretory pulse by deconvolution analysis (copied from Xu et al., 2011).

Mathematically, deconvolution analysis should be able to obtain valid and reliable results for a given hormone's basal and pulsatile secretion pattern as well as simultaneous hormone elimination. As such, there are several assumptions to be made in order to utilize deconvolution analysis, the most important of these being: 1) the half-life and distribution volume of the hormone do not change during the observation interval; 2) basal secretion is at a fixed value; and 3) the pulses are instantaneous secretion events or finite bursts of a homogeneous symmetric or asymmetric shape (Veldhuis & Johnson, 1988; 1992; Xu et al., 2011). Each of these assumptions have their own inherent challenges which are listed below.

The first assumption, that the half-life and distribution volume of a hormone are unchanging over a period of time has not been rigorously tested. However, one study demonstrated that the half-life of injected growth hormone in young healthy men was shorter in the morning (~23 min) than in the evening (~26 min), despite distribution volume being equal (Holl et al., 1993). Additionally, there is an assumption that for a given hormone, half-life is identical across all individual participants. Again, data reflecting this assumption are not extensive. In one study, the recombinant human growth hormone (rhGH) half-life was reduced and metabolic clearance rate increased by ~33% in obese women (Langendonk et al., 1999), another study demonstrated an ~65% increase in the metabolic clearance rate of rhGH with age (Vahl, Møller, Lauritzen, Christiansen, & Jørgensen, 1997b). Given this, it is important for an investigator to consider inter-individual variability within the subject population and account for this

within the study design by sampling hormones at frequent enough time-points that deconvolution analysis is still a valid measure of hormone metabolism.

The second assumption regarding basal secretion of a hormone is equally ambiguous. The *in vivo* basal secretion rate for most hormones is unknown. In order to accurately assess basal secretion of a hormone, the following is suggested: 1) sample the hormone of interest over a 24-h period to determine when pulses are least frequent and when non-pulsatile release is most clear; then, 2) sample frequently during this "best time" to obtain a minimum of 3-5 consecutive unchanging values in-between the pulses; and finally, 3) selectively antagonize the endogenous activator of the given hormone that drives pulsatility (Xu et al., 2011).

Finally, the assumption regarding the secretory burst duration and shape should allow for further postulations to be made regarding the timing of upstream signaling, endocrine and target organ response, and feedback (Xu et al., 2011). It should be assumed that the size and shape of the secretory event should indicate the magnitude of the hormone response. However, if basal secretion rates (see above) are overestimated, pulse amplitude will resultantly be underestimated by reducing the size of each pulse (Xu et al., 2011). In theory, an endocrine gland pulse will result in a large increase in bloodstream concentration of that hormone. The concentration of the hormone within the bloodstream is dependent upon a) the rate of diffusion within the bloodstream (random molecular dispersion), b) advection, or the transport of the hormone due to the pumping action of the heart, and c) convection, the combination of (a) and (b) due to fluid turbulence (Xu et al., 2011).

CHAPTER III

METHODS

Participants

Ten young women between the ages of 18 and 35 years were recruited for this study. Participants were eligible for participation if they had been sedentary for the previous 3 months. Sedentary was defined as less than 3 days per week of moderate-intensity [46-64%$\dot{V}O_{2max}$] or greater physical activity (American College of Sports Medicine, 2014a) with less than 30 min per exercise session. Participants were required to have a body mass index between 23.0 – 39.9 kg/m^2 and be weight-stable (± 2 kg change in body weight in previous 6 months). Control (CON) and exercise (MOD and HIE) trials took place when women were in the early follicular phase of their menstrual cycle (i.e., within 2–8 days after onset of menstrual bleeding). The order of these trials (CON, MOD, and HIE) was randomized and separated by at least one normal menstrual cycle (27–35 days).

Exclusionary criteria for this study was: any known or diagnosed renal, hepatic, hematologic, metabolic, or cardiovascular disorder; smoking (either a current smoker or quit within past 6 months); greater than moderate alcohol consumption (> 1 drink per day) (U.S. Department of AgricultureU.S. Department of Health and Human Services, 2010); and/or regular physical activity (>3 days/week of moderate-intensity activity). Further, women who were taking birth control (other than oral contraceptives), hormone replacement therapy, or recombinant hGH were excluded. Women with diagnosed

anemia, or who have irregular menstrual cycles were also excluded from participation in this study. A regular menstrual cycle was defined as menses occurring every 21 – 35 days (Bulun & Adashi, 2003). Finally, individuals who had a history of extreme dietary patterns (such as eating disorder) were excluded from this study.

Study Design

This experiment was a randomized crossover design. Each participant completed a descriptive data collection day (Day 1). The trial days (CON, MOD, or HIE) occurred when the participant identified they were in the early follicular phase of their cycle (day 2 – 8 after the onset of menses). On these days, participants were randomly assigned to complete either a control session (CON), a moderate-intensity exercise (MOD) session, or a high-intensity interval exercise (HIE) session. Each participant completed all three trials, and each trial was separated by a minimum of 1 menstrual cycle (27–35 days).

On Day 1, following completion of the informed consent and other forms, blood was collected from an antecubital vein to verify that hepatic, renal, metabolic, and hematologic function were normal and for measurement of lipid profile. Participants' anthropometric measurements (height, weight, and waist circumference) were taken and they completed a dual x-ray absorptiometry (DXA) scan for measurement of body composition and distribution of body fat. Lastly, participants completed an incremental maximal oxygen uptake ($\dot{V}O_{2max}$) test on an electronically braked cycle ergometer (Velotron; RacerMate, Seattle, WA).

For the trial days (CON, MOD, or HIE), participants were asked to return to the exercise physiology lab (Pioneer Hall 112) following a day of rest (no structured exercise) at 1700h. Beginning at 1730h participants would: 1) rest quietly in a chair for 30 minutes (CON); 2) complete 30 minutes of cycling at 50% of maximum workload achieved during the $\dot{V}O_{2max}$ test (MOD), or 3) complete 4 Wingate tests on the cycle ergometer (HIE) during a 30 min time period. Participants were then able to relax in the exercise physiology lab until approximately 0700 the next morning. During this time (1730h – 0600h) blood samples were drawn in 10-min intervals for assessment of pulsatile growth hormone secretion. From 0630 to 0700, resting metabolic rate (RMR), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and respiratory exchange ratio (RER) were measured by indirect calorimetry (True One 2400 Metabolic System; ParvoMedics, Sandy, UT). Participants were then asked to return to complete the other trial days when they were again in the early follicular phase of their menstrual cycle.

Day 1 Testing

Blood Draw

Participants were asked to arrive at the lab in the morning following an overnight fast and having not exercised for the previous 24h. A 10 mL serum sample, using standard venipuncture technique, was collected to determine if baseline measures of hepatic, renal, metabolic, and hematologic function were normal (Quest Diagnostics; Denton, TX). The comprehensive metabolic panel included: albumin, albumin/globulin ratio (calculated), alkaline phosphatase, ALT, AST, BUN/creatinine ratio (calculated), calcium, carbon dioxide, chloride, creatinine with GFR estimated, globulin (calculated),

glucose, potassium, sodium, total bilirubin, total protein, and urea nitrogen. Additionally, the sample was analyzed for the participant's lipid profile: total cholesterol, HDL Cholesterol, triglycerides, and LDL-Cholesterol (calculated).

Anthropometric Measurements

While wearing their workout clothing, but no shoes, each participant's height was measured to the nearest 0.1 cm using a stadiometer (Perspective Enterprises; Kalamazoo, MI) and weight was measured to the nearest 0.1 kg using a digital scale (Tanita Corp.; Arlington Heights, IL). Body mass index (BMI) was calculated using the following equation (equation 2):

$$\text{BMI} = \frac{\text{kg}}{\text{m}^2}$$

Equation 2.

Waist circumference was measured to the nearest 1 mm using a spring-loaded tape measure (Gulick II Tape Measure; Lafayette, IN) and was identified as the narrowest part of the torso (above the umbilicus and below the xiphoid process). A horizontal measurement was taken with the participant standing, arms at the side, feet together, and abdomen relaxed (American College of Sports Medicine, 2014a).

Body composition was determined using dual-energy x-ray absorptiometry (DXA; Lunar Prodigy; GE Healthcare, Madison, WI) and visceral fat was quantified using the CoreScan software (GE Healthcare; Madison, WI). Participants were scanned in a supine position, and the scan took approximately 6 minutes to complete. The DXA uses two very low dose x-radiation beams, which travel from the anterior to the posterior

region of interest, to measure bone mineral density. X-rays that are not absorbed are detected on the posterior side of the body; the greater the absorption, the less energy is detected and therefore, bone mineral density is greater. The DXA is able to separate the body into compartments (bone mineral content, fat, and non-bone lean tissue) based on internal algorithms. It is from these algorithms that body composition is derived.

Cardiovascular Fitness

Cardiovascular fitness ($\dot{V}O_{2max}$) was determined using a ramp cycling protocol on an electronically braked cycle ergometer (Velotron; RacerMate, Seattle, WA). Thirty-second averages of expired gasses ($\dot{V}O_2$ and $\dot{V}CO_2$) and expired ventilation ($\dot{V}_E$) were continuously collected by indirect calorimetry (TrueOne 2400; ParvoMedics, Sandy, UT). Heart rate was monitored during the test by 12-lead electrocardiogram (Quinton Q-Stress; Cardiac Science, Milwaukee, WI).

Briefly, the participant started pedaling at 50 W at a comfortable cadence (minimum of 50 rpm) for 60 seconds as a warm-up. At the start of Minute 2 the load increased by 1 W every 2 seconds until the participant reached volitional exhaustion or their pedal cadence dropped below 40 rpm. A successful test ($\dot{V}O_{2max}$) was determined by achievement of a plateau in $\dot{V}O_2$ with increasing work (primary criteria); or if a plateau was not reached, participants needed to achieve the following: (1) heart rate within 10 bpm of age-predicted HR_{max}; (2) RER > 1.10; and (3) blood lactate concentration > 8.0 mM measured by capillary finger-stick 2 min after the cessation of exercise (Howley, Bassett, & Welch, 1995). If not all criteria were achieved; the test was recorded as a $\dot{V}O_{2peak}$ instead of $\dot{V}O_{2max}$. Only one participant (GH-001) did not achieve

$\dot{V}O_{2max}$, as HR was unable to be measured at the end of the test due to electrodes falling off. Participants were given verbal encouragement throughout the exercise test.

Trial Days

For all trial days, participants were asked to arrive at the lab at least 3 hours postprandial at 1700h (5:00 PM). Participants were fed dinner at 1930h (7:30 PM), and ate the same meal during each trial (Icon Meals, Frisco, TX).

Control Day (CON)

For the CON trial, participants sat quietly from 1730 – 1800h. Participants were allowed to watch movies, read, or do homework during this time period. At 0-min, the end of the 30-minute period, 60-min, 90-min, and 120-min, a 25 μL sample of venous blood was taken to measure lactate concentration (YSI 2300; Yellow Springs, OH).

Moderate Intensity Steady-State Exercise (MOD)

For the MOD trial, beginning at 1730h (5:30 PM), participants cycled at ~50% of maximum power output (determined from the $\dot{V}O_{2max}$ test) continuously for 30 minutes. Actual wattage was decreased if necessary to ensure the participant's could complete the full 30 minutes of exercise. During the last 5 min of the trial, indirect calorimetry was used to determine $\dot{V}O_2$, $\dot{V}CO_2$, and RER steady-state values. Heart rate was monitored during exercise using a Polar® heart rate monitor that was fitted around the chest. At 0-min, 5-min time points throughout the MOD trial, 30-min (end of exercise), 32-min, 34-min, 36-min, 38-min, 40-min, 45-min, 50-min, 60-min, 90-min, and 120-min, a 25 μL sample of venous blood was taken to measure lactate.

Activity energy expenditure during MOD was calculated using the following equation:

$$\text{kj} = \text{average power (W)} \times \text{ duration (h)} \times 3.6\ \frac{ksec}{h}$$

Equation 3.

To convert kilojoules to energy expenditure (kcal) one would multiply by 4.184 kcal/kj and multiply that by 25% (assumed efficiency of human work).

$$kcal = kj \times 4.184 \frac{kcal}{kj} \times 0.25$$

Equation 4.

High Intensity Interval Exercise (HIE)

For the HIE trial, beginning at 1730h (5:30 PM), each participant was asked to complete four, 30-s sprints on an electronically-braked cycle ergometer following a light warm-up (5 min at 50 W). The resistance set for the 30-second sprint was equal to 6.5% of the participant's body mass for a total high-intensity exercise time of 2 minutes. The recovery interval between Wingate tests was 4.5 minutes, during which the participant was asked to cycle at a low cadence (30 - 40 rpm) and a light resistance (30 W) to reduce venous pooling in the lower extremities and minimize feelings of nausea or light-headedness. At the end of the fourth sprint, participants continued to cycle at 30 W at an easy cadence (30–40 rpm) for 4.5 minutes of recovery. The total exercise time during the HIE protocol was 30 minutes (including warm-up and recovery intervals). Heart rate was monitored during exercise using a Polar® heart rate monitor (Polar; Lake Success, NY) that

was fitted around the chest. At 0-min, the end of each 30-second sprint, 2-min after the last sprint, 30-min (end of exercise), 32-min, 34-min, 36-min, 38-min, 40-min, 45-min, 50-min, 60-min, 90-min, and 120-min, a 25 μL sample of venous blood was taken to measure lactate.

Activity energy expenditure for HIE was calculated using the same equations as for MOD. The Velotron Wingate software provided total kilojoules for each sprint.

Resting Metabolic Rate (RMR) and Substrate Utilization

Resting metabolic rate was measured by indirect calorimetry using the ventilated hood technique as described by the American Dietetic Association (Compher, Frankenfield, Keim, Roth-Yousey, Evidence Analysis Working Group, 2006). Briefly, upon waking, participants were placed in a semi-recumbent position and asked to lie quietly while a rigid, clear, plastic canopy was placed over the participant's head and sealed for the collection of $\dot{V}O_2$ and $\dot{V}CO_2$ for 30 minutes. The first ten minutes of data was discarded and during the last twenty minutes, a 10-minute steady-state ($\leq$ 10% coefficient of variation in $\dot{V}O_2$ and $\dot{V}CO_2$, calculated as the standard deviation divided by the mean) was used for calculation of RMR and respiratory exchange ratio (RER) after completion of the test. Additionally, throughout the overnight trial period participant's urine was collected for determination of urinary nitrogen (uN_2) and calculation of non-protein RQ (Equation 5, NPRQ) (Simonson & DeFronzo, 1990).

$$\mathrm{NPRQ} = \frac{\mathrm{VCO_2} - 4.84\ (\mathrm{uN_2}\left[\frac{\mathrm{g}}{\mathrm{min}}\right])}{\mathrm{VO_2} - 6.04\ (\mathrm{uN_2}\left[\frac{\mathrm{g}}{\mathrm{min}}\right])}$$

Equation 5.

Blood Collection and Analysis

Blood Sampling

Upon arrival to the exercise physiology laboratory at 1700h, an indwelling catheter was inserted into a forearm or hand vein and attached to a saline drip to prevent clotting. Prior to beginning a trial at 1730h (CON, MOD, or HIE), a baseline blood sample was taken (at approximately 1720h) for measurement of estradiol (E_2), growth hormone (GH), and lactate; 10 mL of serum was collected in a ('red-top') vacutainer (GH), 6 mL of plasma was collected in an EDTA-coated ('purple-top') vacutainer (E_2). Approximately 1 mL of blood was placed in a microcentrifuge tube containing a lysing agent for preservation of blood lactate (YSI 2315 Lactate Preservative Kit) and immediately vortexed. Starting at 1730h, 3-mL serum blood samples were drawn at 10-min intervals for measurement of pulsatile GH until 0600h the following morning (12.5 hours). Saline was infused (~ 80 mL/h) for replacement of fluid lost during blood sampling. There was approximately 1 month separating the participant's trial days (CON, MOD, HIE).

Serum blood samples were allowed to clot for 10-30 minutes and then centrifuged at 3000 rpm (4 ± 2 °C) for 10 minutes. Plasma blood samples were centrifuged immediately at 3000 rpm (4 ± 2 °C) for 10 minutes. Serum and plasma aliquots were separated into pre-labeled cryule vials and frozen at -80 °C until analyzed for hormone concentrations using commercially-available ELISA kits. All samples from a participant were analyzed together to eliminate any interassay variability.

Human Growth Hormone ELISA Kit (LDN, Germany)

Approximately 100 μL of serum was needed for duplicate determination of hGH with this ELISA kit. This ELISA kit follows a one-step 'sandwich' type procedure for assessment of human growth hormone (hGH) in serum samples. In short, a monoclonal antibody specific to hGH is adhered to the provided 96-well plate and following addition of standards, calibrators and unknown samples, another monoclonal antibody specific for a different binding site on hGH is added to each well (conjugated to horse radish peroxidase [HRP]). The hGH found in the sample is bound between the two antibodies effectively creating an 'hGH sandwich.' Any unbound HRP is removed during plate washing and decanting. Following the washing step, an enzyme is added (tetramethylbenzidine and hydrogen peroxide, TMB substrate) and the proceeding reaction is stopped after 10-15 minutes with the provided stop solution (sulfuric acid). The intensity of the color formed by the enzymatic reaction is proportional to the concentration of hGH in a given sample; such that a darker color achieved will equal a higher hGH concentration. The sensitivity of this assay has been determined to be 0.2 ng/mL. Samples collected in the proposed study with a concentration < 0.2 ng/mL will be assigned a value of 0.2 ng/mL for statistical analysis. The inter-assay %CV for all GH plates (n = 31) was 15% for the low control and 16% for the high control. Because we were not able to get all plates were from the same lot, inter-assay variability was re-analyzed by each individual kit lot – lot #160575: low control 13% and high control 11% (n = 13 plates); lot#161218: low control 3% and high control 4% (n = 9 plates); and lot#161122: low control 4% and high control 4% (n = 9 plates).

17β-Estradiol ELISA Kit (LDN, Germany)

25 μL of plasma was used in duplicate for each sample determination of 17β-estradiol. This ELISA kit is based on the principle of competitive binding. Briefly, the microtiter wells are coated with an anti-Estradiol antibody. The estradiol present in the sample competes with an estradiol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of estradiol in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of estradiol in the sample. The measured sensitivity of this assay is 6.2 pg/mL and the range of this assay is from 25 – 2000 pg/mL.

Pulsatile Growth Hormone Secretion

Serial blood sampling (3 mL) began at 1730 hr and continued in 10-min intervals until 0600 hr (12.5 hours) for determination of pulsatile growth hormone secretion. Following the CON, MOD, and HIE trials, participants remained in the seated or semi-recumbent position and were allowed to watch movies, read, do homework, and sleep during this time. Pulsatile growth hormone secretion was estimated by deconvolution analysis (Veldhuis & Johnson, 1988; 1992) using the AutoDecon Software (Johnson et al., 2009). Pulse parameters compared between the three trials were: mean GH concentration ($ng \cdot mL^{-1}$), total pulsatile secretion ($ng \cdot mL^{-1} \cdot 12.5\ h^{-1}$), number of detected pulses, interpulse interval (min), pulse height (ng), and pulse area ($ng \cdot nL^{-1} \cdot min^{-1}$).

Urine Collection and Analysis

Participant's urine was collected throughout the entire 12.5h trial in a 3L urine collection container. The container was kept in an ice chest with freezer blocks to keep cool. In the morning the sample volume was measured and 4.0 mL was aliquoted to 2 cryule vials and stored in the -80°C freezer until analysis. Urea nitrogen was measured using a colorimetric assay (Stanbio, Boerne, TX). Procedures for the urea nitrogen assay and calculation of NPRQ can be found in Appendix J.

Statistical Analysis

The sample size calculations were based on the number of participants needed to detect statistically significant changes in growth hormone secretion from published data (Wideman et al., 1999). Based on these calculations, at a power of 0.80 and $\alpha = 0.05$, approximately 10 participants were needed (Faul, Erdfelder, Lang, & Buchner, 2007); thus, recruitment of 10–12 participants should provide ample power to determine differences between the three trials (CON, MOD, and HIE). It should be noted that this sample size calculation was based on only a control trial (no exercise) and a 30-min steady-state exercise trial at ~70% of maximum power output in young women (Wideman et al., 1999). There are no available data that examine GH secretion rates following a high-intensity interval training exercise bout.

All results are reported as mean ± standard deviation. The dependent variables: GH AUC, estradiol, and NPRQ were analyzed using a repeated measures ANOVA with an $\alpha = 0.05$. Growth hormone and lactate responses for the first 120 minutes were analyzed by a repeated measures factorial ANOVA (condition x time) with a $p < .05$

indicating statistical significance. Due to a large amount of variability in GH secretion among individuals, the deconvolution parameters were log-transformed for analysis. A repeated measures ANOVA was used to determine the effect of exercise intensity (CON, MOD, HIE) on the subsequent GH secretory responses (deconvolution parameters) using a p value < .10. When appropriate, post hoc analyses were conducted using a Bonferroni correction with an $\alpha \leq .05$. Statistical computations were carried out using an SPSS Statistical software package (IBM; Armonk, NY).

CHAPTER IV

PRESENTATION OF FINDINGS

Participant Characteristics

Texas Woman's University campus-wide recruitment emails were sent on February 1, 2016 and July 26, 2016. From these two emails, 253 women responded with interest in participation. Of these women, 20 were immediately disqualified due to location (e.g., Dallas or Houston campus), age (> 35 y), or training status (i.e., regular exerciser, $\geq$ 3 days per week). A follow-up screening email that also included more detailed study information as well as the time commitment was sent to 233 women, of which 71 responded. Twenty-six of these responders qualified for participation in this study, and 13 women scheduled a first visit to the lab. Three women chose not to participate prior to signing the informed consent, and 10 women were consented to participate in the study. Five of these women were dropped from participation due to fear of catheter insertion (n = 1), could not keep catheter line patent (n = 1), and non-response to scheduled appointments (n = 3). Thus, five women completed this study. Table 1 presents the mean descriptive characteristics of the study participants. The raw data for all 5 participants can be found in Appendix K and raw growth hormone data for each trial can be found in Appendix L. All data are presented as mean ± standard deviation (SD) unless otherwise stated.

Table 1

Participant Descriptive Characteristics

	Mean	±	SD
Age (y)	22.6	±	1.3
Height (cm)	159.2	±	5.4
Weight (kg)	69.2	±	7.6
BMI (kg/m^2)	27.4	±	3.1
Waist Circumference (cm)	78.1	±	5.0
Body Fat (%)	39.2	±	1.7
VO_{2max} (L/min)	2.03	±	0.41
VO_{2max} (mL/kg/min)	29.43	±	5.69
HR_{max} (bpm)	189.5	±	10.5
Max Power Output (W)	183.8	±	22.6

Note. n = 5. SD = standard deviation; BMI = body mass index; VO_{2max} = maximal oxygen consumption

A sedentary lifestyle was defined by $\leq$ 2 days per week or $\leq$ 150 min/week of structured/planned physical activity (American College of Sports Medicine, 2014a). In the previous month prior to entry in this study, these women participated in approximately 412 ± 239 total minutes of structured physical activity. This was an average of approximately 91 ± 53 minutes/week, verifying that these participants did not meet the ACSM recommended amount of physical activity. Additionally, the mean VO_{2max} of these participants was 29.4 ± 5.7 mL/kg/min, giving them a "very poor" classification based on ACSM guidelines (American College of Sports Medicine, 2014a). It is believed that all participants achieved VO_{2max} during the cycling ramp protocol: (1) four out of the 5 women achieved a plateau in oxygen uptake within the final minute of the test (< 150 mL/min change in VO_2 with increasing work rate) using 30 second averages (Howley et al., 1995); (2) all women achieved an RER > 1.10 and lactate > 8.0 mmol/L during the VO_{2max} test, and (3) three out of the five women achieved a HR within 10 bpm of age-predicted maximum heart rate.

All participants' trials were completed during the (self-reported) follicular phase of the menstrual cycle. Mean estradiol concentrations ($pg \cdot mL^{-1}$) between the three trials were not different (CON: 84.12 ± 72.8; MOD: 73.3 ± 45.7; HIE: 70.8 ± 44.7; $p = .923$).

Exercise Responses

The mean power output on the cycle ergometer for the MOD trial was 80.6 ± 6.3 W, and measured VO_2 during the last 5-min elicited a value equal to approximately 68.2 ± 9.7% of VO_{2max} (1.36 ± 0.11 $L \cdot min^{-1}$). Average heart rate during the MOD trial was 160 ± 17 bpm, which was equal to 81.2 ± 8.9% of age-predicted HR_{max}. The mean RER during the MOD trial was 0.94 ± 0.06 suggesting participants were oxidizing primarily carbohydrate during the 30-min protocol. Average peak lactate concentration during the MOD trial was 4.7 ± 0.9 $mmol \cdot L^{-1}$.

The peak and mean power output for each sprint during the HIE trial can be found in Table 2. Peak power output decreased compared to the first sprint ($F(3, 12) = 7.82$, $p = .004$, $\omega^2 = .13$). Compared to Sprint 1 (520.4 ± 95.6 W), peak power out was significantly lower for Sprint 3 (460.8 ± 90.7 W, $p = .039$) and Sprint 4 (431.6 ± 67.0 W, $p = .017$), but was not different between Sprint 1 (520.4 ± 95.6 W) and Sprint 2 (495.2 ± 67.4 W, $p = .256$). There was also a main effect for sprint number on mean power output ($F(3, 12) = 14.35$, $p < .01$, $\omega^2 = .50$). Compared to Sprint 1 (389.8 ± 45.7 W), mean power output was significantly lower for Sprint 2 (328.2 ± 38.7 W, $p = .004$), Sprint 3 (283.0 ± 43.5 W, $p = .010$), and Sprint 4 (298.8 ± 25.6 W, $p = .012$). Peak HR measured during the HIE trial was 183 ± 11 bpm, which is equal to approximately 93% ± 6% of age-predicted HR_{max}. Both the peak HR (183 ± 11 bpm) and mean HR (180 ± 11 bpm)

achieved during HIE were significantly greater than the mean HR during MOD (160 ± 17 bpm; $p < .02$). Peak lactate concentration during HIE was 11.1 ± 2.2 mmol·L^{-1} which was significantly higher than peak lactate concentrations during the MOD trial (4.7 ± 0.9 mmol·L^{-1}; $p = .007$).

Table 2

Power Output for Each Sprint During HIE Trial

	PEAK POWER (W)			MEAN POWER (W)		
	Mean	±	SD	MEAN	±	SD
Sprint 1	520.4	±	95.6	389.8	±	45.7
Sprint 2	495.2	±	67.4	328.2	±	38.7†
Sprint 3	460.8	±	90.7*	283.0	±	43.5†
Sprint 4	431.6	±	67.0*	298.8	±	25.6*

Note. n = 5. SD = standard deviation.
* Significantly lower power output from sprint 1 ($p < .05$).
† Significantly lower power output from sprint 1 ($p = .01$).

Exercise Lactate Responses

There was a significant main effect for exercise intensity on lactate response during the first 120 min of each trial ($p < .01$). There was also a significant interaction between exercise intensity and lactate response over time such that MOD resulted in a higher lactate than CON ($p = .008$) and HIE resulted in a higher lactate response than MOD ($p = .025$) and CON ($p = .004$) (Figure 1a). Calculated AUC (n = 5) demonstrated an intensity-dependent increase in lactate response with exercise ($p = .002$) (Figure 7).

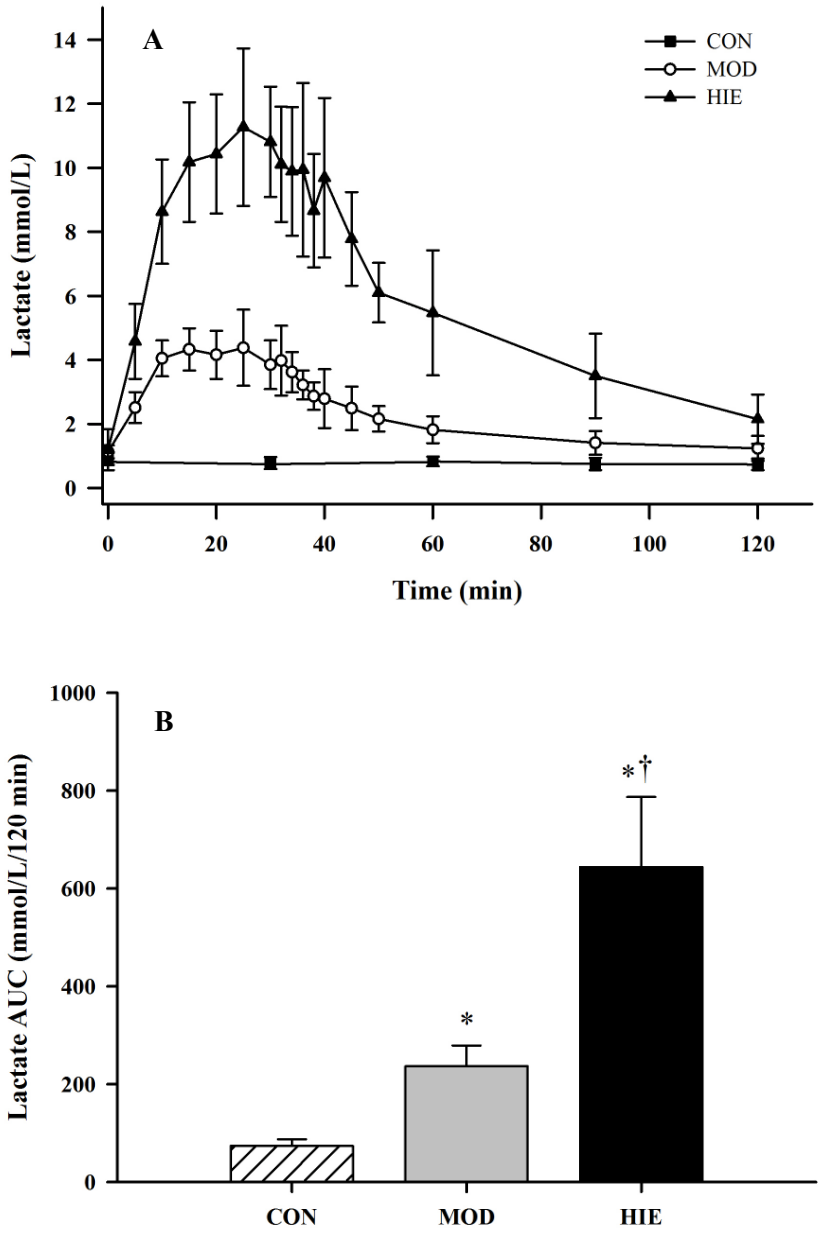

Figure 7. Lactate Response. Lactate response to CON, MOD, and HIE during the first 120-min of each trial. (A) HIE had the highest lactate response compared to MOD and CON ($p < .01$). (B) Mean lactate AUC by trial. * Compared to CON ($p < .01$), † Compared to MOD ($p = .025$). Note. Error bars represent ± SD. n = 5.

Estimated Exercising Energy Expenditure

Energy expenditure from both the MOD and HIE trial was estimated using power output data from the cycle ergometer. Mean energy expenditure from MOD (145.1 ± 11.2 kcal) was significantly higher than the HIE trial (97.4 ± 3.87 kcal; $p = .001$).

Non-Protein Respiratory Quotient (NPRQ)

At 0600 h, following the last blood draw, participant's resting metabolic rate was measured for determination of substrate oxidation 12 h after the end of exercise. Urine was collected throughout the duration of each trial (12.5 h) for measurement of urinary nitrogen excretion and subsequent calculation of NPRQ. There was no difference in urinary nitrogen excretion (g $N_2 \cdot h^{-1}$) between the three trials (CON: 0.339 ± 0.07; MOD: 0.466 ± 0.10; HIE: 0.438 ± 0.11, $p = .181$). Figure 8 shows calculated NPRQ for each trial. NPRQ 12h after HIE (0.77 ± 0.02) was lower than NPRQ measured after MOD (0.80 ± 0.08) and CON (0.82 ± 0.02), but this difference was not significant (V = 0.80, $F(2, 3) = 6.13$, $p = .087$, $\omega^2 = .05$).

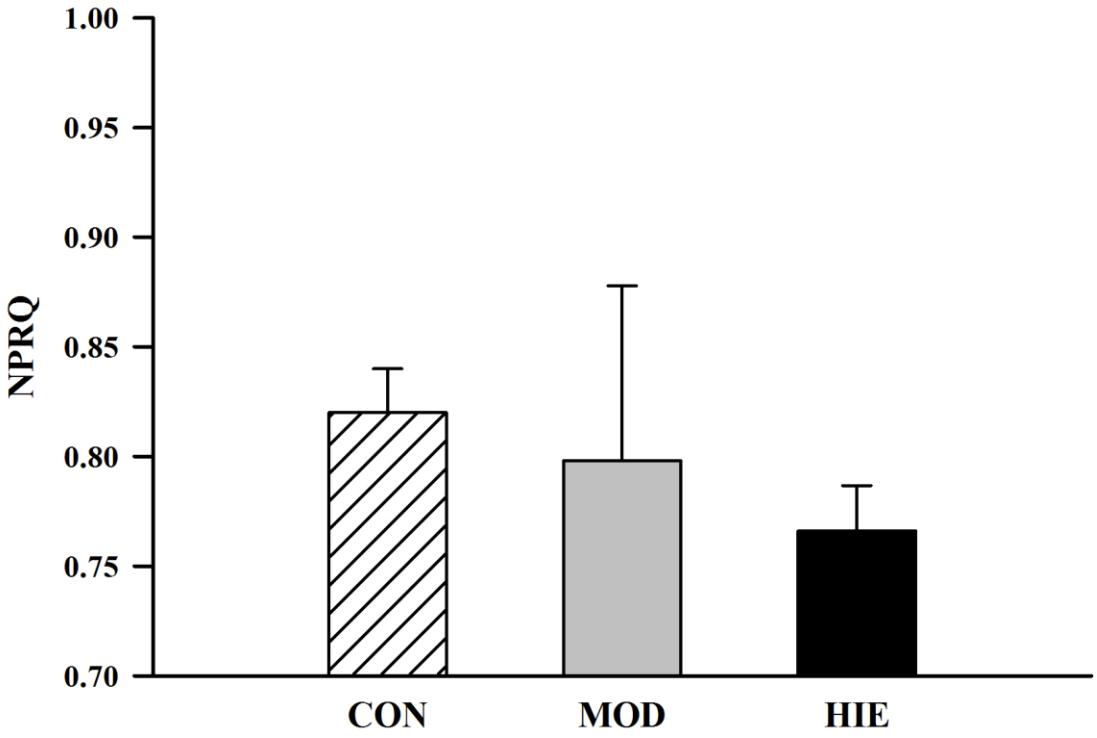

Figure 8. NPRQ. Comparison of calculated non-protein respiratory quotient (NPRQ) from resting metabolic rate (RMR) measurement 12 h after the completion of exercise. Participant's RMR was measured upon waking (0600h) for 30 min. The HIE trial had a non-significantly lower NPRQ compared to CON, indicating a greater reliance on fat for oxidation the morning after an HIE bout. *Note.* Error bars represent ± SD. n = 5.

Exercise Growth Hormone Response

Figure 9 shows the mean serum GH concentrations (± SD) from blood sampled at 10-min intervals over 2 hours for the three conditions (CON, MOD, HIE). Raw GH data for each participant can be found in Appendix L. The peak GH concentration measured over the 120-min period was 3.22 ± 4.00 $ng \cdot mL^{-1}$ for CON (at 40 min), 11.54 ± 2.96 $ng \cdot mL^{-1}$ for MOD (at 30 min), and 18.56 ± 9.90 $ng \cdot mL^{-1}$ for HIE (at 30 min). Peak GH concentration was greater following HIE compared to CON, but this did not quite reach

significance (p = .054). There was no significant difference in peak GH between CON and MOD (p = .106) and MOD and HIE (p = .560). During the 120 minutes there was a significant effect for trial (V = 0.98, $F(2, 3) = 87.22$, p = .002, ω^2 = .21), such that GH concentration was higher during MOD compared to CON (p = .120) and significantly higher in HIE compared to CON (p = .042) (Figure 9). There was no significant difference in GH response between MOD and HIE (p = .641). There was also a significant effect for time on GH response ($F(1.42, 5.70) = 12.45$, p = .011, partial eta squared = .78), however, due to large participant variability in GH response, individual pairwise comparisons between time points did not reach significance.

Calculated GH area under the curve values for 0 – 120 min are shown in Figure 10. Mauchly's test indicated that the assumption of sphericity was violated (X^2 (2) = 8.11, p = .017); so multivariate tests are reported (ε = 0.52). The 120-min GH AUC response to exercise was significantly affected by intensity (V = 0.98, $F(2, 3) = 78.68$, p = .003, ω^2 = .485). Growth hormone AUC for CON (181.70 ± 138.99 $ng \cdot mL^{-1} \cdot 120\ min^{-1}$) was not significantly different from MOD (544.75 ± 160.68 $ng \cdot mL^{-1} \cdot 120\ min^{-1}$, p = .107), but was significantly lower than HIE (1018.23 ± 576.11 $ng \cdot mL^{-1} \cdot 120\ min^{-1}$, p = .046). There was no difference in calculated GH AUC between MOD and HIE (p = .617).

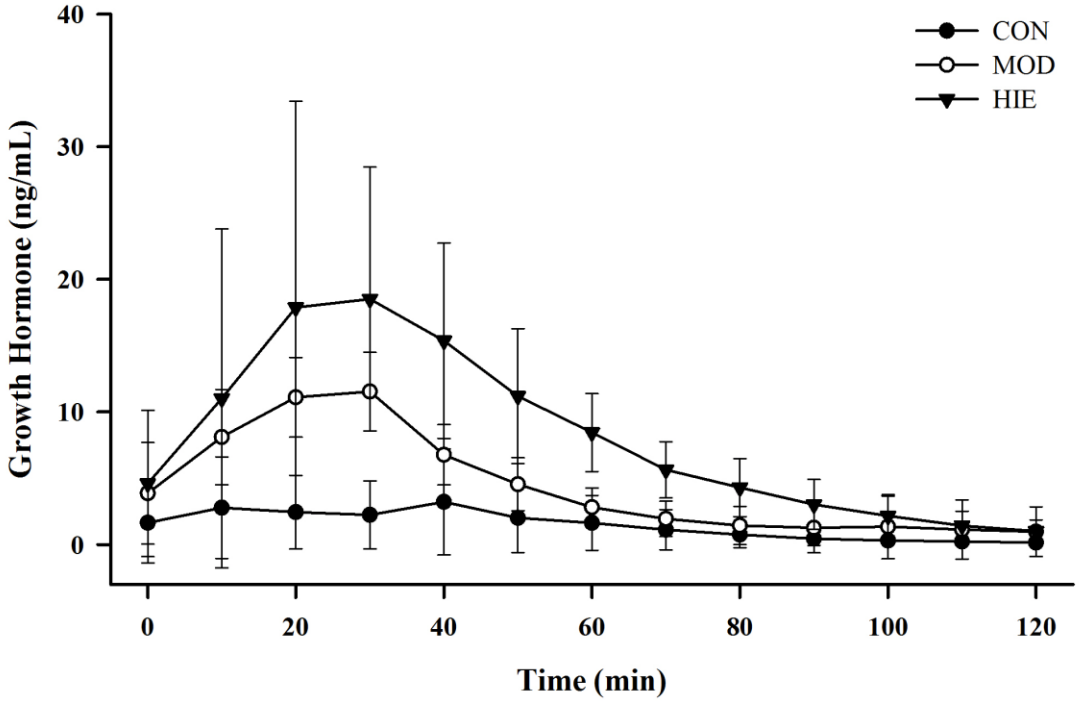

Figure 9. Mean serum growth hormone response for 120-min during CON, MOD, and HIE trials. Participants exercised from 0 – 30 minutes during the MOD and HIE trials. During the 0 – 30 min period (CON only) and the 30 – 120 min period (all trials) participants sat and watched TV or did homework. *Note:* Error bars represent mean data ± standard deviation. n = 5.

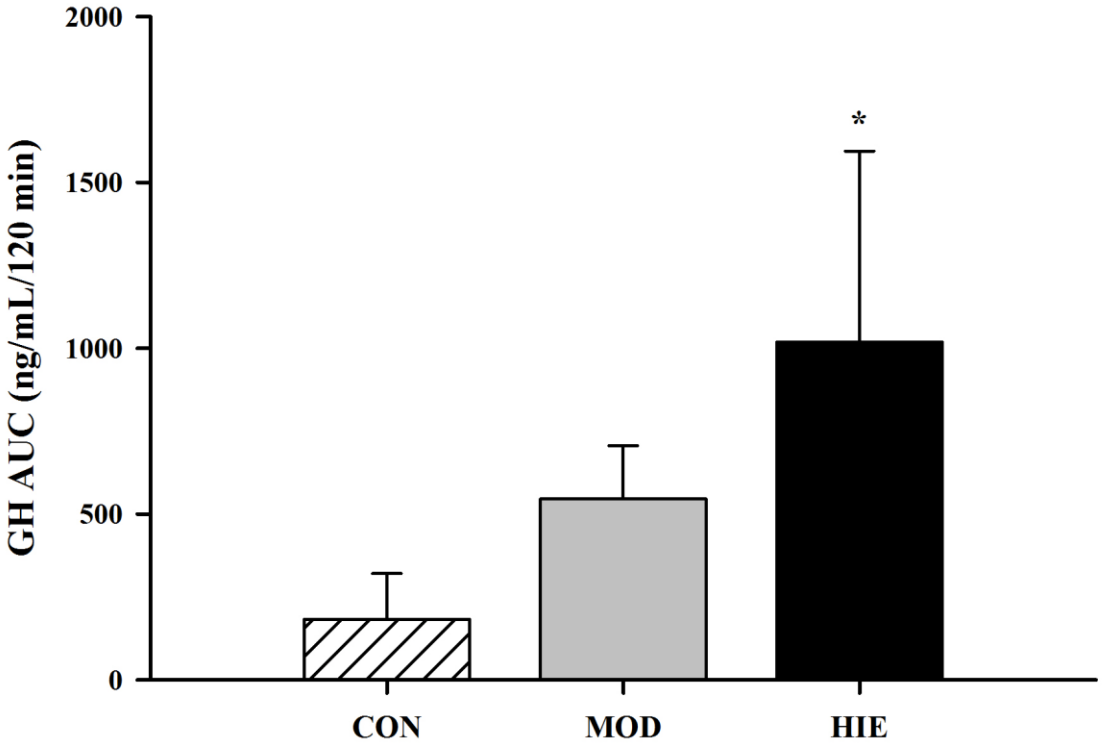

Figure 10. Calculated serum growth hormone area under the curve (AUC) from 10-min sampling over 120 minutes for CON, MOD, and HIE. The calculated AUC over the 120-min period was significantly greater after HIE compared to CON. *Compared to CON (p = .046).
Note: AUC is reported as mean ± SD. n = 5.

Growth Hormone Deconvolution Analysis

Deconvolution parameters (mean GH concentration [$ng \cdot mL^{-1}$], total pulsatile secretion rate [$ng \cdot mL^{-1} \cdot 12.5h^{-1}$], number of bursts, interval between bursts [min], mean burst height [ng], and mean area under bursts [$ng \cdot mL^{-1}$] are presented in Table 3. The mean GH concentration was observably higher during the HIE trial (2.51 ± 1.36 $ng \cdot mL^{-1}$) compared to the CON trial (1.40 ± 0.31 $ng \cdot mL^{-1}$), but this difference was not significant ($p = .37$). The estimated total pulsatile secretion rate for GH during the 12.5 h was highest during the HIE trial (1831.20 ± 873.88 $ng \cdot mL^{-1} \cdot 12.5h^{-1}$) compared to MOD ($1429.22 \pm 205.94$ $ng \cdot mL^{-1} \cdot 12.5h^{-1}$) and CON ($1040.33 \pm 241.96$ $ng \cdot mL^{-1} \cdot 12.5h^{-1}$), but the difference between these trials was not significant ($F(2, 8) = 2.531$, $p = .107$, $\omega^2 = .18$). Due to large variability in individual responses, the deconvolution parameter data was log-transformed. Using the log-transformed data, total pulsatile secretion was significantly affected by trial ($F(2, 8) = 3.495$, $p = .081$, $\omega^2 = .25$) such that total GH pulsatile secretion during HIE was significantly elevated compared to CON ($p = .050$) (Figure 11). Estimated pulsatile secretion during MOD was higher than CON ($p = .083$), but this did not reach significance; and there was no difference in pulsatile secretion between MOD and HIE ($p = .518$). Other deconvolution parameters measured were not different between the three trials.

Table 3

Deconvolution Analysis Parameters for GH Concentrations Across Each Trial

	CON	MOD	HIE
Mean GH	1.40 ± 0.31	1.93 ± 0.25	2.51 ± 1.36
Total Secretion	1040.33 ± 241.96	1429.22 ± 205.94	1831.20 ± 873.88†
Pulse #	10.0 ± 3.7	8.8 ± 1.3	9.2 ± 4.0
Interpulse Interval	74.19 ± 46.78	80.00 ± 12.21	89.80 ± 42.14
Pulse Height	0.61 ± 0.50	0.35 ± 0.09	0.45 ± 0.18
Pulse Area	10.94 ± 7.17	9.42 ± 4.24	12.05 ± 4.89

Note. Mean ± SD. n = 5. Units for mean GH ($ng \cdot mL^{-1}$); total secretion = total *pulsatile* secretion rate ($ng \cdot mL^{-1} \cdot 12.5h^{-1}$); pulse height (ng); interpulse interval (min); and pulse area ($ng \cdot mL^{-1} \cdot min^{-1}$). * Compared to CON ($p = 0.083$); † Different from CON ($p = 0.050$).

To remove the effect of exercise on total pulsatile secretion, data were analyzed for the time frame during which participants were in bed (2230h – 0600h). There was no difference between trials in total pulsatile GH secretion rate for sleep only ($F(2, 8) = 0.419$, $p = .671$, $\omega^2 = 0$) (Figure 11). There were also no differences in other deconvolution parameters between the three trials during sleep. Raw hormone data and estimated GH secretory rate for each participant can be seen in Figures 12 - 16.

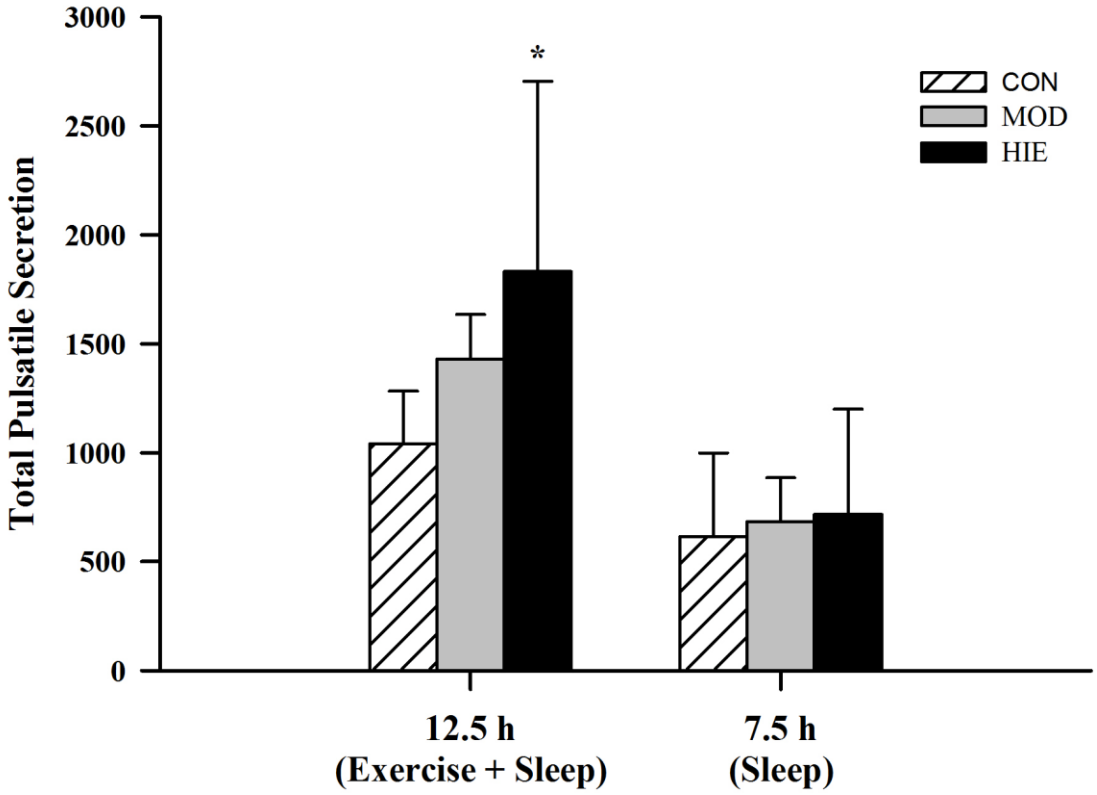

Figure 11. Estimated GH secretory rate for the total time frame (12.5h) and during sleep only (7.5h). Total pulsatile GH secretion ($ng \cdot mL^{-1} \cdot 12.5h^{-1}$) was greater in HIE compared to CON for the total time (12.5h) (p = .05). There were no differences in GH secretory rate ($ng \cdot mL^{-1} \cdot 7.5h^{-1}$) during sleeping between the three trials. *Note.* Values reported are mean ± SD. n = 5.

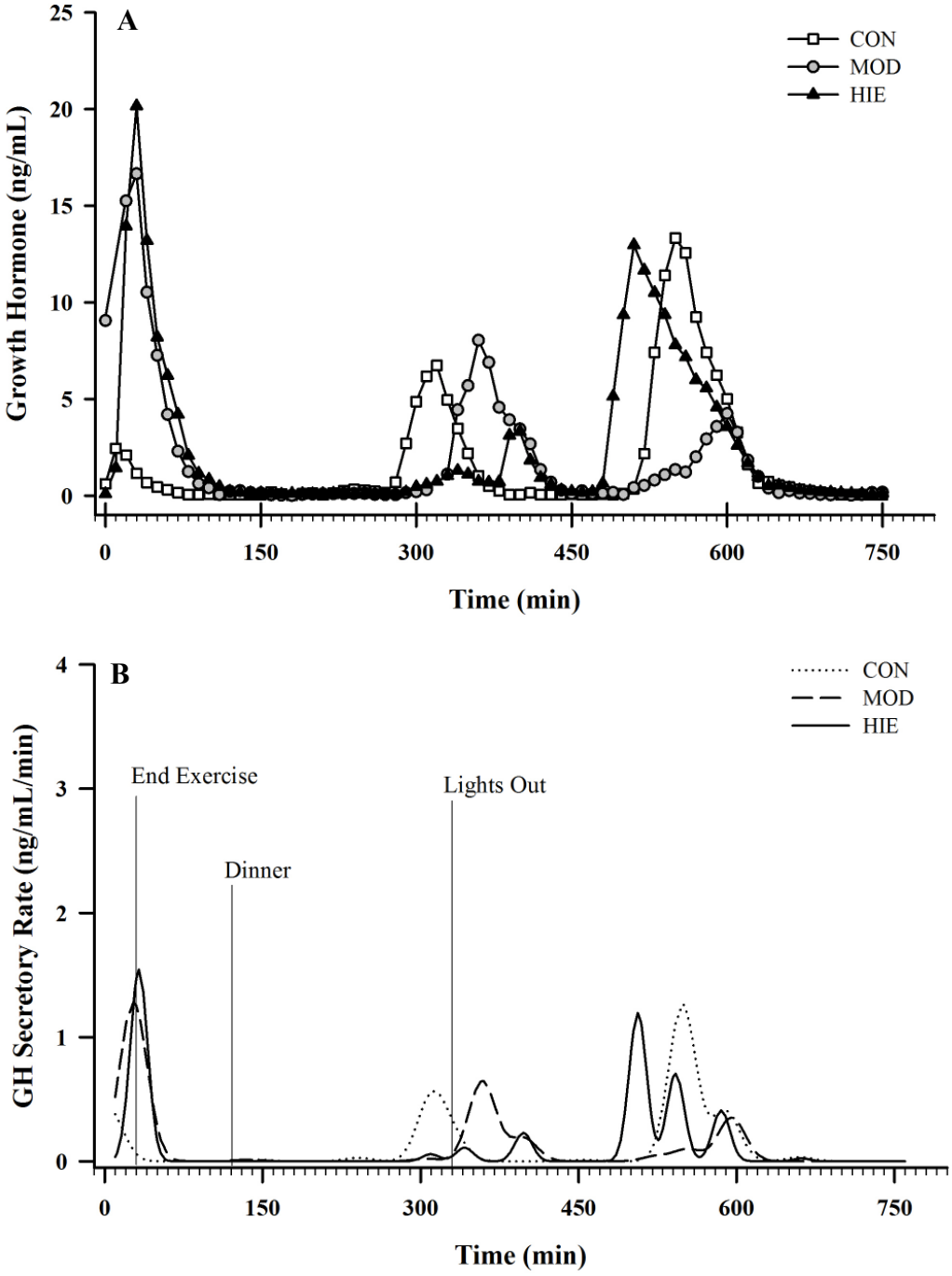

Figure 12. (A) Raw GH secretion data for GH-001 and (B) the deconvolution-estimated secretion rate.

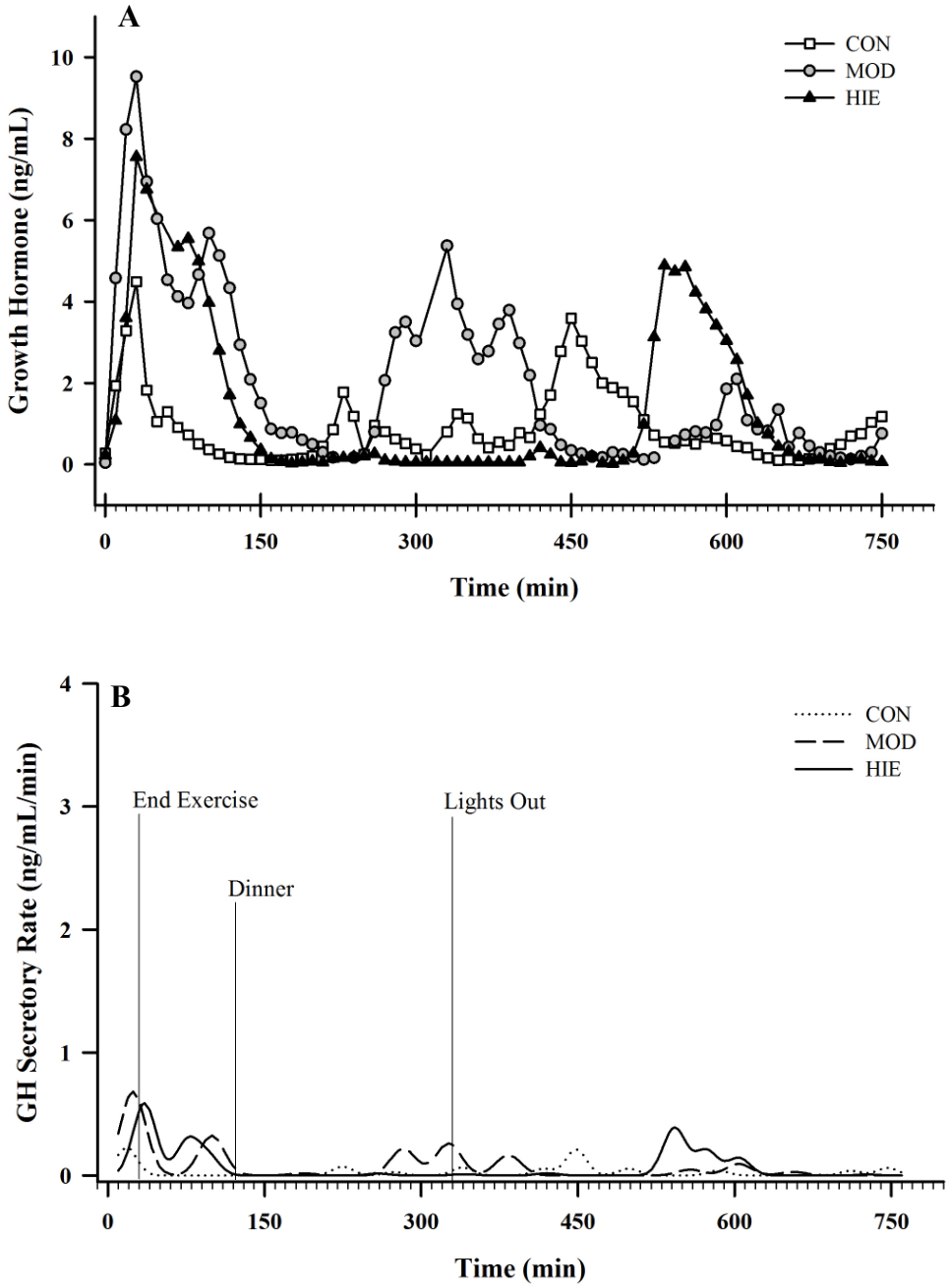

Figure 13. (A) Raw GH secretion data for GH-002 and (B) the deconvolution-estimated secretion rate.

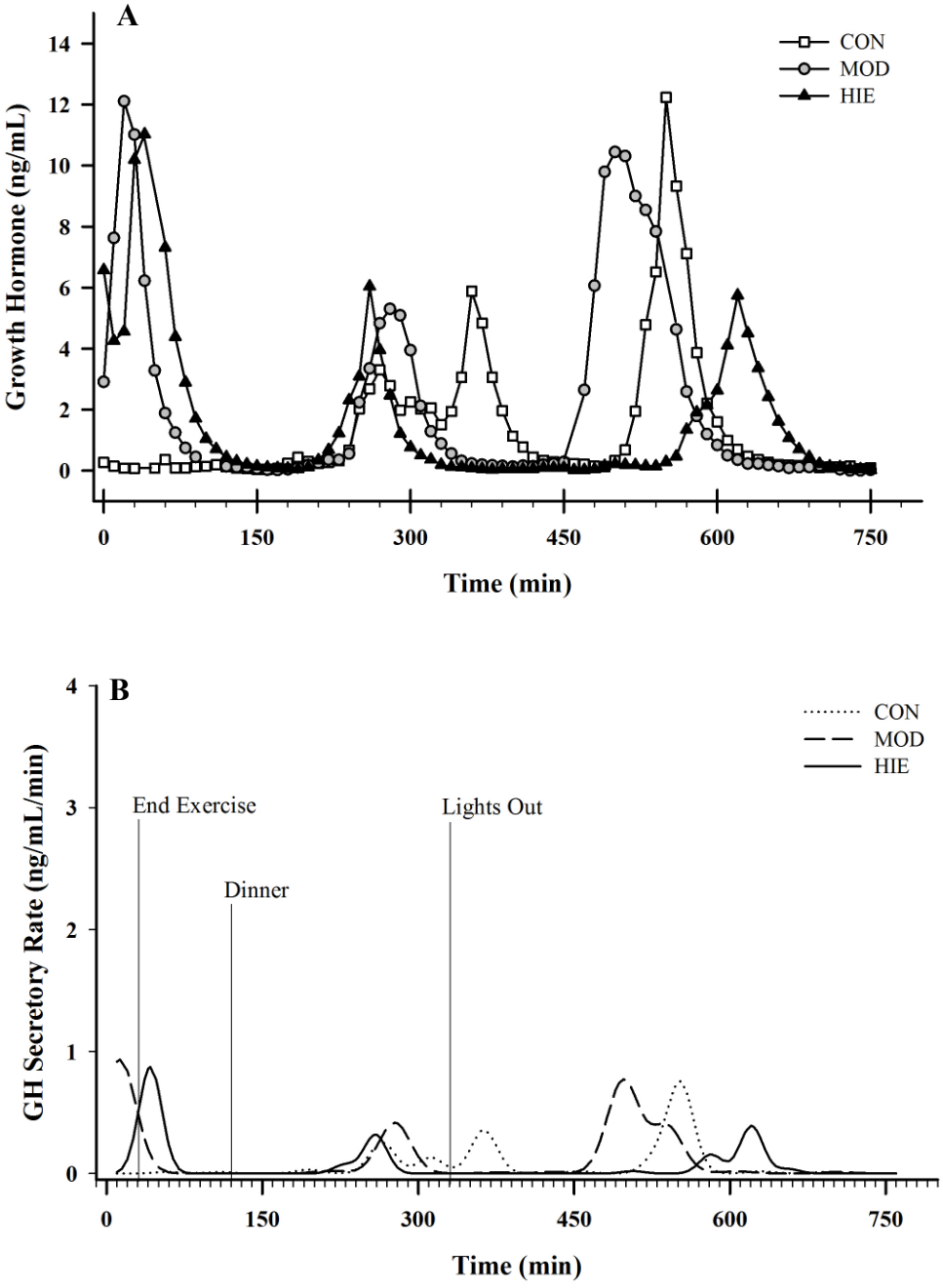

Figure 14. (A) Raw GH secretion data for GH-008 and (B) the deconvolution-estimated secretion rate.

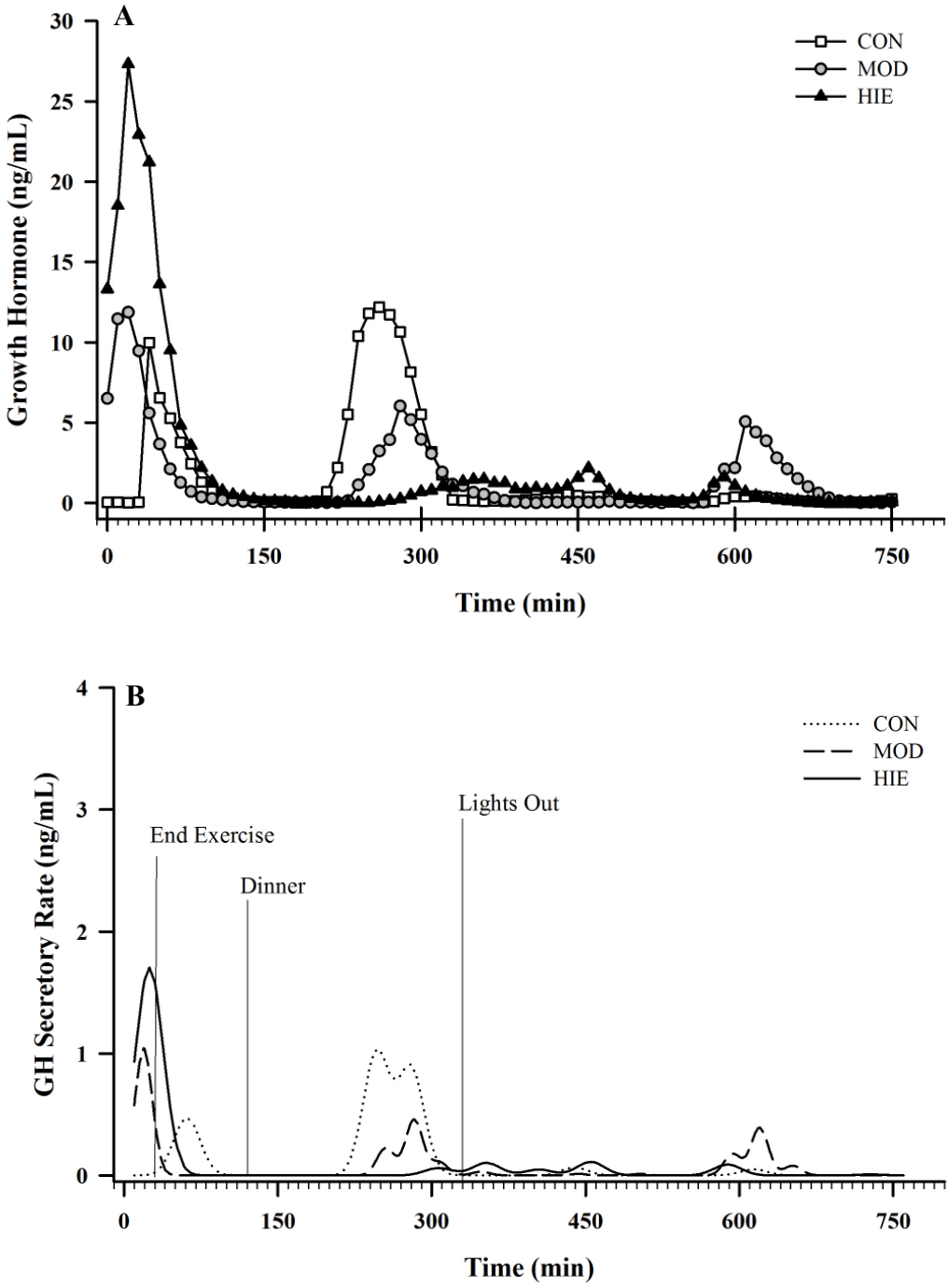

Figure 15. (A) Raw GH secretion data for GH-009 and (B) the deconvolution-estimated secretion rate.

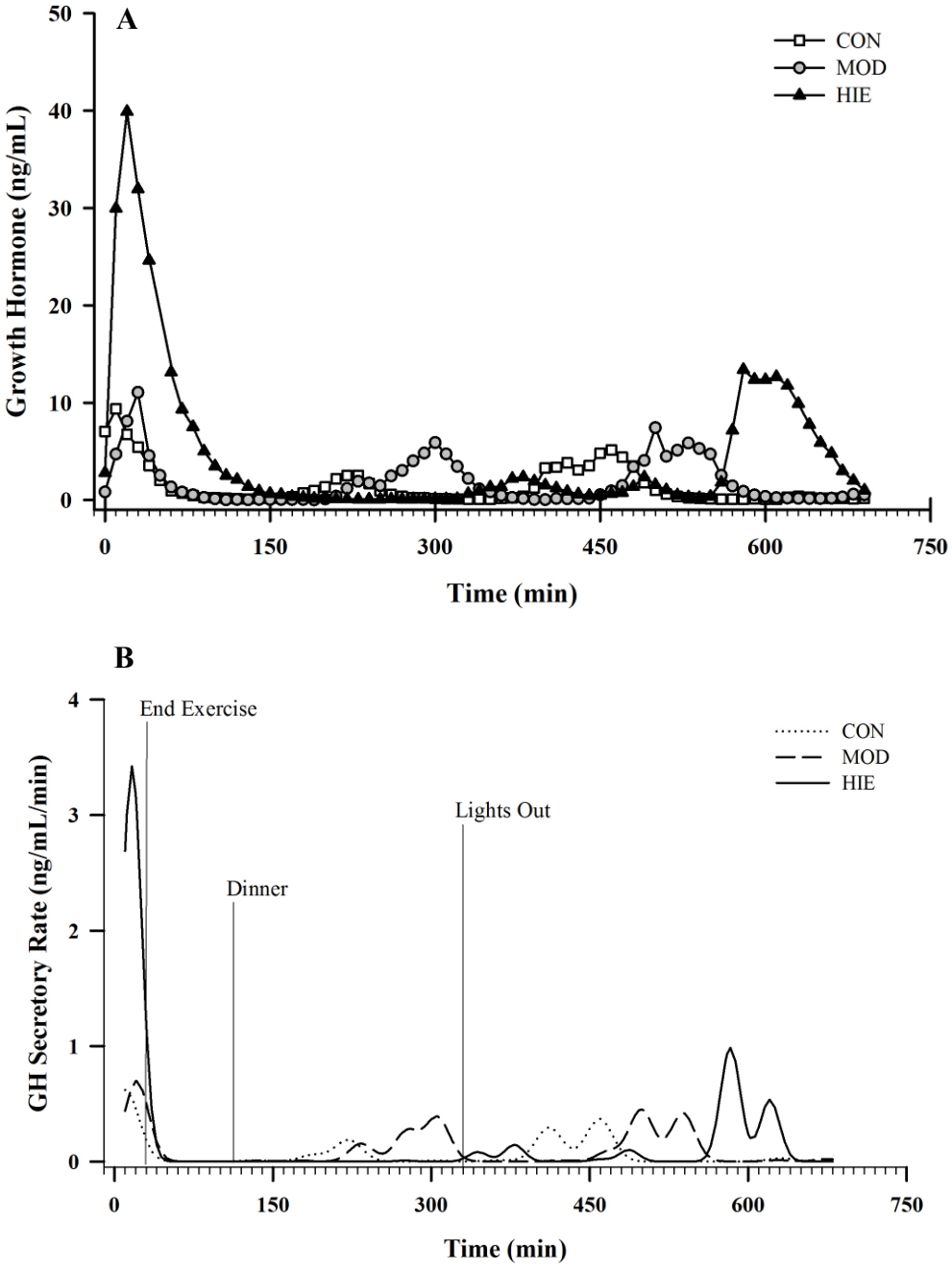

Figure 16. (A) Raw GH secretion data for GH-010 and (B) the deconvolution-estimated secretion rate.

CHAPTER V

DISCUSSION AND SUMMARY

Exercise training is an effective non-pharmacological method through which an increase in GH pulsatile secretion can be achieved. The purpose of this study was to (1) determine if pulsatile GH secretion was increased by high-intensity interval exercise (HIE) compared to moderate-intensity exercise (MOD) and no exercise (CON), and (2) measure the effect of an acute bout of exercise on substrate oxidation (NPRQ) 12h post-exercise. A major action of growth hormone (GH) is to stimulate free fatty acid mobilization from adipose tissue through pulsatile secretion, which leads to an increase in circulating fat available for oxidation (Cersosimo, Danou, Persson, & Miles, 1996; Surya et al., 2009).

The major findings of this study were: (1) lactate response to exercise intensity was dose-dependent; (2) the GH response to HIE was augmented compared to CON, but not different from MOD; (3) total pulsatile GH was increased in HIE compared to CON; (4) overnight GH pulsatile secretion was not influenced by MOD or HIE; and (5) resting fat oxidation was not significantly changed 12h after a single bout of HIE or MOD compared to CON.

Participant Characteristics

Women recruited for this study were required to be sedentary and normal weight (23.0 kg/m^2) to obese (35.0 kg/m^2). The mean BMI of this group of women was 27.4 ± 3.0 kg/m^2, with a range of 23.1 – 30.8 kg/m^2. Thus, by BMI standards, these women

would be classified as overweight (American College of Sports Medicine, 2014a). However, mean percent body fat (%BF) measured by DXA was 39.2% ± 1.7% (range: 37.0% – 40.9%), which coincides with data presented by Gallagher et al. (2000) suggesting that these women would be classified as right on the cusp of overweight and obese (American College of Sports Medicine, 2014a; Gallagher et al., 2000). Only one participant had a BMI in the normal classification (23.1 kg/m^2), but her estimated %BF was 37.0%. The American College of Sports Medicine recommends a %BF between 20%-32% as satisfactory for good health.

Growth hormone secretion is highly influenced by obesity. Even though these women had a high %BF, basal GH concentration was within normal ranges for pre-menopausal women (< 10 ng/mL, Melmed & Kleinberg, 2003) with mean baseline value for all 3 trials equal to 3.37 ± 4.15 ng/mL. These women also had a low amount of visceral fat mass. The mean value was less than 1 lb (± 0.2 lb) of visceral fat measured by DXA. Thus, it may be safe to assume that growth hormone secretion in this group of women was within a normal, healthy range. However, it is difficult to justify this, as we did not test a "lean" group for comparison. It is well characterized that obesity, especially visceral adiposity, is associated with a reduction in circulating GH (Rasmussen et al., 1995; Steyn et al., 2013; Vahl et al., 1997a). Pre-menopausal women with a large visceral fat area (identified by MRI) had a 4-fold reduction in mean plasma GH concentration, basal GH secretion rate, and total daily secretion compared to normal weight women and women with a small visceral fat area (Pijl et al., 2001). Interestingly, there were no

differences in GH secretion between normal weight women and obese women with a small visceral fat area (Pijl et al., 2001), and following a 50% reduction in body weight, women with a large amount of visceral fat continued to have blunted GH secretion compared to the other two groups, despite a 40% reduction in visceral fat mass (Pijl et al., 2001). Clearly visceral adiposity plays an important role in GH secretion regulation. In fact, in GH-deficient adults, treatment with recombinant human growth-hormone redistributed fat from android to gynoid (Zachmann, Fernandez, Tassinari, Thakker, & Prader, 1980), and in a 6 month placebo-controlled trial, GH-deficient adults had a decrease in total fat mass (including trunk fat) and waist-to-hip ratio (Cuneo et al., 1998). However, within the first 3 months of the study, 84% of the patients reported adverse effects to the GH-treatment (Cuneo et al., 1998).

GH Response to Exercise

The mechanism of action of GH on peripheral tissues is dependent upon its pulsatile delivery from the hypothalamus (Surya et al., 2009). Loss of pulsatility in GH secretion is characteristic of the diminished GH concentrations observed in obesity (Langendonk et al., 1999; Veldhuis et al., 1995). Exercise training can increase plasma GH release (Chang, Dodds, Sullivan, Kim, & Malarkey, 1986; Chwalbinska-Moneta et al., 1996; Felsing et al., 1992; Godfrey et al., 2003; Kanaley et al., 1997; Kindermann et al., 1982; Lassarre et al., 1974; Pritzlaff et al., 1999; 2000; Pritzlaff-Roy et al., 2002; D. L. Thompson et al., 1993; Weltman et al., 1992; 2000; 2006; Wideman et al., 1999), and it appears that the magnitude of the response of GH to exercise is intensity-dependent (Pritzlaff et al., 1999; Pritzlaff-Roy et al., 2002). Growth hormone calculated AUC (0-

120 min) after HIE was significantly greater than the no exercise trial in this group of women. Interestingly, the moderate-intensity bout was not significantly elevated from CON or different from HIE. There is much discussion surrounding a minimum threshold of exercise intensity to promote GH release. Pooled data from 29 studies identified a minimum threshold for GH secretion at an intensity above 40% VO_{2max} (Godfrey et al., 2003). Yet, research more consistently shows that exercise above the lactate threshold amplifies pulsatile release of GH, while exercise below the threshold does not (Godfrey et al., 2003; Luger et al., 1992; Weltman et al., 1992). However, in young, recreationally-active men, pulsatile GH release increased linearly above baseline values with increasing exercise intensities both below and above the lactate threshold (Pritzlaff et al., 1999). The lowest measured exercise intensity for Pritzlaff et al. (1999) was at 25% between the VO_2 measured at rest and the VO_2 at lactate threshold (LT), while the highest exercise intensity was at 75% of the difference between VO_2 at LT and VO_{2peak} (Pritzlaff et al., 1999). In the current study, lactate threshold was not measured, but 3 out of the 5 participants were at a VO_2 above their estimated ventilatory threshold (determined from the breakpoint in V_E plotted against VO_2 during the VO_{2max} test) which is highly correlated with lactate threshold (Brooks, Fahey, & Baldwin, 2005e). Mean VO_2 during the MOD trial was at approximately 68% VO_{2max}, and mean lactate concentration during the 30 min bout was 3.9 ± 0.7 mmol/L, which is considered around the onset of blood lactate accumulation (Brooks, Fahey, & Baldwin, 2005e), further suggesting the work rate of these women was at an intensity high enough to promote GH secretion. Two of the three women that were at an exercise intensity above their ventilatory threshold had

mean lactate concentrations during the moderate-intensity session above 4.0 mmol/L, with one participant's value at 4.9 mmol/L. Thus, it is interesting that we did not see a significant increase in GH following our moderate-intensity exercise. Obesity has been shown to attenuate exercise-stimulated GH release (Kanaley, Weatherup-Dentes, Jaynes, & Hartman, 1999; Veldhuis et al., 1995; Weltman et al., 2008), but visceral fat has a much stronger relationship to this response than overall obesity (r = -.68) (Vahl et al., 1997a). Given that our women did not have a large amount of visceral fat, as estimated by DXA, the non-significant increase in GH AUC following MOD is more likely due to a lack of power resulting from a small sample size. Indeed, the measured effect size (ω^2 = .485) for this data (GH AUC) was considered large (Okada, 2013), and given that we were able to reject our null hypothesis, this study is underpowered.

One of the novel aspects of this study was the quantification of pulsatile GH secretion following high-intensity interval exercise by using deconvolution analysis. For a given hormone, the concentration in circulation is dependent upon three variables: (1) the secretion rate of the hormone, (2) the half-life of elimination, and (3) the distribution volume of the hormone (Veldhuis & Johnson, 1992). The pulsatile release of GH from the pituitary is largely dependent on the interplay between growth hormone releasing hormone (GHRH) and somatostatin (Friend, Iranmanesh, & Veldhuis, 1996; Frohman & Kineman, 2010; Jansson & Dickson, 1999; Veldhuis et al., 1991). Pulsatile delivery (0.5 $mg/m^2 \cdot d^{-1}$) of GH in obese adults more than doubled the rate of lipolysis compared to baseline values (Surya et al., 2009). Few studies have assessed how exercise can influence pulsatile secretion and to what extent physical activity could potentially

optimize GH secretory profile in a GH-deficient population (e.g., obesity). The results of this study demonstrated that total pulsatile GH secretion was increased with HIE compared to no exercise. Moderate-intensity exercise also increased total pulsatile secretion, but this increase was not significant. The difference in GH pulsatile secretion was not dependent on total work done, as the HIE trial burned significantly fewer calories during exercise than MOD. Two hours of aerobic exercise at ~70% VO_{2peak} was sufficient to increase GH pulsatile secretion over a 20h period, but this increase was not observed after 1h of aerobic exercise at the same intensity or resistance exercise for 1 and 2 hours (Nindl et al., 2014). Additionally, three 30-min bouts of exercise at 70% VO_{2max} separated throughout the day was sufficient to increase 24h GH secretion (Kanaley et al., 1997) and intermittent exercise (10 min bouts at 50% between LT and VO_{2peak}) scattered throughout the day similarly increased 24h-GH secretion as a single 30-min bout at the same intensity compared to control (Weltman et al., 2008). As expected, the magnitude of this response was attenuated in obese participants, but not different between males and females (Weltman et al., 2008).

While a few studies have examined the GH response to high-intensity interval exercise, this is one of the first to measure the GH secretory response following exercise that also includes overnight secretion data. Nocturnal GH secretion accounts for approximately 85% of total daily GH output (Veldhuis et al., 2004), and it was therefore hypothesized that GH secretion would be augmented overnight following an acute bout of high-intensity interval exercise compared to control. However, there was no difference in overnight GH secretion between the three trials. Kern et al. (1995) found a

compensatory reduction in nighttime GH secretion with a single bout of moderate-intensity long-duration (4 h) exercise such that the 24h GH concentration measured after exercise was not different from control (Kern, Perras, Wodick, Fehm, & Born, 1995). Conversely, 16 weeks of training in participants with the metabolic syndrome increased nocturnal GH secretion in both low-intensity training and high-intensity training groups compared to control (Irving et al., 2009). One of the major contributors to GH secretion at night is achievement of deep (slow-wave) sleep (Van Cauter, 2000). We did not measure sleep quality in the current study, nor did we provide a "familiarization" sleep session in our lab prior to participation. It is possible that participants in this study had "disturbed" sleep (e.g., they did not enter deep sleep), and thus the overnight secretory profile may not be as robust as what the normal secretory profile would be.

Regulation of Substrate Oxidation During and After Exercise

Mobilization of fat from adipose tissue can be stimulated by β-adrenergic activation and α-adrenergic inhibition, insulin concentrations, and GH secretion. Adipocytes have both α- and β-receptors on the plasma membrane that respond to catecholamines in an opposite fashion, with α-receptor stimulation inhibiting lipolysis, and β-receptor stimulation increasing lipolysis (Galitzky, Lafontan, Nordenström, & Arner, 1993; Lafontan et al., 1997). Increased insulin following a meal increases the activity of lipoprotein lipase, leading to an increase in fat deposition by stimulating uptake of circulating fatty acids and re-esterification in the adipocyte (Frayn et al., 1994; Wang & Eckel, 2009). Growth hormone stimulates release of FFA from adipocytes

thereby increasing the circulation of FFA and subsequent oxidation in the liver (Godfrey et al., 2003; Johannsson, 1999).

A single bout of exercise can have a large influence on lipid metabolism through catecholamine secretion, insulin suppression, and GH release. It is well documented that moderate-intensity exercise can increase fat oxidation both during and up to 24h post-exercise (Magkos, Mohammed, Patterson, & Mittendorfer, 2009; Mulla, Simonsen, & Bülow, 2000). Growth hormone is an important mediator of post-exercise FFA mobilization, as inhibition of GH secretion by octreotide infusion had no effect on lipolysis during moderate-intensity exercise (50% VO_{2max}) but post-exercise lipolysis rates were suppressed (Enevoldsen et al., 2007). In contrast, during vigorous intensity exercise, fatty acid mobilization from adipocytes is suppressed despite an increase in catecholamine and GH secretion. This is most likely attributable to catecholamine-induced vasoconstriction leading to a decrease in adipose tissue blood flow, but this hypothesis is difficult to test with current methodologies (Hodgetts, Coppack, Frayn, & Hockaday, 1991; Romijn et al., 1993; Romijn, Coyle, Sidossis, Zhang, & Wolfe, 1995; D. Thompson, Karpe, Lafontan, & Frayn, 2012). Furthermore, lactate is a strong inhibitor of lipolysis, and lactate production will increase with exercise intensity (Hawley, 2002). However, lactate is also known to promote GH secretion (Chwalbinska-Moneta et al., 1996; Elias et al., 1997). The post-exercise lipolytic rate following high-intensity exercise has not been fully elucidated, but it stands to reason (given the aforementioned roles of catecholamines and growth hormone) that there would be an increase in fat oxidation and mobilization following high-intensity exercise. Magkos et al. (2009) demonstrated a

positive correlation between the rate of appearance (Ra) of FFA and exercise intensity, supporting the hypothesis that exercise intensity is an important determinant of FFA mobilization after the cessation of exercise (Magkos et al., 2009). However, this response may be sex-dependent, as men have increased Ra glycerol (Ra_{GL}) concentrations during exercise at 45% VO_{2max} compared to 65% VO_{2max} where women did not demonstrate a difference between the two exercise intensities for Ra_{GL} (Henderson et al., 2007). Additionally, men demonstrated an elevated Ra_{GL} during the 3h post-exercise recovery period unlike women (Henderson et al., 2007). Although a difference in Ra_{GL} existed between the two exercise intensities in males, with the higher exercise intensity eliciting a lower rate of FFA mobilization, exercise at 65% VO_{2max} is not considered vigorous or high-intensity and thus interpretation of these results for high–intensity is limited.

For this study, measurement of substrate oxidation following exercise (by NPRQ) 12h after a single bout of exercise demonstrated a non-significant intensity-dependent increase in reliance on fat following MOD and HIE. Measurement of resting fat oxidation can be complicated by the effect of energy balance; in general, a negative energy balance is associated with an increase in fat oxidation, and a positive energy balance associated with increased carbohydrate oxidation (Rumpler, Seale, Miles, & Bodwell, 1991; D. Thompson et al., 2012). It can be assumed that given the mean energy expenditure of each exercise session (approximately 145 kcal for MOD and 97 kcals for HIE) and the mean energy intake for dinner (397 kcal), that the reduction in NPRQ following HIE is not due to a negative energy balance. Therefore, it is reasonable to speculate that the

increased tendency to utilize fat following HIE in these women may be related to an increase in circulating FFA, and thus increased fat available for oxidation.

Summary

This is the first study to examine the influence of high intensity interval exercise on pulsatile growth hormone secretion that includes overnight GH measurements. We found that HIE increased total GH pulsatile secretion compared to control, but did not influence the overnight GH secretory pattern. The HIE also resulted in a significant increase in GH AUC measured up to 1.5h post exercise. While MOD pulsatile GH secretion was higher compared to control, this difference was not significant. Further research into the effect that HIE has on GH secretion, and whether HIE training (e.g. 2+ weeks) would elicit a significant response requires additional investigation.

Recommendations for Future Study

1. Measure the effect of a 2-week program of high-intensity interval exercise on GH secretory profile in normal weight and obese adults.

Trained individuals demonstrate a blunted GH response to acute exercise compared with untrained controls when intensity is held constant (Hartley, 1975; Hartley et al., 1972; Koivisto, Hendler, Nadel, & Felig, 1982; Weltman et al., 1997). Measured GH secretion from 20-min of high-intensity constant load aerobic exercise was decreased significantly (45%) over a 6 week training program (Weltman et al., 1997), further supporting the idea that a minimum intensity must be achieved to stimulate GH release. It is possible that chronic exercise training leads to GH negative feedback at the hypothalamus such that a reduced GH burst is seen in response to a given exercise

stimulus. High intensity interval exercise may help diminish the "blunting" effect of training due to the nature of the exercise generally being an "all-out" intensity, and therefore a person is always training "maximally." This type of training in obese individuals may benefit them 2-fold: first, HIIT is associated with a significant reduction in fat mass (Perry, Heigenhauser, Bonen, & Spriet, 2008; Talanian et al., 2007; Tremblay et al., 1994; L. J. Whyte, Gill, & Cathcart, 2010; H. Zhang et al., 2017); second, HIIT may result in augmented GH secretion, which can improve cardiometabolic profile (Blüher et al., 2017; Gillen & Gibala, 2014) and increase lipolysis, potentially providing a mechanism for fat loss with HIIT.

2. How does high-intensity interval exercise influence the physiology of the adipocyte?

Adipocytes function as both storage vesicles for triglycerides and as an endocrine organ releasing various hormones that play an important role in metabolic homeostasis. Fat loss is accelerated with high-intensity interval training (Gillen et al., 2013; Gillen & Gibala, 2014), but the influence of HIIT on the structure and function of the adipocyte has not been quantified. Catecholamines, in particular epinephrine stimulate fat mobilization from adipose tissue, and given that high-intensity exercise results in an augmented catecholamine response (Weltman et al., 1994), it is possible that the tissue becomes more sensitive to it's effects. Furthermore, insulin sensitivity is improved with two weeks of HIIT (Richards et al., 2010) and exercise is known to decrease circulating insulin, which has a strong inhibitory role in fat mobilization. Finally, HIIT (and GH) seem to have more of an effect on visceral compared to subcutaneous mobilization of

FFA from adipose tissue; the mechanisms behind this "preference" need to be fully elucidated.

3. Try to understand what influences "somatostatin tone."

As has been stated in the above paragraphs, obesity is associated with blunted GH secretion that is sometimes attributed to a greater "somatostatin tone." However, an exhaustive search of the literature has not been able to fully elucidate what factors of obesity may influence this higher amount of somatostatin that would blunt GH release.

4. Examine the effect a high protein meal/drink has on overnight GH secretion pattern.

Recently a study published out of Michael Ormsbee's lab at Florida State University demonstrated a reduction in fat mass with exercise training combined with protein intake prior to bed (Ormsbee et al., 2015). It would be interesting to examine the mechanisms behind this response. A quick search of the literature revealed no published studies that examine the role that protein intake has on GH secretion. However, protein intake has been associated with increased IGF-1 secretion. Given that IGF-1 is a downstream hormone of GH, protein intake before sleep may augment GH pulsatile release (when it is already stimulated).

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APPENDIX A

Institutional Review Board Approval Letter

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940-898-3378
email: IRB@twu.edu
http://www.twu.edu/irb.html

DATE: October 15, 2015

TO: Ms. Sarah Deemer
Kinesiology

FROM: Institutional Review Board (IRB) - Denton

Re: Approval for The Effect of an Acute Bout of High Intensity Interval Exercise Compared to Steady State Moderate Intensity Exercise on Growth Hormone Secretion in Young, Sedentary Women (Protocol #: 18537)

The above referenced study has been reviewed and approved at a fully convened meeting of the Denton IRB (operating under FWA00000178) on 10/14/2015. This approval is valid for one year and expires on 10/13/2016. The IRB will send an email notification 45 days prior to the expiration date with instructions to extend or close the study. It is your responsibility to request an extension for the study if it is not yet complete, to close the protocol file when the study is complete, and to make certain that the study is not conducted beyond the expiration date.

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. A copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc. Dr. David Nichols, Kinesiology
Dr. Kyle Biggerstaff, Kinesiology
Graduate School

APPENDIX B

Participant Recruitment Materials

Research Participants Needed!!!

Can H.I.I.T increase your ability to burn fat?

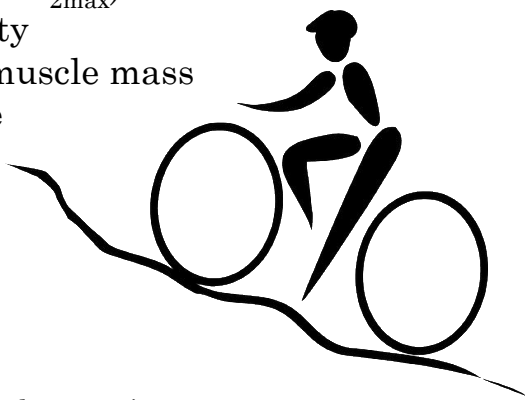
Female participants are needed to examine the influence of high-intensity interval exercise compared to moderate-intensity exercise on growth hormone secretion and fat metabolism.

If you are:

- Woman aged 18-35 years old
- Exercise less than 3 days per week
- BMI 23 – 35 kg/m^2 (normal weight – obese)

Benefits of Participation Include:

- $100 upon completion of study
- Your aerobic fitness level (VO_{2max})
- Your measured bone density
- Your % body fat and lean muscle mass
- Lipid and metabolic profile



If interested, please contact:
Sarah Deemer
sdeemer@twu.edu

Participation is voluntary and may be discontinued at any time.
All email conversations are confidential and kept between the addressee and research team member. There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions.

Verbal Recruitment Script

Hello,

I am recruiting women to participate in a study looking at how the concentration of a hormone in your body (growth hormone) is influenced by exercise intensity. After an initial visit, you will be asked to come back to the lab on 3 different occasions, where you will be asked to stay overnight. These overnight stays will involve either no exercise, high-intensity exercise, or moderate-intensity exercise followed by blood sampling overnight to look at your growth hormone secretion. We will also measure your resting metabolic rate and if your body prefers to burn fat or carbohydrate calories. If this sounds like something you would be willing to help me with, may I give you this flyer? My email address is on the flyer, please feel free to contact me with any questions you may have.

Thank you.

Email Recruitment Script

Subject Line: Looking for a Few Good Women - Kinesiology Research Study

High-intensity exercise has been in the media a lot lately, and it has become a very popular form of fitness. For example, CrossFit, OrangeTheory Fitness, and Insanity are all types of popular high-intensity workout programs. This study wants to look at how a certain hormone in your body (growth hormone) is influenced by high intensity exercise. The reason why is because growth hormone helps burn fat, and we want to see if growth hormone concentrations increase with high intensity exercise compared to moderate intensity or no exercise.

After an initial visit, you will be asked to come back to the lab on 3 different occasions, where you will be asked to stay overnight. Each of the 3 visits will involve one of the following scenarios: no exercise, high-intensity exercise, or moderate-intensity exercise. After we will take samples of your blood overnight to look at your growth hormone levels. We will also measure your resting metabolic rate (RMR) and if your body prefers to burn fat or carbohydrate calories.

To take part in this study, you must be a healthy female adult between the ages of 18 – 35 years of age and are not pregnant. You cannot have type 1 or type 2 diabetes, a history of any chronic illness such as cardiovascular, neurological, or impaired renal function. We would like for your body mass index to be between 23 – 35 kg/m^2. Here is a link to see what your BMI is:

http://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/english_bmi_calculator/bmi_calculator.html

If you are interested in participating or have any questions about the study, please email Sarah Deemer at sdeemer@twu.edu.

Thank you for your time!

Sarah Deemer, M.S., C.S.C.S.
PhD Candidate
Department of Kinesiology
Texas Woman's University

Please note, that there is a potential risk of loss of confidentiality in all email, downloading, and internet transactions.

Email Response Recruitment Script

Hello {Name},

Thank you for your interest in our study. This study is looking to determine how a certain hormone (growth hormone) concentration in your body is altered by exercise intensity. High-intensity interval training has been in the media a lot lately, and is suggested to be a great way to improve fitness and lose weight in a short amount of time. We are trying to understand why high-intensity exercise results in better weight loss than moderate-intensity (steady-state) exercise. Hopefully you will be willing to help.

If you have any of the following medical diagnoses (listed below), unfortunately you do not qualify to participate in this study. Thank you for your interest in our study.

- Diabetes
- Cardiovascular Disease
- Cancer
- Hypothyroid/Hyperthyroid Disorder
- Uncontrolled Asthma

If you do not have any of the above conditions, please answer the following questions to further determine your eligibility for participation:

1. Do you currently exercise? ☐ Yes ☐ No
2. Has a doctor ever told you that you cannot exercise? ☐ Yes ☐ No
3. Do you have any [known] adverse effects to exercise? ☐ Yes ☐ No

Thank you again for your time and interest, I will be in contact with you regarding your qualification for this study. Please let me know if there are any questions I may be able to answer in the meantime.

Sarah Deemer
Department of Kinesiology
Texas Woman's University

Note: Participation is voluntary and may be discontinued at any time.
All email conversations are confidential and kept between the addressee and research team member.

APPENDIX C

Participant Screening Form

PARTICIPANT NAME:		**SCREENER:**
HOW DID YOU HEAR ABOUT STUDY?		**DATE:**
		QUALIFY: ☐ YES ☐ NO
AGE (18 – 35)		**PARTICIPANT ID:**
YEAR BORN (1981-1998)		
APPROXIMATE HEIGHT		
APPROXIMATE WEIGHT		
CALCULATED BMI (KG/M^2)		
PREGNANT	☐ YES ☐ NO	
REGULAR PERIODS	☐ YES ☐ NO	
~ DATE OF LAST PERIOD		
MEDICAL CONDITION		
MEDICAL DISABILITY		
MEDICATIONS		
SUPPLEMENTS		
BLOOD DRAWING OK?	☐ YES ☐ NO	
DO YOU EXERCISE?	☐ YES ☐ NO	
IF YES, HOW MANY D/WK	☐ 1 DAY ☐ 2 DAYS ☐ 3 DAYS ☐ >3 DAYS	
IF YES, ~ TIME SPENT?		

SCHEDULED VISIT 1:	
PARTICIPANT ID:	
PHONE #:	
EMAIL:	
POST ADDRESS:	

NOTES:

APPENDIX D

Informed Consent Form

Texas Woman's University

Informed Consent to Participate in a Research Project

TITLE OF THE STUDY: The Effect of an Acute Bout of High Intensity Interval Exercise Compared to Steady State Moderate Intensity Exercise on Growth Hormone Secretion in Young, Sedentary Women

PRINCIPAL INVESTIGATOR: Sarah E Deemer sdeemer@twu.edu (940) 898-2549
ADVISOR: Kyle Biggerstaff, PhD kbiggerstaff@twu.edu (940) 898-2596

WHO IS DOING THE STUDY? Department of Kinesiology, Texas Woman's University

PURPOSE: You are being invited to participate in a study that is examining the effect of exercise intensity on growth hormone secretion (the amount of growth hormone that circulates in your body in the blood). High-intensity interval exercise is a type of exercise that is usually done at maximal or supra-maximal intensity for a very short amount of time. A typical high intensity interval exercise session could last anywhere from 5-20 minutes compared to the traditional mode of aerobic training which lasts 30-60 minutes. This form will explain the procedures, risks, and benefits of participation in this study. If you have questions about the information found in this form, please ask a member of the research team before signing.

WHERE IS THIS STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST?
The study will take place on the Texas Woman's University Denton campus in the Department of Kinesiology (Exercise Physiology laboratory, room 112) located in Pioneer Hall. The entire study should last approximately 3 months.

PROCEDURES

You are being asked to participate in this study which is designed to see how exercise intensity (high-intensity interval exercise or moderate-intensity steady state exercise) influences growth hormone secretion. If you qualify to participate, you will be asked to come to the lab on 4 different occasions spread out over a minimum of 3 months. Visit 1 will last approximately 1 – 2 hours and visits 2, 3, and 4 will each require 14 hours of your time. We will ask that you do not eat or drink anything (besides water) for at least 3 hours before each session. During this study several different measurements will be made: 1) anthropometric measurements; 2) body fat and bone density; 3) maximal aerobic fitness (VO_{2max}); 4) lactate response to HIIE and moderate intensity exercise; 5) hormone response to high-intensity interval exercise and moderate-intensity exercise; and 6) resting metabolic rate and substrate oxidation in response to high-intensity interval exercise or moderate-intensity exercise. Each of these is explained in more detail below:

Health History & Physical Activity Questionnaire

To assess your general health and physical activity status, you will be asked to complete a short questionnaire. When answering questions, please be as honest and accurate as possible. This form should take approximately 10-15 minutes to complete.

Anthropometric Measurements

These tests include: measurement of height, measurement of weight, and measurement of the distance around your waist and hips. These measurements will provide valuable information about your body fatness and distribution of your body fat. These tests should take approximately 10 minutes to complete.

Participant's Initials: _____ Date: _____

Approved by the
Texas Woman's University
Institutional Review Board

Approved: June 5, 2015
Modifications Approved:
January 15, 2016

Bone Density and Body Fat Percentage

The DEXA is a machine that will take an x-ray of your entire body and measure your bone density and body fat percentage (BF%). During this procedure you will lie on your back on a padded table for approximately 6 minutes. The amount of radiation you will be exposed to is very small. This test should take approximately 10 minutes to complete.

Maximal Aerobic Fitness (VO_{2max}) Test

This test measures your body's peak ability to use oxygen. The test involves exercising on a bicycle beginning with an easy intensity and gradually increasing to a point of maximal intensity at which you can no longer continue. The intensity of the exercise will get harder every couple of seconds. You will breathe in regular room air through a snorkel-like mouthpiece during this test, and a computer will analyze the air that you breathe out. Your heart rate and blood pressure will be monitored throughout this test. Your heart rate during this test will be measured by electrocardiogram (ECG). Small sticky patches (electrodes) will be applied to the skin on your chest to measure your heart's electrical signals. Before application of the electrodes, your skin will be cleaned with an alcohol swab and wiped down with a piece of gauze. Placement of these electrodes will take approximately 5 minutes to complete. At the end of this test a small blood sample (about a drop) will be taken by finger stick technique to test your blood lactate. This test should take approximately 15-20 minutes to complete.

Trial Days (Control, HIIE, Moderate-Intensity Exercise)

On 3 different days, you will come to the lab to complete one of three trials: no exercise (CON), high-intensity interval exercise (EX_{HIE}), or moderate-intensity steady state exercise (EX_{SS}). During these test days you will be asked to arrive at the lab at 5:00 PM and stay overnight until 7 AM the next morning.
Each of these visits will be separated by one menstrual cycle (~ 1 month). Each visit is explained in more detail below:

Control Day (CON)

During this day, you will be asked to arrive at the lab at 5:00 PM, at least 3 hours after your last meal. An intravenous catheter will be placed in a forearm or hand vein for blood sampling during the study. After this, you will be asked to relax; and you will be allowed to read, do homework, watch TV or movies, and sleep. There will be no exercise involved on this trial day. At 7:30 PM you will be fed dinner. At 11:00 PM the lights will be turned out, and you will be asked to go to sleep. At 6:00 AM we will wake you up and remove the intravenous catheter. From 6:30 AM – 7:00 AM we will measure the calories your body uses at rest (RMR) as well as whether your body prefers *burning* fat or carbohydrate (RER) at rest. At the end of this test, we will feed you breakfast. The total time requirement for the CON day is 14 hours.

High-Intensity Interval Exercise Day (EX_{HIE})

During this day, you will be asked to arrive at the lab at 5:00 PM, at least 3 hours after your last meal. An intravenous catheter will be placed in a forearm or hand vein for blood sampling during the study. At 5:30 PM you will begin exercise. During this exercise you will be asked to pedal the stationary bike as fast as you can for 30 seconds against a resistance equal to 7.5% of your body weight (kg). In between each sprint you will pedal slowly against a minimum resistance on the bike for 4.5 minutes while you recover. This exercise session will consist of 4 sprints for a total high-intensity exercise time of 2 minutes. Including warm-up and recovery, the total exercise time for the EX_{HIE} day will be 30 minutes. After this, you will be asked to relax; and you will be allowed to read, do homework, watch TV or movies, and sleep. At 7:30 PM you will be fed dinner. At 11:00 PM the lights will be turned out, and you will be asked to go to sleep. At 6:00 AM we will wake you up and remove the intravenous catheter. From 6:30 AM – 7:00 AM we will measure the calories your body uses at rest (RMR) as well as whether your body prefers *burning* fat or carbohydrate (RER) at rest. At the end of this test, we will feed you breakfast. The total time requirement for the EX_{HIE} day is 14 hours.

Participant's Initials: _____ Date: _____

Moderate-Intensity Steady-State Exercise Day (EX_{SS})
During this day, you will be asked to arrive at the lab at 5:00 PM, at least 3 hours after your last meal. An intravenous catheter will be placed in a forearm or hand vein for blood sampling during the study. At 5:30 PM you will begin exercise. During this exercise you will be asked to pedal the stationary bike at a comfortable pace for you at a power output equal to 70% of what your max power output was measured at. The total exercise time for the EX_{SS} day will be 30 minutes. After this, you will be asked to relax; and you will be allowed to read, do homework, watch TV or movies, and sleep. At 7:30 PM you will be fed dinner. At 11:00 PM the lights will be turned out, and you will be asked to go to sleep. At 6:00 AM we will wake you up and remove the intravenous catheter. From 6:30 AM – 7:00 AM we will measure the calories your body uses at rest (RMR) as well as whether your body prefers *burning* fat or carbohydrate (RER) at rest. At the end of this test, we will feed you breakfast. The total time requirement for the EX_{SS} day is 14 hours.

Blood Measurements

On the first day you come visit us, we will draw 10 mL of blood from your arm to make sure you are healthy and able to participate in our study. This sample will also be tested for your lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides). These results will be available to you when you have completed the study.

During the trial days (CON, EX_{HIE}, EX_{SS}) an intravenous catheter will be placed in a forearm or hand vein and will remain in place until the next morning. Before the start of exercise a blood sample will be drawn (16 mL) for measurement of your estrogen, lactate concentration, and your growth hormone concentration. During the exercise, at 5-min increments, your blood will be tested for lactate and other exercise-related hormones. After the exercise (or control session), beginning at 6:00 PM, a small amount of blood (3 mL) will be drawn at 10-min time intervals to measure the amount of growth hormone your body is secreting. The total amount of blood drawn on a given trial day (CON, EX_{HIE}, or EX_{SS}) is approximately 22-25 tbsp (310-360 mL). The total amount of blood drawn for the entire study will be approximately 73 tbsp (1.06 L).

The total time commitment from your participation in this study is approximately 44 hours.

BENEFITS OF PARTICIPATION

From the results of these tests you will be told your maximal aerobic capacity (VO_{2max}), your bone mineral density, your body fat percentage, your lipid profile, your resting metabolic rate, and whether your body preferentially burns fats or carbohydrates. Some of your results will be immediately available at the time of testing (body fat for example) while others will be provided later. Following completion of data collection for the entire study, you may be provided with the generalizable study results upon request. Additionally, after you have completed all study visits, you will be paid $100.

POTENTIAL RISKS OF PARTICIPATION

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

There are potential risks that may occur while participating in this research. For purposes of privacy, the doors to the testing rooms will be locked while you are being tested. There are no known physical risks to any of the surveys and anthropometric measurements.

Participant's Initials: _____ Date: _____

DEXA Scan: The risks associated with the DEXA to measure body fat and bone density are very low. The radiation you will receive in this study is less than 1/3000th of the FDA limit for annual exposure. Put another way, you could receive 3000 DEXA scans in a single year and still not meet the FDA limit for radiation exposure. In this study, you will receive one scan. The more radiation you receive over the course of your life, the more is the risk of having cancerous tumors or causing changes in genes. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risks is not known. Women who are pregnant or could be pregnant should receive no unnecessary radiation and should not participate in this study.

Exercise Testing (VO_{2max}): Exercise rarely causes any problems in normal participants, but in individuals with known or hidden heart disease, exercise may cause chest pain, dizziness, or bouts of irregular heart rhythms. Exercise will be stopped immediately if there are any signs of excessive strain. There is no additional risk involved with completing the peak exercise test beyond that normally associated with exercise and hard physical exertion. With any type of exercise testing or training, there is the possibility of injury or discomfort. During intense training and/or testing, the risk of having a heart attack or even dying goes up slightly; however, the risk in a patient with no history of heart disease is low, and your overall risk of heart attack or death over the time of exercise training will actually decrease. Exercise may also occasionally be accompanied by abnormal blood pressure, nausea, fainting, muscle soreness, joint and bone injury, and in rare instances, heart attack, stroke, or death. Every precaution will be taken to minimize these risks by closely monitoring your vital signs (heart rate and blood pressure) throughout exercise, and all technicians are certified in CPR and AED techniques.

Shortness of Breath, lightheadedness, nausea (EX_{HIE}): High-intensity exercise training has been associated with shortness of breath, fatigue, light-headedness, and in some cases nausea. In order to minimize these effects, you will do a 5-minute warm-up and be instructed through an active recovery during each exercise session. Active recovery has been associated with a reduction in the afore-mentioned risks. Furthermore, each exercise session will be supervised by the PI and both heart rate and rating of perceived exertion (RPE) will be recorded for each session.

Muscle soreness/joint soreness/muscle fatigue (VO_{2max}, EX_{HIE}, EX_{SS}): You may also experience local muscle and/or joint soreness following exercise. To reduce (but not completely avoid) the effect of muscle/joint soreness, you will be allowed a time for a warm-up and cool-down, and will be advised about stretching exercises.

Finger Stick (Lactate measurement, day 1): This will feel like a little pin-prick that may be associated with some discomfort and/or bruising. To minimize bruising, pressure will be applied to the site for approximately one minute after the stick.

Standard Venipuncture (Day 1): The risks of collecting a blood sample include the possibility of requiring more than one attempt to obtain the blood sample, local discomfort (pinch when the needle enters your skin), minor bruising or bleeding at the site (10%), possible temporary lightheadedness, infection (<0.01%), or development of a blood clot (<0.01%). A trained and experienced individual will perform the technique and your blood will be collected in a hygienic setting with sterile materials and biohazard protection measures to minimize these risks.

Participant's Initials: _____ Date: _____

Peripheral Venous Catheter (CON, EX_{HIE}, EX_{SS}): The risks of collecting a blood sample will include the possibility of requiring more than one attempt to obtain the blood sample, local discomfort (pinch when the needle enters your skin), minor bruising or bleeding at the site (10%), or possible temporary lightheadedness, infection (<0.01%), or development of a blood clot (< 0.01%). These risks are slightly increased compared to a standard blood draw. A trained and an experienced individual will perform the technique and your blood will be collected in a hygienic setting with sterile materials and biohazard protection measures to minimize these risks.

Discomfort from Venous Catheter (CON, EX_{HIE}, EX_{SS}): There is a small risk of the catheter perforating (going through) the vein or not being inserted into a blood vessel. Also, you may experience discomfort, bleeding, and/or bruising. On a rare occasion, you may feel dizzy or faint. The likelihood of you experiencing these complications is very remote (about 1 in 10,000) when the procedure is carried out by trained personnel, and proper equipment is used. Universal precautions will be used during all blood draw procedures. Sites for blood draws will be cleaned with alcohol immediately prior to each venipuncture or catheter insertion. Each new needle that is opened will be disposed of in biohazard boxes immediately after use. A registered nurse or a trained phlebotomist will obtain all blood samples.

Embarrassment (anthropometric measurements, day 1): Some people feel embarrassed or are uncomfortable with their shirt off. To minimize this risk, trained technicians will complete all anthropometric measurements (height, weight, waist and hip circumference), and when requested, the technician can be of the same gender as you. Screens can also be placed around you to maintain privacy during these measurements.

Loss of Confidentiality: There is potential for a loss of confidentiality during communication through email, downloading, other Internet transactions, or data stored offline. To minimize this risk, all collected data will be stored in a password-protected computer and locked filing cabinet in the PI's office (Pioneer Hall, 213). Further, you will be coded with a combination of letters/numbers that cannot identify you (e.g. GH-001, GH-002, etc...). Confidentiality will be protected to the extent that is allowed by law.

Loss of Time: You will be allocating time to be available to participate in this research study. To minimize unwanted loss of your time during the study, schedules will be made and given to both the research team and to you (the participant). These schedules will inform both parties of the day, and time of day that you are scheduled to be in the lab. These schedules will also outline what you will be doing during your time in the lab. This will allow the research team to plan in advance to ensure that everything is performed and completed in the available time frame.

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

CAN MY TAKING PART IN THIS STUDY END EARLY?

As mentioned, we are aware that this study requires a significant time commitment from you as a volunteer. It is very important to the study that you not miss scheduled visits with study personnel. In the event that something comes up that will make you miss a visit, please call and let us know. Please also note that we may call you if a visit is missed, simply to check and make sure that everything is OK. If you have conflicts that require you to miss more than 10% of your scheduled visits, we will have to remove you from the study. If this happens, we will contact you and let you know the reason why you will not be allowed to continue, and make arrangements to send you the study results you have completed. Should our testing reveal information that suggests you need to be referred for medical care, we will refer you to your health care provider.

Participant's Initials: _____ Date: _____

RIGHT TO ASK QUESTIONS AND/OR WITHDRAW FROM THE STUDY

If you have questions or concerns at any time during the course of this investigation or after you complete the study, you may contact Kyle Biggerstaff, PhD at (940) 898-2596. Dr. Biggerstaff's office is located in room 116B Pioneer Hall.

You will be given a copy of this signed and dated consent form to keep. If you have any questions about the research study you should ask the researchers; their phone numbers are at the top of this form. If you have questions about your rights as a participant in this research or the way this study has been conducted, you may contact the Texas Woman's University Office of Research and Sponsored Programs at 940-898-3378 or via e-mail at IRB@twu.edu.

PARTICIPATION

Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled. Your signature acknowledges that you have read the information stated and willingly signed this consent form.

PRIVACY OF RECORDS

Only you, Sarah Deemer, Dr. Biggerstaff, and members of the research team will have access to your results. All data collected in this study will kept in a locked file cabinet on the TWU campus and will be coded by subject number rather than by name. The results of the research will be published; however, no publication will contain information which will allow you to be identified. Five years following the completion of this study, all collected data sheets and signed forms will be shredded and discarded.

AUTHORIZATION

Signature of Study Participant: _____

Printed Name of Study Participant: _____ Date: _____

Approved by the
Texas Woman's University
Institutional Review Board

Approved: June 5, 2015
Modifications Approved:
January 15, 2016

APPENDIX E

Health History Form

LIFESTYLE SURVEY

The questions in this survey are designed to help us understand your health. Try to answer each question truthfully. There are no right or wrong answers. If you are not sure, try your best guess. If you have any questions please ask the research assistant. You do not have to answer any of the questions if they make you uncomfortable. YOUR ANSWERS WILL BE KEPT STRICTLY CONFIDENTIAL.

HEALTH HABITS

1. Now thinking about your physical health, which includes physical illness and injury, for how many days during the past 30 days did you have poor physical health?	Number of days	
2. Now thinking about your mental health, which includes stress, depression, and emotional problems, for how many days during the past 30 days did you have poor mental health?	Number of days	
3. During the past 30 days, for about how many days did poor physical or mental health keep you from doing your usual activities, such as self-care, work or recreation?	Number of days	
4. Have you smoked at least 100 cigarettes (5 packs) in your whole life? **(IF NO, GO TO QUESTION #7)**	YES	NO
5. About how many cigarettes do you now smoke each day?	Cigarettes /day	
a. If 0, how long has it been since you quite smoking?	years /months	
6. About how old were you when you started smoking cigarettes regularly? (at least one cigarette per week)	years old	
7. A drink is 1 can or bottle of beer, 1 glass of wine, 1 can or bottle of wine cooler, 1 cocktail or 1 shot of liquor. About how many days a week do you have an alcoholic drink?	days /week	
8. On the days that you drink, about how many drinks do you have (on average)?	drinks each time I drink	

9. How would you consider your health? (Please mark one only)

VERY GOOD	GOOD	AVERAGE	POOR	VERY POOR
1	2	3	4	5

10. Have you ever had or do you have the following?
If you check YES, please provide an additional explanation.

YES	NO	DON'T KNOW	(CHECK ALL THAT APPLY)
			Free or easy bleeding (hemophilia) Please Explain:
			Low blood iron (anemia) Please Explain:
			Sickle cell anemia Please Explain:
			Rheumatic fever Please Explain:
			Heart murmur Please Explain:
			Irregular heart beat Please Explain:
			Mitral valve prolapse Please Explain:
			Any heart problem Please Explain:
			Pacemaker or implanted defibrillator Please Explain:
			High blood pressure Please Explain:
			High blood cholesterol Please Explain:
			Varicose veins Please Explain:
			Cancer Please Explain:
			Emphysema Please Explain:
			Chronic bronchitis Please Explain:
			Asthma Please Explain:

YES	NO	DON'T KNOW	(CHECK ALL THAT APPLY)
			Lung disease Please Explain:
			Seizures Please Explain:
			Stroke Please Explain:
			Low blood sugar Please Explain:
			Diabetes Please Explain:
			Kidney Disease Please Explain:
			Hepatitis Please Explain:
			Liver Disease Please Explain:
			Eye problems Please Explain:
			Hearing problems Please Explain:
			Thyroid problems Please Explain:
			Orthopedic problems Please Explain:
			Back problems Please Explain:
			Joint problems Please Explain:
			Arthritis Please Explain:
			AIDS Please Explain:
			Alcoholism Please Explain:
			Other medical problems Please Explain:

11. Have you recently (within the last year) had any of the following symptoms?
If you check YES, please provide an additional explanation.

YES	NO	DON'T KNOW	(CHECK ALL THAT APPLY)
			Chest pain Please Explain:
			Shortness of breath Please Explain:
			Heart palpations or fast heartbeat Please Explain:
			Arm or shoulder pain Please Explain:
			Burning sensations Please Explain:
			Unusual fatigue with slight exertion Please Explain:
			Severe headache Please Explain:
			Blurred vision Please Explain:
			Low or high blood sugar Please Explain:
			Frequent urination Please Explain:
			Blood in urine Please Explain:
			Coughing of blood Please Explain:
			Feeling faint or dizzy Please Explain:
			Difficulty walking Please Explain:
			Low bone density (osteoporosis) Please Explain:
			Leg or ankle swelling Please Explain:

YES	NO	DON'T KNOW	(CHECK ALL THAT APPLY)
			Swelling in your joints Please Explain:
			Low-back pain Please Explain:
			Weakness in arm Please Explain:
			Leg numbness Please Explain:
			Significant emotional problem Please Explain:
			Other medical problems Please Explain:

12. Are you taking any medications or supplements? If yes, please list and describe below.	**YES**	**NO**	**DON'T KNOW**

13. Of the following members of your family, describe any cardiovascular disease, heart disease, stroke, or diabetes each has (or had).

YES	NO	DON'T KNOW	
			Mother Please Explain:
			Father Please Explain:
			Mother's Mother Please Explain:
			Mother's Father Please Explain:
			Father's Mother Please Explain:
			Father's Father Please Explain:

MENSTRUAL HISTORY			
14. What is your date of birth?	MM	DD	YY
15. At approximately what age (year and months) did you begin menstruating?	AGE		MM
16. Do you currently have regular menstrual cycles (i.e., regularly spaced periods of menstrual bleeding)?	YES		NO
If you answered "**YES**" to question #16:			
a. Approximately how many days separate your periods?	Less than 25 days	25-32 days	More than 32 days
b. Approximately how many days does your bleeding last?	Less than 3 days	3-7 days	More than 7 days
c. When did your most recent period begin?	MM	DD	YY
d. Is there any reason to believe that you are pregnant?	YES		NO
If you answered "**NO**" to question #16:			
a. Have your menstrual periods stopped completely?	YES		NO
b. When did your most recent period begin?	MM	DD	YY
c. If you still occasionally have menstrual bleeding, describe the pattern.			

17. How would you consider your eating habits? (PLEASE MARK ONE ONLY)				
VERY GOOD	GOOD	AVERAGE	POOR	VERY POOR
1	2	3	4	5

18. How often do you eat the following foods? (CIRCLE ONE NUMBER FOR EACH ITEM)							
	never or few times a year	**about once a month**	**several times a month**	**few times a week**	**once a day**	**3 or 4 times a day**	**5 times a day or more**
FRESH FRUITS AND VEGETABLES	1	2	3	4	5	6	7
PASTRIES (pie, cake, cookies, brownies, sweet rolls, donuts)	1	2	3	4	5	6	7
BREAD (bread, pasta, tortilla)	1	2	3	4	5	6	7
POULTRY	1	2	3	4	5	6	7
FISH	1	2	3	4	5	6	7
PORK	1	2	3	4	5	6	7
RED MEATS (beef, lamb, lunch meats)	1	2	3	4	5	6	7
WHOLE MILK	1	2	3	4	5	6	7
FRIED FOOD	1	2	3	4	5	6	7
HOW OFTEN DO YOU EAT AT RESTAURANTS	1	2	3	4	5	6	7

These next few questions are about exercise, recreation and physical activities other than your regular job duties. For each of the following activities, please tell us how many times you did them in the past MONTH. For each activity that you did in the last MONTH, please tell us how much time on average in hours or minutes you spent doing the activity EACH TIME YOU DID IT.

19. First we will start with typical sport activities like basketball, volleyball and soccer and fitness related activities like jogging, running, riding a bike, dancing, and aerobics. These are NOT activities you do during work. These are NOT activities you do with your children either; only with other adults or by yourself.

a. In the past MONTH, have you done any typical sport or fitness related activities? (if "NO", go to Question #20)	**YES**	**NO**
b. If Yes, please tell us how many times in the past MONTH you have done the sport or fitness related activities listed below. If you did the activity every day then you would say you did it 30 times in the last MONTH. Then tell us how much time ON AVERAGE in minutes or hours you spent doing each sport or fitness related activity each time you did it.	**Times in last MONTH**	**Average Hours/ Minutes Each Time**
Aerobics/Aerobic Dance (using a video during a class in a gym)		
Other Dancing (for fun with friends and family)		
Jogging or Running		
Golf		
Bowling		
Tennis		
Basketball		
Baseball or Softball		
Soccer		
Weight lifting/training		
Riding a bike outside or a stationary bike inside		
Swimming		
Yoga		
Roller skating or blading		
Other1:		
Other2:		
Other3:		

20. Now we would like to know about recreational activities that you do when you have free time like watching television, reading, and spending time with your family. These are NOT activities you do during work.		
a. In the past MONTH, have you participated in any recreational activities? (IF "NO" go to Question #21)	**YES**	**NO**
b. If Yes, please tell us how many times in the past MONTH you have done the following recreational activities. If you did the activity once every day then you would say you did it 30 times in the last MONTH. Then tell us how much time ON AVERAGE in minutes or hours you spent doing each recreational activity each time you did it.	**Times in past MONTH**	**Avg Hours/ Minutes Each Time**
Using a computer		
Going to church		
Quiet play with children (board games, drawing, coloring, reading stories)		
Active play with children (playing tag, soccer, baseball, hide and seek)		
Talking on the telephone		
Listening to music		
Watching television or videos		
Watching a movie in a theater		
Visiting with friends (in person, not on the phone)		
Playing games (dominos, checkers, boardgames, cards)		
Reading		
Other 1:		
Other 2:		
Other 3:		
Other 4:		

21. Now we would like to know about household activities that you do such as cleaning, gardening, and taking care of children. These are NOT activities you do during work.		
a. In the past MONTH, have you done any household activities? (if "NO", go to Question 22)	**YES**	**NO**
b. If Yes, please tell us how many times in the past MONTH you have done the following household activities. If you did the activity once every day then you would say you did it 30 times in the last MONTH. Then tell us how much time ON AVERAGE in minutes or hours you spent doing each household activity each time you did it.	**Times in past MONTH**	**Average Hours/ Minutes Each Time**
Light Cleaning (picking up the house, dusting, sweeping, ironing, dishes)		
Hard Cleaning (scrub floors, move objects, carry loads up stairs)		
Childcare (feeding, bathing, dressing)		
Gardening or Yardwork		
Shopping (grocery, clothes)		
Light home repair/maintenance (changing light bulbs, fixing loose fixtures)		
Heavy home repair/maintenance (carpentry, lifting large objects)		
Laundry (time loading, unloading, hanging, folding only)		
Food Preparation (cooking, serving)		
Other 1:		
Other 2:		
Other 3:		
Other 4:		

22. ON AVERAGE , how would you describe your walking pace when you walk? (PLEASE CHECK ONLY ONE)	
	Slow, like taking a leisurely stroll.
	Moderate, like you had somewhere to go.
	Fast, like walking (without jogging) to catch a bus or make an appointment.

23. ON AVERAGE , how would you describe the intensity with which you clean the house or do other work around the house? (PLEASE CHECK ONLY ONE)	
	Light, like taking a leisurely stroll.
	Moderate, like walking to get somewhere.
	Hard, like fast walking (without jogging) to catch a bus or make an appointment.

24. Compared to others of your age and gender, would you say you are: (PLEASE CHECK ONE ONLY)	
	MUCH LESS ACTIVE
	SOMEWHAT LESS ACTIVE
	ABOUT AS ACTIVE
	SOMEWHAT MORE ACTIVE
	MUCH MORE ACTIVE

25. For each of the following statements, please indicate how often they prevent you from exercising on a scale of one to five. One represents Never, three represents Sometimes and five represents Always. Two is in between Never and Sometimes. Four is in between Sometimes and Always.

	Never		Sometimes		Always
a. I do not have a safe place to exercise.	1	2	3	4	5
b. I feel self-conscious about exercising because I am too overweight.	1	2	3	4	5
c. My health prevents me from exercising. Please Explain:	1	2	3	4	5
d. I do not enjoy exercising.	1	2	3	4	5
e. I cannot motivate myself to exercise.	1	2	3	4	5
f. I do not have anyone to exercise with.	1	2	3	4	5
g. I do not have time to exercise.	1	2	3	4	5
h. I do not have the energy to exercise.	1	2	3	4	5
i. I am afraid I will hurt myself when I exercise.	1	2	3	4	5
j. It is too hot or too cold to exercise.	1	2	3	4	5
k. My spouse/significant other does not want me to exercise.	1	2	3	4	5
l. I cannot find anyone to watch my children while I exercise.	1	2	3	4	5
m. I cannot walk/jog/run in my neighborhood. Please Explain:	1	2	3	4	5
n. I do not have clothes and/or shoes to exercise in.	1	2	3	4	5
o. Other. Please Explain:	1	2	3	4	5

PERSONAL INFORMATION

1. Please check the ethnic group that you most identify with:

If you are Hispanic, please check one of the following:

☐ Mexican National ☐ Mexican American ☐ Chicana/o

☐ Latina/o ☐ Other, please detail: _____

If you are **NOT** Hispanic, please check one of the following:

☐ American Indian ☐ Alaskan Native ☐ Asian or Pacific Islander

☐ African American, not of Hispanic Origin ☐ Caucasian, not of Hispanic Origin

☐ Other, please detail: _____

2. When were you born? _____/_____/_____
Month Day Year

3. What is your gender? ☐ Female ☐ Male

4. How tall are you? _____ Feet **AND** _____ inches **OR** _____ meters

5. How much do you weigh? _____ pounds **OR** _____ kilograms

6. Please check marital status: ☐ SINGLE ☐ MARRIED ☐ DIVORCED

☐ WIDOWED ☐ COMMON LAW ☐ OTHER: _____

7. Please check highest level of education COMPLETED:

☐ Less than 7th Grade
☐ Junior High/Secondary School (9th Grade)
☐ Some High School (10th or 11th Grade)
☐ Completed High School
☐ Some College or Vocational Training
☐ Completed Associate Degree
☐ Completed Bachelor Degree
☐ Completed Graduate Degree

8. Status of employment: ☐ Full Time ☐ Part Time ☐ Unemployed

9. If student, give status of schooling: ☐ Full Time ☐ Part Time _____

APPENDIX F

Dietary Record Sheet with Instructions

Growth Hormone & Exercise Study 3-Day Diet Record

Instructions: Please fill out the following form as completely and accurately as possible. You will be asked to replicate these meals as best as you can each time you return to the lab for a prolonged trial visit. Be sure to include things like sugar or cream in coffee, spreads and condiments (such as mayo or mustard on sandwiches, or ketchup with your French fries), and other important details about food prep that you might forget. If you eat out at a restaurant, please be sure to also include the restaurant in the food description.

Date: _____ (circle one) DAY 1 DAY 2 DAY 3

MEAL	TIME OF DAY	WHAT DID I EAT?
Breakfast		
Morning Snack		
Lunch		
Afternoon Snack		
Dinner		
Late-Night Snack		

APPENDIX G

Data Collection Sheets

GH Secretion Study **ID #:** _____

Data Collection Sheet - First Visit

Date: _____

Time of Blood Draw: _____

Age (y): _____ Height (cm): _____ Weight (kg): _____

Waist Circumference (cm)

Trial 1	Trial 2	Trial 3	Trial 4	Average

DEXA Scan

Total BMD (g/cm^2)	Total BMC (g)	Total Mass (kg)	Total Fat (%)	Legs Fat (%)	Trunk Fat (%)	*Est.* Visceral AT	
						in^3	lbs

HR_{rest} (bpm): _____ BP_{rest}(mmHg): _____

$VO_{2max\ TEST}$

VO_2 (L/min):	VCO_2 (L/min):	RER:
Lactate (mmol/L):	HR_{max} (bpm):	RPE:
VO_{2max} (mL/kg/min):	Max Work Rate (W):	

50% $Work_{max}$ (W): _____

GH Secretion Study Date: _____

ID#: _____ **Time Arrived at lab:** _____

Weight (kg): _____ **Condition:** CON

Catheter Placement *(circle one)*: Right arm Left arm **Time Placed:** _____

(Min)	Time	Actual Time	[La$^-$]	10 mL RED	6 mL PRPLE	3 mL RED	HR (bpm)	RPE
0	5:20 PM							
start rest	5:30 PM		***Start Rest***					
5	5:35 PM							
10	5:40 PM							
15	5:45 PM							
20	5:50 PM							
25	5:55 PM							
30	6:00 PM							
35	6:05 PM							
40	6:10 PM							
45	6:15 PM							
50	6:20 PM							
60	6:30 PM							
70	6:40 PM							
80	6:50 PM							
90	7:00 PM							
100	7:10 PM							
110	7:20 PM							
120	7:30 PM							
Feed dinner								
130	7:40 PM							
140	7:50 PM							
150	8:00 PM							
160	8:10 PM							
170	8:20 PM							
180	8:30 PM							
190	8:40 PM							
200	8:50 PM							
210	9:00 PM							
220	9:10 PM							
230	9:20 PM							
240	9:30 PM							
250	9:40 PM							
260	9:50 PM							
270	10:00 PM							

1

GH Secretion Study

Date: _____

(Min)	Time	Actual Time	[La-]	10 mL RED	6 mL PRPLE	3 mL RED	HR (bpm)	RPE
280	10:10 PM							
290	10:20 PM							
300	10:30 PM							
310	10:40 PM							
320	10:50 PM							
330	11:00 PM							
				Lights Out				
340	11:10 PM							
350	11:20 PM							
360	11:30 PM							
370	11:40 PM							
380	11:50 PM							
390	12:00 AM							
400	12:10 AM							
410	12:20 AM							
420	12:30 AM							
430	12:40 AM							
440	12:50 AM							
450	1:00 AM							
460	1:10 AM							
470	1:20 AM							
480	1:30 AM							
490	1:40 AM							
500	1:50 AM							
510	2:00 AM							
520	2:10 AM							
530	2:20 AM							
540	2:30 AM							
550	2:40 AM							
560	2:50 AM							
570	3:00 AM							
580	3:10 AM							
590	3:20 AM							
600	3:30 AM							
610	3:40 AM							
620	3:50 AM							
630	4:00 AM							
640	4:10 AM							
650	4:20 AM							
660	4:30 AM							
670	4:40 AM							
680	4:50 AM							
690	5:00 AM							

2

GH Secretion Study

Date: _____

(Min)	Time	Actual Time	[La-]	10 mL RED	6 mL PRPLE	3 mL RED	HR (bpm)	RPE
700	5:10 AM							
710	5:20 AM							
720	5:30 AM							
730	5:40 AM							
740	5:50 AM							
750	6:00 AM							
Wake Up								

Collect ALL urine throught visit ☐

(record times below)

	Start Time	Actual Time
Start RMR	6:30 AM	
End RMR	7:00 AM	

Notes:

3

GH Secretion Study

Date: _____

ID#: _____ **Time Arrived at lab:** _____

Weight (kg): _____ **Condition *(circle one)*:** EX_{MOD} EX_{HIE}

Catheter Placement *(circle one)*: Right arm Left arm **Time Placed:** _____

(Min)	Time	Actual Time	[La$^-$]	10 mL RED	6 mL PRPLE	3 mL RED	HR (bpm)	RPE
0	5:20 PM							
start ex	5:30 PM		***Start Exercise***					
5	5:35 PM							
10	5:40 PM							
15	5:45 PM							
20	5:50 PM							
25	5:55 PM							
30	6:00 PM							
32	6:02 PM							
34	6:04 PM							
36	6:06 PM							
38	6:08 PM							
40	6:10 PM							
45	6:15 PM							
50	6:20 PM							
60	6:30 PM							
70	6:40 PM							
80	6:50 PM							
90	7:00 PM							
100	7:10 PM							
110	7:20 PM							
120	7:30 PM							
Feed dinner								
130	7:40 PM							
140	7:50 PM							
150	8:00 PM							
160	8:10 PM							
170	8:20 PM							
180	8:30 PM							
190	8:40 PM							
200	8:50 PM							
210	9:00 PM							
220	9:10 PM							
230	9:20 PM							
240	9:30 PM							

1

GH Secretion Study

Date: _____

(Min)	Time	Actual Time	[La-]	10 mL RED	6 mL PRPLE	3 mL RED	HR (bpm)	RPE
250	9:40 PM							
260	9:50 PM							
270	10:00 PM							
280	10:10 PM							
290	10:20 PM							
300	10:30 PM							
310	10:40 PM							
320	10:50 PM							
330	11:00 PM							
				Lights Out				
340	11:10 PM							
350	11:20 PM							
360	11:30 PM							
370	11:40 PM							
380	11:50 PM							
390	12:00 AM							
400	12:10 AM							
410	12:20 AM							
420	12:30 AM							
430	12:40 AM							
440	12:50 AM							
450	1:00 AM							
460	1:10 AM							
470	1:20 AM							
480	1:30 AM							
490	1:40 AM							
500	1:50 AM							
510	2:00 AM							
520	2:10 AM							
530	2:20 AM							
540	2:30 AM							
550	2:40 AM							
560	2:50 AM							
570	3:00 AM							
580	3:10 AM							
590	3:20 AM							
600	3:30 AM							
610	3:40 AM							
620	3:50 AM							
630	4:00 AM							
640	4:10 AM							
650	4:20 AM							
660	4:30 AM							

2

GH Secretion Study

Date: _____

(Min)	Time	Actual Time	[La⁻]	10 mL RED	6 mL PRPLE	3 mL RED	HR (bpm)	RPE
670	4:40 AM							
680	4:50 AM							
690	5:00 AM							
700	5:10 AM							
710	5:20 AM							
720	5:30 AM							
730	5:40 AM							
740	5:50 AM							
750	6:00 AM							
Wake Up								

Collect ALL urine throught visit ☐

(record times below)

	Start Time	Actual Time
Start RMR	6:30 AM	
End RMR	7:00 AM	

Notes:

3

APPENDIX H

Analysis of Variance Tables

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Sprint	Sphericity Assumed	33298.550	3	11099.517	14.353	.000	.782	43.060	.998
	Greenhouse-Geisser	33298.550	1.967	16929.801	14.353	.002	.782	28.231	.978
	Huynh-Feldt	33298.550	3.000	11099.517	14.353	.000	.782	43.060	.998
	Lower-bound	33298.550	1.000	33298.550	14.353	.019	.782	14.353	.805
Error(Sprint)	Sphericity Assumed	9279.700	12	773.308					
	Greenhouse-Geisser	9279.700	7.867	1179.507					
	Huynh-Feldt	9279.700	12.000	773.308					
	Lower-bound	9279.700	4.000	2319.925					

a. Computed using alpha = .05

b. Mean Power output for each sprint

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Sprint	Sphericity Assumed	22692.000	3	7564.000	7.824	.004	.662	23.471	.944
	Greenhouse-Geisser	22692.000	1.877	12092.497	7.824	.015	.662	14.682	.811
	Huynh-Feldt	22692.000	3.000	7564.000	7.824	.004	.662	23.471	.944
	Lower-bound	22692.000	1.000	22692.000	7.824	.049	.662	7.824	.563
Error(Sprint)	Sphericity Assumed	11601.500	12	966.792					
	Greenhouse-Geisser	11601.500	7.506	1545.601					
	Huynh-Feldt	11601.500	12.000	966.792					
	Lower-bound	11601.500	4.000	2900.375					

a. Computed using alpha = .05

b. One-way repeated measures ANOVA for peak power output measured for each sprint.

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	1035.341	1	1035.341	29.240	.006	.880	29.240	.975
	Greenhouse-Geisser	1035.341	1.000	1035.341	29.240	.006	.880	29.240	.975
	Huynh-Feldt	1035.341	1.000	1035.341	29.240	.006	.880	29.240	.975
	Lower-bound	1035.341	1.000	1035.341	29.240	.006	.880	29.240	.975
Error(Trial)	Sphericity Assumed	141.635	4	35.409					
	Greenhouse-Geisser	141.635	4.000	35.409					
	Huynh-Feldt	141.635	4.000	35.409					
	Lower-bound	141.635	4.000	35.409					
Time	Sphericity Assumed	713.992	16	44.625	95.713	.000	.960	1531.405	1.000
	Greenhouse-Geisser	713.992	2.784	256.424	95.713	.000	.960	266.505	1.000
	Huynh-Feldt	713.992	9.808	72.799	95.713	.000	.960	938.726	1.000
	Lower-bound	713.992	1.000	713.992	95.713	.001	.960	95.713	1.000
Error(Time)	Sphericity Assumed	29.839	64	.466					
	Greenhouse-Geisser	29.839	11.138	2.679					
	Huynh-Feldt	29.839	39.231	.761					
	Lower-bound	29.839	4.000	7.460					
Trial * Time	Sphericity Assumed	209.551	16	13.097	22.528	.000	.849	360.447	1.000
	Greenhouse-Geisser	209.551	2.309	90.772	22.528	.000	.849	52.007	1.000
	Huynh-Feldt	209.551	5.642	37.143	22.528	.000	.849	127.096	1.000
	Lower-bound	209.551	1.000	209.551	22.528	.009	.849	22.528	.936
Error(Trial*Time)	Sphericity Assumed	37.207	64	.581					
	Greenhouse-Geisser	37.207	9.234	4.029					
	Huynh-Feldt	37.207	22.567	1.649					
	Lower-bound	37.207	4.000	9.302					

a. Computed using alpha = .05

b. Repeated measures two-way ANOVA (trial x time): MOD vs HIE only

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	197.167	2	98.583	40.703	.000	.911	81.406	1.000
	Greenhouse-Geisser	197.167	1.094	180.168	40.703	.002	.911	44.543	.998
	Huynh-Feldt	197.167	1.195	165.018	40.703	.001	.911	48.632	.999
	Lower-bound	197.167	1.000	197.167	40.703	.003	.911	40.703	.996
Error(Trial)	Sphericity Assumed	19.376	8	2.422					
	Greenhouse-Geisser	19.376	4.377	4.426					
	Huynh-Feldt	19.376	4.779	4.054					
	Lower-bound	19.376	4.000	4.844					
Time	Sphericity Assumed	160.610	4	40.153	172.042	.000	.977	688.168	1.000
	Greenhouse-Geisser	160.610	2.200	73.000	172.042	.000	.977	378.516	1.000
	Huynh-Feldt	160.610	4.000	40.153	172.042	.000	.977	688.168	1.000
	Lower-bound	160.610	1.000	160.610	172.042	.000	.977	172.042	1.000
Error(Time)	Sphericity Assumed	3.734	16	.233					
	Greenhouse-Geisser	3.734	8.801	.424					
	Huynh-Feldt	3.734	16.000	.233					
	Lower-bound	3.734	4.000	.934					
Trial * Time	Sphericity Assumed	156.799	8	19.600	48.948	.000	.924	391.582	1.000
	Greenhouse-Geisser	156.799	2.040	76.878	48.948	.000	.924	99.833	1.000
	Huynh-Feldt	156.799	4.182	37.496	48.948	.000	.924	204.686	1.000
	Lower-bound	156.799	1.000	156.799	48.948	.002	.924	48.948	.999
Error(Trial*Time)	Sphericity Assumed	12.814	32	.400					
	Greenhouse-Geisser	12.814	8.158	1.571					
	Huynh-Feldt	12.814	16.727	.766					
	Lower-bound	12.814	4.000	3.203					

a. Computed using alpha = .05

b. Repeated measures two-way ANOVA (condition x time): CON, MOD, and HIE

Tests of Within-Subjects Effects[b]

MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	862661.384	2	431330.692	46.594	.000	.921	93.188	1.000
	Greenhouse-Geisser	862661.384	1.061	812915.915	46.594	.002	.921	49.445	.999
	Huynh-Feldt	862661.384	1.125	766853.623	46.594	.001	.921	52.415	.999
	Lower-bound	862661.384	1.000	862661.384	46.594	.002	.921	46.594	.998
Error(Trial)	Sphericity Assumed	74057.485	8	9257.186					
	Greenhouse-Geisser	74057.485	4.245	17446.738					
	Huynh-Feldt	74057.485	4.500	16458.153					
	Lower-bound	74057.485	4.000	18514.371					

a. Computed using alpha = .05

b. Repeated Measures one-way ANOVA for lactate area under the curve (0 - 120 min).

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	.045	2	.022	2.135	.181	.348	4.270	.316
	Greenhouse-Geisser	.045	1.392	.032	2.135	.203	.348	2.972	.250
	Huynh-Feldt	.045	1.902	.023	2.135	.184	.348	4.060	.306
	Lower-bound	.045	1.000	.045	2.135	.218	.348	2.135	.205
Error(Trial)	Sphericity Assumed	.084	8	.010					
	Greenhouse-Geisser	.084	5.568	.015					
	Huynh-Feldt	.084	7.607	.011					
	Lower-bound	.084	4.000	.021					

a. Computed using alpha = .05

b. One-way ANOVA for urinary nitrogen content from CON, MOD, and HIE

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	.007	2	.004	1.435	.293	.264	2.869	.224
	Greenhouse-Geisser	.007	1.191	.006	1.435	.298	.264	1.708	.168
	Huynh-Feldt	.007	1.407	.005	1.435	.298	.264	2.019	.184
	Lower-bound	.007	1.000	.007	1.435	.297	.264	1.435	.154
Error(Trial)	Sphericity Assumed	.021	8	.003					
	Greenhouse-Geisser	.021	4.763	.004					
	Huynh-Feldt	.021	5.629	.004					
	Lower-bound	.021	4.000	.005					

a. Computed using alpha = .05

b. One-way ANOVA for measured NPRQ

Multivariate Tests[a,e]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[d]
Trial	Pillai's Trace	.983	87.223[b]	2.000	3.000	.002	.983	174.445	1.000
	Wilks' Lambda	.017	87.223[b]	2.000	3.000	.002	.983	174.445	1.000
	Hotelling's Trace	58.148	87.223[b]	2.000	3.000	.002	.983	174.445	1.000
	Roy's Largest Root	58.148	87.223[b]	2.000	3.000	.002	.983	174.445	1.000
Time	Pillai's Trace	.[c]	.	.	.	.	.	.	.
	Wilks' Lambda	.[c]	.	.	.	.	.	.	.
	Hotelling's Trace	.[c]	.	.	.	.	.	.	.
	Roy's Largest Root	.[c]	.	.	.	.	.	.	.
Trial * Time	Pillai's Trace	.[c]	.	.	.	.	.	.	.
	Wilks' Lambda	.[c]	.	.	.	.	.	.	.
	Hotelling's Trace	.[c]	.	.	.	.	.	.	.
	Roy's Largest Root	.[c]	.	.	.	.	.	.	.

a. Design: Intercept
Within Subjects Design: Trial + Time + Trial * Time

b. Exact statistic

c. Cannot produce multivariate test statistics because of insufficient residual degrees of freedom.

d. Computed using alpha = .05

e. Two-way repeated measures ANOVA (trial x time) for Growth Hormone (0 - 120 min)

Multivariate Tests[a,d]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[c]
trial	Pillai's Trace	.981	78.681[b]	2.000	3.000	.003	.981	157.362	1.000
	Wilks' Lambda	.019	78.681[b]	2.000	3.000	.003	.981	157.362	1.000
	Hotelling's Trace	52.454	78.681[b]	2.000	3.000	.003	.981	157.362	1.000
	Roy's Largest Root	52.454	78.681[b]	2.000	3.000	.003	.981	157.362	1.000

a. Design: Intercept
Within Subjects Design: trial

b. Exact statistic

c. Computed using alpha = .05

d. One-way ANOVA for calculated Area Under the Curve for GH (CON, MOD, HIE)

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	3.081	2	1.541	2.357	.157	.371	4.715	.345
	Greenhouse-Geisser	3.081	1.152	2.675	2.357	.193	.371	2.715	.241
	Huynh-Feldt	3.081	1.320	2.335	2.357	.185	.371	3.111	.263
	Lower-bound	3.081	1.000	3.081	2.357	.199	.371	2.357	.221
Error(Trial)	Sphericity Assumed	5.229	8	.654					
	Greenhouse-Geisser	5.229	4.607	1.135					
	Huynh-Feldt	5.229	5.279	.990					
	Lower-bound	5.229	4.000	1.307					

a. Computed using alpha = .05

b. One-way repeated measures ANOVA for mean GH concentration for each trial (CON, MOD, HIE)

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	1563823.32	2	781911.659	2.531	.141	.388	5.062	.367
	Greenhouse-Geisser	1563823.32	1.170	1336788.53	2.531	.178	.388	2.961	.258
	Huynh-Feldt	1563823.32	1.360	1149823.23	2.531	.169	.388	3.442	.284
	Lower-bound	1563823.32	1.000	1563823.32	2.531	.187	.388	2.531	.234
Error(Trial)	Sphericity Assumed	2471654.24	8	308956.780					
	Greenhouse-Geisser	2471654.24	4.679	528205.295					
	Huynh-Feldt	2471654.24	5.440	454329.693					
	Lower-bound	2471654.24	4.000	617913.560					

a. Computed using alpha = .05

b. One-way ANOVA for total pulsatile secretion (not log-transformed data)

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	3.733	2	1.867	.235	.796	.055	.470	.076
	Greenhouse-Geisser	3.733	1.690	2.208	.235	.762	.055	.397	.073
	Huynh-Feldt	3.733	2.000	1.867	.235	.796	.055	.470	.076
	Lower-bound	3.733	1.000	3.733	.235	.653	.055	.235	.067
Error(Trial)	Sphericity Assumed	63.600	8	7.950					
	Greenhouse-Geisser	63.600	6.762	9.406					
	Huynh-Feldt	63.600	8.000	7.950					
	Lower-bound	63.600	4.000	15.900					

a. Computed using alpha = .05

b. One-way ANOVA for # GH Pulses

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	622.672	2	311.336	.370	.702	.085	.741	.091
	Greenhouse-Geisser	622.672	1.908	326.411	.370	.693	.085	.706	.090
	Huynh-Feldt	622.672	2.000	311.336	.370	.702	.085	.741	.091
	Lower-bound	622.672	1.000	622.672	.370	.576	.085	.370	.077
Error(Trial)	Sphericity Assumed	6726.344	8	840.793					
	Greenhouse-Geisser	6726.344	7.631	881.505					
	Huynh-Feldt	6726.344	8.000	840.793					
	Lower-bound	6726.344	4.000	1681.586					

a. Computed using alpha = .05

b. One-way ANOVA for GH Interpulse Interval

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	17.509	2	8.755	.280	.763	.065	.560	.081
	Greenhouse-Geisser	17.509	1.958	8.943	.280	.759	.065	.548	.080
	Huynh-Feldt	17.509	2.000	8.755	.280	.763	.065	.560	.081
	Lower-bound	17.509	1.000	17.509	.280	.625	.065	.280	.070
Error(Trial)	Sphericity Assumed	250.052	8	31.256					
	Greenhouse-Geisser	250.052	7.831	31.931					
	Huynh-Feldt	250.052	8.000	31.256					
	Lower-bound	250.052	4.000	62.513					

a. Computed using alpha = .05

b. One-way repeated measures ANOVA for GH mean pulse area (CON, MOD, HIE)

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	.166	2	.083	.761	.498	.160	1.523	.138
	Greenhouse-Geisser	.166	1.153	.144	.761	.446	.160	.878	.111
	Huynh-Feldt	.166	1.322	.126	.761	.459	.160	1.006	.116
	Lower-bound	.166	1.000	.166	.761	.432	.160	.761	.105
Error(Trial)	Sphericity Assumed	.871	8	.109					
	Greenhouse-Geisser	.871	4.611	.189					
	Huynh-Feldt	.871	5.287	.165					
	Lower-bound	.871	4.000	.218					

a. Computed using alpha = .05

b. One-way repeated measures ANOVA for GH mean burst height (CON, MOD, HIE)

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	.125	2	.063	3.495	.081	.466	6.990	.484
	Greenhouse-Geisser	.125	1.471	.085	3.495	.106	.466	5.143	.393
	Huynh-Feldt	.125	2.000	.063	3.495	.081	.466	6.990	.484
	Lower-bound	.125	1.000	.125	3.495	.135	.466	3.495	.302
Error(Trial)	Sphericity Assumed	.143	8	.018					
	Greenhouse-Geisser	.143	5.886	.024					
	Huynh-Feldt	.143	8.000	.018					
	Lower-bound	.143	4.000	.036					

a. Computed using alpha = .05

b. One-way repeated measures ANOVA for total pulsatile secretion (Log-transformed data)

Tests of Within-Subjects Effects[b]

MeasureMEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Trial	Sphericity Assumed	27436.364	2	13718.182	.149	.864	.036	.298	.066
	Greenhouse-Geisser	27436.364	1.539	17829.395	.149	.813	.036	.229	.064
	Huynh-Feldt	27436.364	2.000	13718.182	.149	.864	.036	.298	.066
	Lower-bound	27436.364	1.000	27436.364	.149	.719	.036	.149	.061
Error(Trial)	Sphericity Assumed	737756.311	8	92219.539					
	Greenhouse-Geisser	737756.311	6.155	119856.886					
	Huynh-Feldt	737756.311	8.000	92219.539					
	Lower-bound	737756.311	4.000	184439.078					

a. Computed using alpha = .05

b. one-way repeated measures ANOVA for total pulsatile GH secretion during sleep (CON, MOD, HIE)

APPENDIX I

Procedures for Blood Analyses (GH and E_2)

Growth Hormone HGH ELISA Protocol

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD A (0)	STD B (1)	STD F (50)	UNK 2	UNK 6	UNK 10	UNK 14	UNK 18	UNK 22	UNK 26	UNK 30	UNK 34
B	STD A (0)	STD B (1)	STD F (50)	UNK 2	UNK 6	UNK 10	UNK 14	UNK 18	UNK 22	UNK 26	UNK 30	UNK 34
C	STD (0.1)	STD C (5)	CON 1	UNK 3	UNK 7	UNK 11	UNK 15	UNK 19	UNK 23	UNK 27	UNK 31	UNK 35
D	STD (0.1)	STD C (5)	CON 1	UNK 3	UNK 7	UNK 11	UNK 15	UNK 19	UNK 23	UNK 27	UNK 31	UNK 35
E	STD (0.3)	STD D (10)	CON 2	UNK 4	UNK 8	UNK 12	UNK 16	UNK 20	UNK 24	UNK 28	UNK 32	UNK 36
F	STD (0.3)	STD D (10)	CON 2	UNK 4	UNK 8	UNK 12	UNK 16	UNK 20	UNK 24	UNK 28	UNK 32	UNK 36
G	STD (0.7)	STD E (25)	UNK 1	UNK 5	UNK 9	UNK 13	UNK 17	UNK 21	UNK 25	UNK 29	UNK 33	UNK 37
H	STD (0.7)	STD E (25)	UNK 1	UNK 5	UNK 9	UNK 13	UNK 17	UNK 21	UNK 25	UNK 29	UNK 33	UNK 37

Note: All reagents must reach room temperature before using.

1. Prepare **ANTI-HGH-HRP-CONJUGATE**: 120 µL HRP + 12 mL ASSAY BUFFER
   * Discard any leftover HRP *
2. Prepare **WASH BUFFER**: 450 mL DI H_2O + 50 mL WASH BUFFER CONCENTRATE

Create 3 new standards:

	1 ng/mL STD	Assay Buffer
0.1 ng/mL	100 µL	900 µL
0.3 ng/mL	300 µL	700 µL
0.7 ng/mL	700 µL	300 µL

Assay Procedure:

1. Pipette 25 µL of Standards (A – F) into appropriate wells in duplicate.
2. Pipette 25 µL of Controls (1 & 2) into appropriate wells in duplicate.
3. Pipette 25 µL of specimen sample (UNK) into appropriate wells in duplicate.
4. Pipette 100 µL of **ANTI-HGH-HRP-CONJUGATE** into all wells using a multichannel pipette.
5. Incubate on a plate shaker at 200 rpm for **1 HOUR** (**ROOM TEMPERATURE**).
6. Wash wells 3 times with 300 µL of **WASH BUFFER**.
7. Tap plate firmly against absorbent paper to ensure all wells are dry.
8. Add 100 µL of **TMB SUBSTRATE** to all wells using a multichannel pipette.
9. Incubate on plate shaker at room temperature for 10-15 minutes.
   Note: or until Standard F achieves a dark blue color
10. Pipette 50 µL of **STOP SOLUTION** into each well.

Read plate within 20 min of adding STOP SOLUTION at **450nm** on a microplate reader.

- Subtract mean absorbance value of STD A (0) from all other samples.
- Use a 4-parameter or 5-parameter curve for standard curve calculation.

Estradiol ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD A	STD E	UNK 1	UNK 5	UNK 9	UNK 13	UNK 17	UNK 21	UNK 25	UNK 29	UNK 33	UNK 37
B	STD A	STD E	UNK 1	UNK 5	UNK 9	UNK 13	UNK 17	UNK 21	UNK 25	UNK 29	UNK 33	UNK 37
C	STD B	STD F	UNK 2	UNK 6	UNK 10	UNK 14	UNK 18	UNK 22	UNK 26	UNK 30	UNK 34	UNK 38
D	STD B	STD F	UNK 2	UNK 6	UNK 10	UNK 14	UNK 18	UNK 22	UNK 26	UNK 30	UNK 34	UNK 38
E	STD C	CON 1	UNK 3	UNK 7	UNK 11	UNK 15	UNK 19	UNK 23	UNK 27	UNK 31	UNK 35	UNK 39
F	STD C	CON 1	UNK 3	UNK 7	UNK 11	UNK 15	UNK 19	UNK 23	UNK 27	UNK 31	UNK 35	UNK 39
G	STD D	CON 2	UNK 4	UNK 8	UNK 12	UNK 16	UNK 20	UNK 24	UNK 28	UNK 32	UNK 36	UNK 40
H	STD D	CON 2	UNK 4	UNK 8	UNK 12	UNK 16	UNK 20	UNK 24	UNK 28	UNK 32	UNK 36	UNK 40

*Note: Allow all reagents and samples to reach room temperature (21°C - 26°C) before starting the assay.

Wash Solution

Dilute 50 mL of 10X concentrated WASH SOLUTION with 450 mL DI H_2O to a final volume of 500 mL.

Assay Procedure

1. Pipette 25 µL of standard, controls, and unknowns into each appropriate well.
2. Incubate for 60 minutes at room temperature on plate shaker (set to > 600 rpm).
3. Add 100 µL of ENZYME CONJUGATE to all wells.
4. Incubate for 60 minutes at room temperature on plate shaker (set to > 600 rpm).
5. Discard content of wells.
6. Wash wells 4 times with wash solution (300 µL per well).
7. Remove as much wash solution as possible from wells by tapping plate on absorbent paper.
8. Add 200 µL SUBSTRATE to all wells.
9. Wrap plate in foil.
10. Incubate 30 minutes (do not put on shaker!).
11. Add 50 µL STOP SOLUTION to all wells.
12. Read absorbance of wells at 450 nm on plate reader within 15 minutes of adding stop solution.

APPENDIX J

Procedures for Urine Analysis (urea N_2 and NPRQ calculation)

STANBIO UREA NITROGEN KIT PROCEDURES

For urine samples:

Add 1.9 mL of water to 100 µL of a well-mixed urine specimen.
Use 20 µL of this dilution (UNKNOWN) for analysis

Procedure:

1. Label 6+ test tubes:
    a. Blank
    b. Standard 1 (25 mg/dL)
    c. Standard 2 (50 mg/dL)
    d. Standard 3 (75 mg/dL)
    e. UNKNOWN 1 (a)
    f. UNKNOWN 1 (b)

    } This is a single sample run in duplicate

2. Pipette 20 µL water into Blank and 20 µL standard into appropriately labeled tubes
3. Pipette 20 µL of UNKNOWN into tubes (a) and (b)
4. Add 1.0 mL of BUN COLOR REAGENT into each tube
5. Add 2.0 mL of BUN ACID REAGENT into each tube
6. Cover and mix well
7. Incubate for exactly 12 minutes in a 100°C heat block
8. Remove from heat block and immerse in cold water bath for 4 minutes
9. Remove from cold water bath and mix well
10. Pipette 500 µL of mixture into 48-well microplate and read at 520 nm
    a. Color is stable for 30 minutes
    b. Alternatively can pipette 1.0 mL into cuvettes

Calculation of Results:

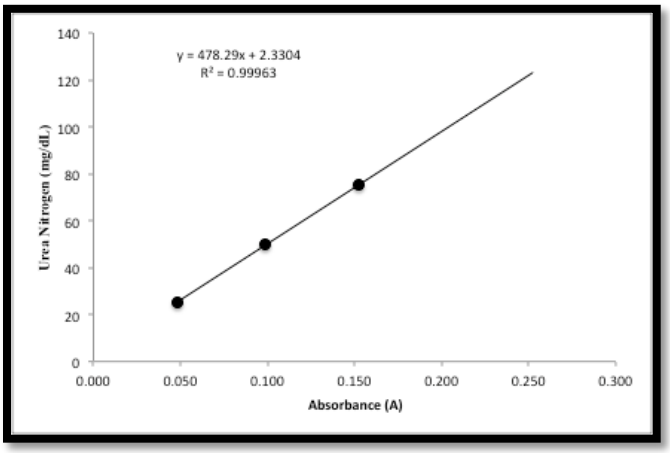
1. Plot absorbance readings vs BUN values (mg/dL) of standards on linear graph paper
2. Draw a line of best fit through the 3 standards
3. Plot absorbance of UNKNOWN sample on line of best fit
4. Determine concentration of UNKNOWN

Calculation of BUN:

1. Multiply observed value by 20
2. (gm/12.5 h) = BUN $\left(\frac{\text{mg}}{\text{dL}}\right) \times \frac{\text{urine volume (mL)}}{100{,}000}$

EXAMPLE CALCULATION FOR NON-PROTEIN RESPIRATORY QUOTIENT (NPRQ)

- Unknown sample with a blank-corrected absorbance = 0.032 nm
- Total urine volume = 900 mL (over 12.5 h)
- RMR VO_2 = 0.18 L/min
- RMR VCO_2 = 0.15 L/min



Step 1: Calculate urine urea nitrogen (UUN)

$$\text{UUN}\left(\frac{\text{mg}}{\text{dL}}\right) = (478.29 \times 0.032) + 2.3304$$

$$\text{UUN}\left(\frac{\text{mg}}{\text{dL}}\right) = 17.40$$

Step 2: Correct for dilution of urine by multiplying by 20

$$\mathbf{UUN}\left(\frac{\mathbf{mg}}{\mathbf{dL}}\right) = \mathbf{17.40 \times 20 = 347.93}$$

Step 3: Calculate BUN

$$\text{BUN}\left(\frac{\text{g}}{12.5\ \text{h}}\right) = 347.93 \times \left(\frac{900\ \text{mL}}{100{,}000}\right)$$

$$\mathbf{BUN}\left(\frac{\mathbf{g}}{\mathbf{12.5\ h}}\right) = \mathbf{3.131}$$

Step 4: Calculate grams of N_2 per hour by dividing BUN by 12.5 = 0.251 g N_2/h

Step 5: Calculate grams of protein per hour → per minute

$$0.251\ \frac{\text{g N}_2}{\text{h}} \times\ 6.25\ \frac{\text{g PRO}}{\text{g N}_2} = 1.566\ \frac{\text{g PRO}}{\text{h}} \times\ \frac{1\ \text{h}}{60\ \text{min}} = \mathbf{0.0261}\ \left(\frac{\mathbf{g\ PRO}}{\mathbf{min}}\right)$$

Step 6: Correct VO_2 and VCO_2 for oxidation of protein:

$$0.0261\ \frac{\text{g PRO}}{\text{min}} \times\ 0.97\ \frac{\text{L O}_2}{\text{g PRO}} = \mathbf{0.0253}\ \frac{\mathbf{L\ O_2}}{\mathbf{min}}$$

$$0.0261\ \frac{\text{g PRO}}{\text{min}} \times\ 0.78\ \frac{\text{L CO}_2}{\text{g PRO}} = \mathbf{0.0204}\ \frac{\mathbf{L\ CO_2}}{\mathbf{min}}$$

Step 7: Calculate total respiratory exchange corrected for the amounts of O_2 consumed (VO_2) and CO_2 produced (VCO_2) by the oxidation of protein:

$$0.1849\ \frac{\text{L O}_2}{\text{min}} - 0.0253\ \frac{\text{L O}_2}{\text{min}} = \mathbf{0.1596}\ \frac{\mathbf{L\ O_2}}{\mathbf{min}}$$

$$0.1532\ \frac{\text{L CO}_2}{\text{min}} - 0.0204\ \frac{\text{L CO}_2}{\text{min}} = \mathbf{0.1328}\ \frac{\mathbf{L\ CO_2}}{\mathbf{min}}$$

Step 8: Calculate NPRQ:

$$\frac{\text{VCO}_2}{\text{VO}_2} = \frac{0.1328}{0.1596} = \mathbf{0.83}$$

APPENDIX K

Raw Data

Table K1
Icon Meals Macronutrient Data

Meal	ID	Total Kcal	CHO (g)	PRO (g)	FAT (g)
BBQ Brisket Baked Potato	GH-001	495	38	46	18
Chicken & Veggies	GH-002	242	11.7	39.1	4.4
Beefy Mac & Cheese	GH-008	470	34	40	18
Buffalo Quesadilla	GH-009	480	38	42	17
Salmon & Broccoli	GH-010	299	9	32	15

Note. Participants ate the same meal for each overnight trial. CHO (carbohydrate); PRO (protein).

Table K2
Participant Descriptive Characteristics

	GH-001	GH-002	GH-008	GH-009	GH-010
Age (y)	22	24	22	21	24
Height (cm)	159.0	160.7	164.5	161.6	150.2
Weight (kg)	73.6	66.0	62.4	80.4	63.8
BMI (kg/m^2)	29.1	25.6	23.1	30.8	28.3
Waist Circumference (cm)	81.7	73.9	72.3	84.0	78.9
Body Fat (%)	40.9	38.2	37.0	40.7	39.4
Max Power (W_{max})	195	218	166	165	175
VO_{2max} (L/min)	2.25	2.57	1.61	2.09	1.65
VO_{2max} (mL/kg/min)	30.6	38.9	25.8	26.0	25.9

Note. BMI = body mass index; VO_{2max} = maximal oxygen consumption. Max power = highest power output achieved during VO_{2max} test.

Table K3
Criteria for Achievement of VO_{2max}

	GH-001	GH-002	GH-008	GH-009	GH-010
Age-Predicted HR_{max} (bpm)	198	196	198	199	196
Heart Rate (bpm)	n/a	189	199	175	195
Lactate (mmol/L)	13.0	10.3	9.2	10.2	9.55
RER	1.13	1.14	1.33	1.15	1.41
RPE	18	20	17	19	17
Plateau in VO_2	No	Yes	Yes	Yes	Yes

Note. (1) Heart rate must be within 10 bpm of age-predicted HRmax; (2) Lactate $\geq$ 8.0 mmol/L; (3) RER $\geq$ 1.10; RPE > 17 (ideal); plateau in VO_2 identified as a < 150 mL/min increase in VO_2 with an increase in work rate every 30 seconds (Howley et al., 1995).

Table K4
Estradiol (pg/mL) Concentration

	GH-001*	GH-002	GH-008	GH-009†	GH-010*
Control	36.41	90.60	43.12	208.39	42.09
Moderate-Intensity Exercise	39.63	86.65	48.18	147.87	43.99
High-Intensity Interval Exercise	53.66	83.61	44.03	142.70	30.03

Note. Estradiol measurements were determined from '0' sample collected in K_2EDTA
* Sronyx Birth control (oral tablets): Levonorgestrel and Ethinyl Estradiol Tablets, USP 0.1 mg/0.02 mg.
† Aubra Birth control (oral tablets): Levonorgestrel and Ethinyl Estradiol Tablets, USP 0.1 mg/0.02 mg.

Table K5
Exercise Variables Measured During MOD Trial

	GH-001	GH-002	GH-008	GH-009	GH-010
VO_2 (L/min)	1.48	1.36	1.25	1.45	1.25
VO_2 (mL/kg/min)	20.1	20.8	20.1	17.6	19.0
$\%VO_{2max}$	66	53	77	69	76
RER	0.85	0.96	0.95	0.94	1.00
EE (kcal)	144	162	135	149	135
Power Output (W)	80	90	75	83	75
% W_{max}	41	41	45	50	43
Mean HR (bpm)	144	171	181	141	164
%HRmax	73	87	91	71	84
Mean Lactate (mmol/L)	3.4	3.5	4.0	5.0	3.6
Peak Lactate (mmol/L)	4.2	4.0	4.5	6.3	4.4

Note. $\%VO_{2max}$ = MOD VO_2/VO_{2max}; RER = VCO_2/VO_2 measured during MOD trial; EE (energy expenditure) was calculated as power output (W) x exercise duration (h) x 3.6 [Broker & Gregor (1994) *MSSE 26*(1): 64-74]; $\%W_{max}$ = average watts during MOD/W_{max}; $\%HR_{max}$ = mean HR/HR_{max}; mean lactate was calculated as average of lactate values measured in 5-min increments during exercise (min 5 - min 30).

Table K6

Heart Rate During MOD Trial and Post-Exercise

Time Point	GH-001	GH-002	GH-008	GH-009	GH-010
(0)	86	80	82	74	83
(5)	148	158	169	132	145
(10)	141	168	183	141	152
(15)	141	170	179	146	163
(20)	145	177	183	145	171
(25)	145	173	181	130	176
(30)	146	179	188	152	178
(32)	116	125		115	
(34)	112	123		96	115
(36)		130	123	91	109
(38)	105	123	120	90	100
(40)	104	118	120	87	98
(45)	90	113	112	93	94
(50)	97	108	115	86	94
(60)	85	100	105	78	89
(90)		104	103	83	84
(120)		105	100	76	84
(750)		75	85	77	74

Note. Heart rate measurements are beats per minute (bpm).

Table K7

Lactate During MOD Trial and Post-Exercise

Time Point	GH-001	GH-002	GH-008	GH-009	GH-010
(0)	0.854	1.08	1.41	1.13	1.17
(5)	2.56	2.35	2.20	3.32	2.10
(10)	4.20	3.81	4.10	4.83	3.30
(15)	3.66	4.05	4.48	5.39	4.06
(20)	3.68	3.67	4.44	5.36	3.66
(25)	3.57	3.44	4.73	6.30	3.85
(30)	2.74	3.41	4.13	4.55	4.39
(32)	3.12	3.06	3.83	5.74	4.16
(34)	3.22	2.91	3.45	4.46	4.04
(36)	3.10	2.60	3.31	3.86	3.22
(38)	2.73	2.24	2.85	3.21	3.31
(40)	2.05	2.21	2.32	4.27	3.09
(45)		1.67	2.33	3.30	2.65
(50)	2.09	1.61	2.04	2.67	2.40
(60)	1.63	1.50	1.47	2.47	2.02
(90)	1.67	1.19	1.16	1.93	1.08
(120)	1.27	0.896	1.31	1.85	0.884
(750)	0.652	0.491	0.374	0.808	0.511

Note. Lactate measurements (mmol/L).

Table K8

Power Output During HIE Trial

Time Point	GH-001	GH-002	GH-008	GH-009	GH-010
Peak Power					
1st sprint	550	588	432	624	409
2nd sprint	471	541	451	588	425
3rd sprint	468	501	336	579	420
4th sprint	391	495	390	513	369
Mean Power					
1st sprint	380	376	365	470	358
2nd sprint	336	292	298	389	326
3rd sprint	322	265	215	306	307
4th sprint	339	274	294	306	281

Note. Power output is expressed in watts (W).

Table K9

Heart Rate During HIE Trial and Post-Exercise

Time Point	GH-001	GH-002	GH-008	GH-009	GH-010
(0)	83	75	78	64	89
(1st)	151	182	191	165	190
(2nd)	178	186	191	168	193
(3rd)	175	183	181	162	193
(4th)	175	181	184	167	191
(30)		159	149	96	125
(32)		158	119	102	116
(34)	110	152	114	96	116
(36)		147	112	90	113
(38)	111	138	113	86	116
(40)	106	123	116	88	113
(45)		118	113	87	118
(50)	102	111	111	82	101
(60)	89	111	103	88	101
(90)	85	97	90	74	88
(120)	81	92	84	70	89
(750)	75	70	82	70	82

Note. Heart rate measurements are beats per minute (bpm). 1st: end of 1st sprint, 2nd: end of 2nd sprint, 3rd: end of 3rd sprint, 4th: end of 4th sprint.

Table K10

Lactate During HIE Trial and Post-Exercise

Time Point	GH-001	GH-002	GH-008	GH-009	GH-010
(0)	1.20	1.42	0.518	0.701	2.14
(1st)	5.09	5.89	5.21	3.26	3.42
(2nd)	8.80	10.32	9.96	7.70	6.36
(3rd)	10.45	12.37	11.50	8.73	7.86
(4th)	10.00	13.1	11.50	8.81	8.74
(2-min)		14.50	11.83	9.10	9.64
(30)	10.25	13.25	11.90	9.12	9.55
(32)	8.43	12.80	10.80	8.54	9.98
(34)	9.16	12.50	11.46	8.53	7.78
(36)	8.24	13.75	11.83	7.48	8.40
(38)	8.02		11.30	7.75	7.57
(40)	8.74	13.25	11.25	7.72	7.47
(45)	7.43			6.52	9.38
(50)	6.73			5.04	6.53
(60)	5.77		7.82	3.09	5.19
(90)	2.98	5.21	4.46	1.90	2.96
(120)	1.74	3.46	2.01	1.46	2.08
(750)	0.479	0.460	0.460	0.604	

Note. Lactate measurements are beats per minute (mmol/L). 1st: end of first sprint, 2nd: end of 2nd sprint, 3rd: end of 3rd sprint, 4th: end of 4th sprint, 2-min: lactate measured 2 minutes after 4th sprint (during recovery).

Table K11

Estimated Energy Expenditure During Exercise Trials

	GH-001	GH-002	GH-008	GH-009	GH-010
Moderate-Intensity Exercise	144	162	135	149	135
High-Intensity Interval Exercise	100	94	93	103	96

Note. Energy Expenditure expressed as total kilocalories expended.

Table K12

Non-Protein Respiratory Quotient (NPRQ) and Substrate Oxidation

Time Point	GH-001	GH-002	GH-008	GH-009	GH-010
CON					
Urea N_2	0.442	0.251	0.335	0.332	0.345
NPRQ	0.79	0.83	0.83	0.81	0.84
% CHO	29.90	43.80	43.80	36.91	47.20
% FAT	70.10	56.20	56.20	63.10	52.80
MOD					
Urea N_2	0.334	0.539	0.387	0.584	0.485
NPRQ	0.77	0.81	0.79	0.92	0.70
% CHO	22.80	36.90	29.90	74.10	0.00
% FAT	77.20	63.10	70.10	25.90	100.00
HIE					
Urea N_2	0.466	0.274	0.437	0.437	0.575
NPRQ	0.77	0.77	0.73	0.78	0.78
% CHO	22.80	22.80	8.40	26.30	26.30
% FAT	77.20	77.20	91.60	73.70	73.70

Note. CON = Control Trial; MOD = moderate-intensity trial; HIE = high-intensity interval exercise trial; urea nitrogen (N_2) is measured as g/h.

Table K13

Calculated Area Under the Curve (0 – 120 min)

	GH-001	GH-002	GH-008	GH-009	GH-010
Control (CON)	80.19	168.53	16.56	307.88	335.34
Moderate-Intensity Exercise (MOD)	752.73	655.82	467.05	503.21	344.96
High-Intensity Interval Exercise (HIE)	720.55	548.92	607.68	1326.87	1887.11

Note. Units for AUC are ng/mL/120 min.

Table K14
Deconvolution Analysis Data – 12.5h CON Trial

	GH-001	GH-002	GH-008	GH-009	GH-010
Mean GH ($ng \cdot mL^{-1}$)	1.6759	0.8875	1.3779	1.6328	1.4196
Pulsatile Secretion ($ng \cdot mL^{-1} \cdot 12.5h^{-1}$)	1273.7	674.5	1047.2	1240.9	965.3
Pulse #	10	4	10	13	13
Interpulse Interval (min)	75.24	154.59	40.29	48.47	52.36
Pulse Height (ng)	0.41	0.53	0.28	1.50	0.33
Pulse Area ($ng \cdot mL^{-1} \cdot min^{-1}$)	13.61	22.23	4.55	8.05	6.24

Table K15
Deconvolution Analysis Data – 12.5h MOD Trial

	GH-001	GH-002	GH-008	GH-009	GH-010
Mean GH ($ng \cdot mL^{-1}$)	2.00	1.98	2.24	1.54	1.86
Pulsatile Secretion ($ng \cdot mL^{-1} \cdot 12.5h^{-1}$)	1522.9	1505.1	1679.0	1170.6	1268.5
Pulse #	8	8	9	11	8
Interpulse Interval (min)	81.89	89.88	71.38	63.99	92.86
Pulse Height (ng)	0.47	0.23	0.39	0.37	0.32
Pulse Area ($ng \cdot mL^{-1} \cdot min^{-1}$)	16.18	6.59	9.99	5.19	9.13

Table K16
Deconvolution Analysis Data – 12.5h HIE Trial

	GH-001	GH-002	GH-008	GH-009	GH-010
Mean GH ($ng \cdot mL^{-1}$)	2.55	1.33	1.63	2.26	4.77
Pulsatile Secretion ($ng \cdot mL^{-1} \cdot 12.5h^{-1}$)	1942.0	1010.8	1239.1	1716.7	3247.4
Pulse #	14	5	7	7	13
Interpulse Interval (min)	48.33	151.99	102.00	93.65	53.05
Pulse Height (ng)	0.55	0.38	0.27	0.34	0.71
Pulse Area ($ng \cdot mL^{-1} \cdot min^{-1}$)	8.47	15.45	7.67	9.77	18.91

Table K17

Deconvolution Analysis Data – 7.5h CON Trial (Sleep Data Only)

	GH-001	GH-002	GH-008	GH-009	GH-010
Mean GH ($ng \cdot mL^{-1}$)	2.49	0.91	1.90	0.42	1.11
Pulsatile Secretion ($ng \cdot mL^{-1} \cdot 7.5h^{-1}$)	1143.80	419.10	872.79	191.81	445.35
Pulse #	4	5	6	2	7
Interpulse Interval (min)	111.36	99.48	46.76	173.12	52.17
Pulse Height (ng)	0.64	0.23	0.43	0.04	0.20
Pulse Area ($ng \cdot mL^{-1} \cdot min^{-1}$)	21.09	13.05	6.13	2.71	3.59

Table K18

Deconvolution Analysis Data – 7.5h MOD Trial (Sleep Data Only)

	GH-001	GH-002	GH-008	GH-009	GH-010
Mean GH ($ng \cdot mL^{-1}$)	1.54	1.35	2.10	0.89	1.79
Pulsatile Secretion ($ng \cdot mL^{-1} \cdot 7.5h^{-1}$)	709.21	620.16	967.57	411.88	715.34
Pulse #	5	4	4	7	7
Interpulse Interval (min)	60.44	108.88	53.65	58.34	60.77
Pulse Height (ng)	0.43	0.65	0.30	0.23	0.38
Pulse Area ($ng \cdot mL^{-1} \cdot min^{-1}$)	15.43	30.11	10.87	3.43	4.68

Table K19

Deconvolution Analysis Data – 7.5h HIE Trial (Sleep Data Only)

	GH-001	GH-002	GH-008	GH-009	GH-010
Mean GH ($ng \cdot mL^{-1}$)	2.61	0.93	0.81	0.65	3.37
Pulsatile Secretion ($ng \cdot mL^{-1} \cdot 7.5h^{-1}$)	1202.90	427.59	372.15	299.93	1282.40
Pulse #	9	2	8	10	4
Interpulse Interval (min)	41.85	47.14	30.92	36.57	81.49
Pulse Height (ng)	0.44	0.28	0.11	0.08	0.25
Pulse Area ($ng \cdot mL^{-1} \cdot min^{-1}$)	8.34	9.71	1.99	1.76	7.87

APPENDIX L

Raw Growth Hormone Data for Each Participant

Table L1. Raw Growth Hormone Data for GH-001: Control Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	0.615	0.604	0.610	0.008	0.006	1.28%
5:40 PM	(10)	2.406	2.483	2.445	0.054	0.039	2.23%
5:50 PM	(20)	1.977	2.213	2.095	0.167	0.118	7.97%
6:00 PM	(30)	1.05	1.251	1.151	0.142	0.101	12.35%
6:10 PM	(40)	0.668	0.689	0.679	0.015	0.011	2.19%
6:20 PM	(50)	0.444	0.432	0.438	0.008	0.006	1.94%
6:30 PM	(60)	0.3	0.314	0.307	0.010	0.007	3.22%
6:40 PM	(70)	0.128	0.188	0.158	0.042	0.030	26.85%
6:50 PM	(80)	0.046	<0.000	0.046			
7:00 PM	(90)	<0.000	<0.000				
7:10 PM	(100)	0.078	<0.000	0.078			
7:20 PM	(110)	<0.000	<0.000				
7:30 PM	(120)	<0.000	<0.000				
7:40 PM	(130)	<0.000	<0.000				
7:50 PM	(140)	<0.000	<0.000				
8:00 PM	(150)	<0.000	<0.000				
8:10 PM	(160)	0.188	<0.000	0.188			
8:20 PM	(170)	0.078	0.105	0.092	0.019	0.014	20.87%
8:30 PM	(180)	0.017	0.017	0.017	0.000	0.000	0.00%
8:40 PM	(190)	0.149	0.046	0.098	0.073	0.052	74.70%
8:50 PM	(200)	0.105	0.149	0.127	0.031	0.022	24.50%
9:00 PM	(210)	0.062	0.017	0.040	0.032	0.023	80.56%
9:10 PM	(220)	0.122	0.122	0.122	0.000	0.000	0.00%
9:20 PM	(230)	0.243	0.243	0.243	0.000	0.000	0.00%
9:30 PM	(240)	0.304	0.335	0.320	0.022	0.016	6.86%
9:40 PM	(250)	0.274	0.289	0.282	0.011	0.007	3.77%
9:50 PM	(260)	0.228	0.213	0.221	0.011	0.008	4.81%
10:00 PM	(270)	0.152	0.167	0.160	0.011	0.008	6.65%
10:10 PM	(280)	0.691	0.691	0.691	0.000	0.000	0.00%
10:20 PM	(290)	2.843	2.544	2.694	0.211	0.150	7.85%
10:30 PM	(300)	4.942	4.784	4.863	0.112	0.079	2.30%
10:40 PM	(310)	5.773	6.576	6.175	0.568	0.402	9.20%
10:50 PM	(320)	6.881	6.598	6.740	0.200	0.142	2.97%
11:00 PM	(330)	5.002	4.902	4.952	0.071	0.050	1.43%
11:10 PM	(340)	3.327	3.621	3.474	0.208	0.147	5.98%
11:20 PM	(350)	2.13	2.25	2.190	0.085	0.060	3.87%
11:30 PM	(360)	1.12	0.928	1.024	0.136	0.096	13.26%
11:40 PM	(370)	0.504	0.489	0.497	0.011	0.008	2.14%
11:50 PM	(380)	0.243	0.228	0.236	0.011	0.007	4.50%
12:00 AM	(390)	0.047	0.047	0.047	0.000	0.000	0.00%
12:10 AM	(400)	0.032	0.062	0.047	0.021	0.015	45.13%
12:20 AM	(410)	0.105	0.188	0.147	0.059	0.042	40.06%
12:30 AM	(420)	<0.000	<0.000				
12:40 AM	(430)	<0.000	0.046	0.046			
12:50 AM	(440)	<0.000	<0.000				
1:00 AM	(450)	0.078	<0.000	0.078			
1:10 AM	(460)	<0.000	<0.000				

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	<0.000	<0.000				
1:30 AM	(480)	<0.000	0.078	0.078			
1:40 AM	(490)	<0.000	<0.000				
1:50 AM	(500)	0.078	0.128	0.103	0.035	0.025	34.33%
2:00 AM	(510)	0.355	0.355	0.355	0.000	0.000	0.00%
2:10 AM	(520)	2.374	1.968	2.171	0.287	0.203	13.22%
2:20 AM	(530)	7.342	7.489	7.416	0.104	0.074	1.40%
2:30 AM	(540)	11.608	11.183	11.396	0.301	0.213	2.64%
2:40 AM	(550)	11.901	14.753	13.327	2.017	1.426	15.13%
2:50 AM	(560)	11.81	13.296	12.553	1.051	0.743	8.37%
3:00 AM	(570)	9.06	9.405	9.233	0.244	0.172	2.64%
3:10 AM	(580)	7.174	7.636	7.405	0.327	0.231	4.41%
3:20 AM	(590)	6.033	6.425	6.229	0.277	0.196	4.45%
3:30 AM	(600)	4.886	5.11	4.998	0.158	0.112	3.17%
3:40 AM	(610)	3.345	3.184	3.265	0.114	0.081	3.49%
3:50 AM	(620)	1.785	1.457	1.621	0.232	0.164	14.31%
4:00 AM	(630)	0.684	0.62	0.652	0.045	0.032	6.94%
4:10 AM	(640)	0.709	0.75	0.730	0.029	0.021	3.97%
4:20 AM	(650)	0.538	0.549	0.544	0.008	0.006	1.43%
4:30 AM	(660)	0.42	0.457	0.439	0.026	0.019	5.97%
4:40 AM	(670)	0.314	0.328	0.321	0.010	0.007	3.08%
4:50 AM	(680)	0.255	0.239	0.247	0.011	0.008	4.58%
5:00 AM	(690)	0.188	0.188	0.188	0.000	0.000	0.00%
5:10 AM	(700)	0.128	0.105	0.117	0.016	0.012	13.96%
5:20 AM	(710)	<0.000	0.046	0.046			
5:30 AM	(720)	0.078	0.046	0.062	0.023	0.016	36.50%
5:40 AM	(730)	<0.000	<0.000				
5:50 AM	(740)	<0.000	<0.000				
6:00 AM	(750)	<0.000	<0.000				

Note: values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L2. Raw Growth Hormone Data for GH-001: Moderate-Intensity Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	9.225	8.878	9.052	0.245	0.174	2.71%
5:40 PM	(10)						
5:50 PM	(20)	13.73	16.767	15.249	2.147	1.519	14.08%
6:00 PM	(30)	16.603	16.701	16.652	0.069	0.049	0.42%
6:10 PM	(40)	10.852	10.193	10.523	0.466	0.330	4.43%
6:20 PM	(50)	7.217	7.295	7.256	0.055	0.039	0.76%
6:30 PM	(60)	3.956	4.44	4.198	0.342	0.242	8.15%
6:40 PM	(70)	2.289	2.304	2.297	0.011	0.007	0.46%
6:50 PM	(80)	1.288	1.197	1.243	0.064	0.046	5.18%
7:00 PM	(90)	0.56	0.683	0.622	0.087	0.062	13.99%
7:10 PM	(100)	0.405	0.389	0.397	0.011	0.008	2.85%
7:20 PM	(110)	0.042	0.06	0.051	0.013	0.009	24.96%
7:30 PM	(120)	0.228	0.219	0.224	0.006	0.005	2.85%
7:40 PM	(130)	0.219	0.286	0.253	0.047	0.033	18.76%
7:50 PM	(140)	0.153	<0.000	0.153			
8:00 PM	(150)	0.093	0.153	0.123	0.042	0.030	34.49%
8:10 PM	(160)	0.025	0.044	0.035	0.013	0.009	38.94%
8:20 PM	(170)	0.025	0.025	0.025	0.000	0.000	0.00%
8:30 PM	(180)	0.003	0.003	0.003	0.000	0.000	0.00%
8:40 PM	(190)	0.077	0.044	0.061	0.023	0.017	38.57%
8:50 PM	(200)	0.061	0.093	0.077	0.023	0.016	29.39%
9:00 PM	(210)	0.093	0.077	0.085	0.011	0.008	13.31%
9:10 PM	(220)	0.109	0.077	0.093	0.023	0.016	24.33%
9:20 PM	(230)	0.093	0.093	0.093	0.000	0.000	0.00%
9:30 PM	(240)	0.093	0.109	0.101	0.011	0.008	11.20%
9:40 PM	(250)	0.093	0.124	0.109	0.022	0.016	20.20%
9:50 PM	(260)	0.061	0.061	0.061	0.000	0.000	0.00%
10:00 PM	(270)	<0.000	0.044	0.044			
10:10 PM	(280)	0.044	0.044	0.044	0.000	0.000	0.00%
10:20 PM	(290)	0.153	0.139	0.146	0.010	0.007	6.78%
10:30 PM	(300)	0.21	0.196	0.203	0.010	0.007	4.88%
10:40 PM	(310)	0.304	0.291	0.298	0.009	0.007	3.09%
10:50 PM	(320)						
11:00 PM	(330)	1.062	1.152	1.107	0.064	0.045	5.75%
11:10 PM	(340)	4.474	4.406	4.440	0.048	0.034	1.08%
11:20 PM	(350)	5.749	5.623	5.686	0.089	0.063	1.57%
11:30 PM	(360)	7.936	8.141	8.039	0.145	0.103	1.80%
11:40 PM	(370)	6.829	6.964	6.897	0.095	0.068	1.38%
11:50 PM	(380)	4.678	4.457	4.568	0.156	0.111	3.42%
12:00 AM	(390)	3.874	3.973	3.924	0.070	0.049	1.78%
12:10 AM	(400)	3.34	3.554	3.447	0.151	0.107	4.39%
12:20 AM	(410)	2.716	2.634	2.675	0.058	0.041	2.17%
12:30 AM	(420)	1.322	1.372	1.347	0.035	0.025	2.62%
12:40 AM	(430)	0.693	0.693	0.693	0.000	0.000	0.00%
12:50 AM	(440)	0.282	0.269	0.276	0.009	0.006	3.34%
1:00 AM	(450)	0.093	0.093	0.093	0.000	0.000	0.00%
1:10 AM	(460)	0.265	0.224	0.245	0.029	0.021	11.86%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	0.0.93	0.109	0.101	0.011	0.008	11.20%
1:30 AM	(480)	0.124	0.109	0.117	0.011	0.008	9.10%
1:40 AM	(490)	0.168	0.153	0.161	0.011	0.008	6.61%
1:50 AM	(500)	0.054	0.067	0.061	0.009	0.007	15.19%
2:00 AM	(510)	0.419	0.407	0.413	0.008	0.006	2.05%
2:10 AM	(520)	0.531	0.519	0.525	0.008	0.006	1.62%
2:20 AM	(530)	0.805	0.793	0.799	0.008	0.006	1.06%
2:30 AM	(540)	1.056	1.106	1.081	0.035	0.025	3.27%
2:40 AM	(550)	1.372	1.322	1.347	0.035	0.025	2.62%
2:50 AM	(560)	1.207	1.233	1.220	0.018	0.013	1.51%
3:00 AM	(570)	2.02	1.993	2.007	0.019	0.014	0.95%
3:10 AM	(580)	2.88	2.977	2.929	0.069	0.049	2.34%
3:20 AM	(590)	3.44	3.712	3.576	0.192	0.136	5.38%
3:30 AM	(600)	4.388	4.105	4.247	0.200	0.142	4.71%
3:40 AM	(610)	3.354	3.2	3.277	0.109	0.077	3.32%
3:50 AM	(620)	1.823	1.849	1.836	0.018	0.013	1.00%
4:00 AM	(630)	0.993	0.993	0.993	0.000	0.000	0.00%
4:10 AM	(640)	0.382	0.394	0.388	0.008	0.006	2.19%
4:20 AM	(650)	0.118	0.182	0.150	0.045	0.032	30.17%
4:30 AM	(660)	0.224	0.278	0.251	0.038	0.027	15.21%
4:40 AM	(670)	0.124	0.139	0.132	0.011	0.008	8.07%
4:50 AM	(680)	0.124	0.093	0.109	0.022	0.016	20.20%
5:00 AM	(690)	0.061	<0.000	0.061			
5:10 AM	(700)	0.044	0.025	0.035	0.013	0.009	38.94%
5:20 AM	(710)	0.061	0.077	0.069	0.011	0.008	16.40%
5:30 AM	(720)	0.025	<0.000	0.025			
5:40 AM	(730)	0.077	0.025	0.051	0.037	0.026	72.10%
5:50 AM	(740)	0.109	0.196	0.153	0.062	0.044	40.34%
6:00 AM	(750)	0.168	0.168	0.168	0.000	0.000	0.00%

Note: Time points highlighted in grey are missing values. Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L3. Raw Growth Hormone Data for GH-001: High-Intensity Interval Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	0.087	0.097	0.092	0.007	0.005	7.69%
5:40 PM	(10)	1.436	1.436	1.436	0.000	0.000	0.00%
5:50 PM	(20)	13.92	13.972	13.946	0.037	0.026	0.26%
6:00 PM	(30)	19.986	20.34	20.163	0.250	0.177	1.24%
6:10 PM	(40)	13.944	12.461	13.203	1.049	0.742	7.94%
6:20 PM	(50)	7.528	8.866	8.197	0.946	0.669	11.54%
6:30 PM	(60)	6.307	6.129	6.218	0.126	0.089	2.02%
6:40 PM	(70)	4.151	4.255	4.203	0.074	0.052	1.75%
6:50 PM	(80)	2.162	2.000	2.081	0.115	0.081	5.50%
7:00 PM	(90)	1.082	1.215	1.149	0.094	0.067	8.19%
7:10 PM	(100)	0.809	0.86	0.835	0.036	0.026	4.32%
7:20 PM	(110)	0.438	0.498	0.468	0.042	0.030	9.07%
7:30 PM	(120)	0.218	0.229	0.224	0.008	0.006	3.48%
7:40 PM	(130)	0.173	0.173	0.173	0.000	0.000	0.00%
7:50 PM	(140)	0.13	0.141	0.136	0.008	0.005	5.74%
8:00 PM	(150)	0.13	0.13	0.130	0.000	0.000	0.00%
8:10 PM	(160)	0.098	0.057	0.078	0.029	0.021	37.41%
8:20 PM	(170)	0.047	0.067	0.057	0.014	0.010	24.81%
8:30 PM	(180)	0.109	0.098	0.104	0.008	0.006	7.52%
8:40 PM	(190)	0.088	0.098	0.093	0.007	0.005	7.60%
8:50 PM	(200)	0.109	0.057	0.083	0.037	0.026	44.30%
9:00 PM	(210)	0.098	0.077	0.088	0.015	0.011	16.97%
9:10 PM	(220)	0.109	0.109	0.109	0.000	0.000	0.00%
9:20 PM	(230)	0.109	0.098	0.104	0.008	0.006	7.52%
9:30 PM	(240)	0.077	0.077	0.077	0.000	0.000	0.00%
9:40 PM	(250)	0.077	0.088	0.083	0.008	0.006	9.43%
9:50 PM	(260)	0.067	0.057	0.062	0.007	0.005	11.40%
10:00 PM	(270)	0.067	0.077	0.072	0.007	0.005	9.82%
10:10 PM	(280)	0.067	0.077	0.072	0.007	0.005	9.82%
10:20 PM	(290)	0.173	0.184	0.179	0.008	0.006	4.36%
10:30 PM	(300)	0.45	0.45	0.450	0.000	0.000	0.00%
10:40 PM	(310)	0.571	0.583	0.577	0.008	0.006	1.47%
10:50 PM	(320)	0.708	0.758	0.733	0.035	0.025	4.82%
11:00 PM	(330)	1.029	1.016	1.023	0.009	0.006	0.90%
11:10 PM	(340)	1.282	1.296	1.289	0.010	0.007	0.77%
11:20 PM	(350)	1.095	1.135	1.115	0.028	0.020	2.54%
11:30 PM	(360)	0.771	0.695	0.733	0.054	0.038	7.33%
11:40 PM	(370)	0.67	0.708	0.689	0.027	0.019	3.90%
11:50 PM	(380)	0.682	0.745	0.714	0.045	0.032	6.24%
12:00 AM	(390)	3.054	3.182	3.118	0.091	0.064	2.90%
12:10 AM	(400)	3.558	3.086	3.322	0.334	0.236	10.05%
12:20 AM	(410)	1.798	1.87	1.834	0.051	0.036	2.78%
12:30 AM	(420)	0.924	0.924	0.924	0.000	0.000	0.00%
12:40 AM	(430)	0.571	0.583	0.577	0.008	0.006	1.47%
12:50 AM	(440)	0.276	0.286	0.281	0.007	0.005	2.52%
1:00 AM	(450)	0.209	0.2	0.205	0.006	0.004	3.11%
1:10 AM	(460)	0.153	0.172	0.163	0.013	0.009	8.27%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	0.209	0.191	0.200	0.013	0.009	6.36%
1:30 AM	(480)	0.629	0.639	0.634	0.007	0.005	1.12%
1:40 AM	(490)	5.353	4.919	5.136	0.307	0.217	5.98%
1:50 AM	(500)	9.829	8.874	9.352	0.675	0.478	7.22%
2:00 AM	(510)	12.435	13.507	12.971	0.758	0.536	5.84%
2:10 AM	(520)	11.992	11.334	11.663	0.465	0.329	3.99%
2:20 AM	(530)	10.654	10.354	10.504	0.212	0.150	2.02%
2:30 AM	(540)	8.954	9.767	9.361	0.575	0.406	6.14%
2:40 AM	(550)	7.212	8.38	7.796	0.826	0.584	10.59%
2:50 AM	(560)	7.59	6.744	7.167	0.598	0.423	8.35%
3:00 AM	(570)	6.008	5.971	5.990	0.026	0.019	0.44%
3:10 AM	(580)	5.516	5.607	5.562	0.064	0.046	1.16%
3:20 AM	(590)	4.341	4.811	4.576	0.332	0.235	7.26%
3:30 AM	(600)	3.58	3.543	3.562	0.026	0.019	0.73%
3:40 AM	(610)	2.597	2.597	2.597	0.000	0.000	0.00%
3:50 AM	(620)	1.633	1.716	1.675	0.059	0.042	3.50%
4:00 AM	(630)	0.839	1.096	0.968	0.182	0.129	18.78%
4:10 AM	(640)	0.478	0.56	0.519	0.058	0.041	11.17%
4:20 AM	(650)	0.549	0.519	0.534	0.021	0.015	3.97%
4:30 AM	(660)	0.402	0.402	0.402	0.000	0.000	0.00%
4:40 AM	(670)	0.286	0.286	0.286	0.000	0.000	0.00%
4:50 AM	(680)	0.228	0.238	0.233	0.007	0.005	3.03%
5:00 AM	(690)	0.162	0.2	0.181	0.027	0.019	14.85%
5:10 AM	(700)	0.172	0.162	0.167	0.007	0.005	4.23%
5:20 AM	(710)	0.125	0.134	0.130	0.006	0.005	4.91%
5:30 AM	(720)	0.097	0.115	0.106	0.013	0.009	12.01%
5:40 AM	(730)	0.097	0.069	0.083	0.020	0.014	23.85%
5:50 AM	(740)	0.069	0.078	0.074	0.006	0.005	8.66%
6:00 AM	(750)	0.087	0.162	0.125	0.053	0.038	42.60%

Note: The values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L4. Raw Growth Hormone Data for GH-002: Control Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	0.27	0.27	0.270	0.000	0.000	0.00%
5:40 PM	(10)	1.993	1.865	1.929	0.091	0.064	4.69%
5:50 PM	(20)	3.372	3.196	3.284	0.124	0.088	3.79%
6:00 PM	(30)	4.585	4.392	4.489	0.136	0.096	3.04%
6:10 PM	(40)	2.111	1.533	1.822	0.409	0.289	22.43%
6:20 PM	(50)	0.864	1.24	1.052	0.266	0.188	25.27%
6:30 PM	(60)	1.287	1.306	1.297	0.013	0.010	1.04%
6:40 PM	(70)	0.925	0.894	0.910	0.022	0.016	2.41%
6:50 PM	(80)	0.75	0.7	0.725	0.035	0.025	4.88%
7:00 PM	(90)	0.49	0.51	0.500	0.014	0.010	2.83%
7:10 PM	(100)	0.363	0.373	0.368	0.007	0.005	1.92%
7:20 PM	(110)	0.257	0.257	0.257	0.000	0.000	0.00%
7:30 PM	(120)	0.172	0.172	0.172	0.000	0.000	0.00%
7:40 PM	(130)	0.134	0.134	0.134	0.000	0.000	0.00%
7:50 PM	(140)	0.125	0.125	0.125	0.000	0.000	0.00%
8:00 PM	(150)	0.115	0.125	0.120	0.007	0.005	5.89%
8:10 PM	(160)	0.106	0.106	0.106	0.000	0.000	0.00%
8:20 PM	(170)	0.097	0.106	0.102	0.006	0.005	6.27%
8:30 PM	(180)	0.115	0.143	0.129	0.020	0.014	15.35%
8:40 PM	(190)	0.143	0.162	0.153	0.013	0.010	8.81%
8:50 PM	(200)	0.209	0.209	0.209	0.000	0.000	0.00%
9:00 PM	(210)	0.441	0.451	0.446	0.007	0.005	1.59%
9:10 PM	(220)	0.682	1.021	0.852	0.240	0.170	28.15%
9:20 PM	(230)	1.581	1.972	1.777	0.276	0.195	15.56%
9:30 PM	(240)	1.224	1.14	1.182	0.059	0.042	5.03%
9:40 PM	(250)	0.177	0.319	0.248	0.100	0.071	40.49%
9:50 PM	(260)	0.936	0.977	0.957	0.029	0.021	3.03%
10:00 PM	(270)	0.791	0.822	0.807	0.022	0.016	2.72%
10:10 PM	(280)	0.609	0.639	0.624	0.021	0.015	3.40%
10:20 PM	(290)	0.51	0.519	0.515	0.006	0.005	1.24%
10:30 PM	(300)	0.344	0.421	0.383	0.054	0.039	14.23%
10:40 PM	(310)	0.238	0.247	0.243	0.006	0.005	2.62%
10:50 PM	(320)						
11:00 PM	(330)	0.781	0.822	0.802	0.029	0.021	3.62%
11:10 PM	(340)	1.224	1.257	1.241	0.023	0.017	1.88%
11:20 PM	(350)	0.952	1.323	1.138	0.262	0.186	23.06%
11:30 PM	(360)	0.587	0.682	0.635	0.067	0.048	10.59%
11:40 PM	(370)	0.385	0.427	0.406	0.030	0.021	7.31%
11:50 PM	(380)	0.606	0.488	0.547	0.083	0.059	15.25%
12:00 AM	(390)	<0.000	0.468	0.468			
12:10 AM	(400)	0.719	0.828	0.774	0.077	0.055	9.96%
12:20 AM	(410)	0.7	0.625	0.663	0.053	0.038	8.00%
12:30 AM	(420)	1.14	1.323	1.232	0.129	0.092	10.51%
12:40 AM	(430)	1.644	1.77	1.707	0.089	0.063	5.22%
12:50 AM	(440)	2.652	2.897	2.775	0.173	0.123	6.24%
1:00 AM	(450)	3.849	3.327	3.588	0.369	0.261	10.29%
1:10 AM	(460)	2.97	3.088	3.029	0.083	0.059	2.75%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	2.482	2.524	2.503	0.030	0.021	1.19%
1:30 AM	(480)	1.917	2.08	1.999	0.115	0.082	5.77%
1:40 AM	(490)	1.731	2.039	1.885	0.218	0.154	11.55%
1:50 AM	(500)	1.757	1.784	1.771	0.019	0.014	1.08%
2:00 AM	(510)	1.573	1.508	1.541	0.046	0.033	2.98%
2:10 AM	(520)	1.087	1.099	1.093	0.008	0.006	0.78%
2:20 AM	(530)	0.741	0.705	0.723	0.025	0.018	3.52%
2:30 AM	(540)	0.561	0.537	0.549	0.017	0.012	3.09%
2:40 AM	(550)	0.49	0.549	0.520	0.042	0.030	8.03%
2:50 AM	(560)	0.585	0.585	0.585	0.000	0.000	0.00%
3:00 AM	(570)	0.513	0.49	0.502	0.016	0.012	3.24%
3:10 AM	(580)	0.633	0.717	0.675	0.059	0.042	8.80%
3:20 AM	(590)	0.621	0.657	0.639	0.025	0.018	3.98%
3:30 AM	(600)	0.561	0.609	0.585	0.034	0.024	5.80%
3:40 AM	(610)	0.407	0.478	0.443	0.050	0.036	11.35%
3:50 AM	(620)	0.442	0.372	0.407	0.049	0.035	12.16%
4:00 AM	(630)	0.209	0.267	0.238	0.041	0.029	17.23%
4:10 AM	(640)	0.152	0.163	0.158	0.008	0.006	4.94%
4:20 AM	(650)	0.106	0.106	0.106	0.000	0.000	0.00%
4:30 AM	(660)	0.106	0.118	0.112	0.008	0.006	7.58%
4:40 AM	(670)	0.095	0.095	0.095	0.000	0.000	0.00%
4:50 AM	(680)	0.14	0.14	0.140	0.000	0.000	0.00%
5:00 AM	(690)	0.163	0.152	0.158	0.008	0.006	4.94%
5:10 AM	(700)	0.407	0.383	0.395	0.017	0.012	4.30%
5:20 AM	(710)	0.501	0.478	0.490	0.016	0.012	3.32%
5:30 AM	(720)	0.693	0.705	0.699	0.008	0.006	1.21%
5:40 AM	(730)	0.79	0.717	0.754	0.052	0.037	6.85%
5:50 AM	(740)	1.049	1.024	1.037	0.018	0.013	1.71%
6:00 AM	(750)	1.187	1.162	1.175	0.018	0.013	1.51%

Note: Time points highlighted in grey are missing values. Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L5. Raw Growth Hormone Data for GH-002: Moderate-Intensity Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	0.044	0.044	0.044	0.000	0.000	0.00%
5:40 PM	(10)	4.538	4.616	4.577	0.055	0.039	1.21%
5:50 PM	(20)	8.618	7.833	8.226	0.555	0.393	6.75%
6:00 PM	(30)	9.416	9.631	9.524	0.152	0.108	1.60%
6:10 PM	(40)	7.16	6.731	6.946	0.303	0.215	4.37%
6:20 PM	(50)	6.472	5.598	6.035	0.618	0.437	10.24%
6:30 PM	(60)	4.59	4.472	4.531	0.083	0.059	1.84%
6:40 PM	(70)	4.076	4.169	4.123	0.066	0.047	1.60%
6:50 PM	(80)	4.01	3.916	3.963	0.066	0.047	1.68%
7:00 PM	(90)	4.811	4.511	4.661	0.212	0.150	4.55%
7:10 PM	(100)	5.623	5.739	5.681	0.082	0.058	1.44%
7:20 PM	(110)	5.109	5.147	5.128	0.027	0.019	0.52%
7:30 PM	(120)	4.222	4.446	4.334	0.158	0.112	3.65%
7:40 PM	(130)	3.047	2.831	2.939	0.153	0.108	5.20%
7:50 PM	(140)	2.145	2.032	2.089	0.080	0.057	3.83%
8:00 PM	(150)	1.514	1.495	1.505	0.013	0.009	0.89%
8:10 PM	(160)	0.974	0.764	0.869	0.148	0.105	17.09%
8:20 PM	(170)	0.813	0.739	0.776	0.052	0.037	6.74%
8:30 PM	(180)	0.767	0.804	0.786	0.026	0.019	3.33%
8:40 PM	(190)	0.583	0.62	0.602	0.026	0.019	4.35%
8:50 PM	(200)	0.509	0.484	0.497	0.018	0.013	3.56%
9:00 PM	(210)	0.291	0.304	0.298	0.009	0.007	3.09%
9:10 PM	(220)	0.182	0.168	0.175	0.010	0.007	5.66%
9:20 PM	(230)						
9:30 PM	(240)	0.153	0.182	0.168	0.021	0.015	12.24%
9:40 PM	(250)	0.251	0.251	0.251	0.000	0.000	0.00%
9:50 PM	(260)	0.792	0.804	0.798	0.008	0.006	1.06%
10:00 PM	(270)	2.048	2.08	2.064	0.023	0.016	1.10%
10:10 PM	(280)	3.189	3.287	3.238	0.069	0.049	2.14%
10:20 PM	(290)	3.357	3.646	3.502	0.204	0.145	5.84%
10:30 PM	(300)	2.99	3.076	3.033	0.061	0.043	2.00%
10:40 PM	(310)						
10:50 PM	(320)						
11:00 PM	(330)	5.147	5.598	5.373	0.319	0.226	5.94%
11:10 PM	(340)	3.956	3.929	3.943	0.019	0.014	0.48%
11:20 PM	(350)	3.203	3.175	3.189	0.020	0.014	0.62%
11:30 PM	(360)	2.579	2.594	2.587	0.011	0.007	0.41%
11:40 PM	(370)	2.801	2.757	2.779	0.031	0.022	1.12%
11:50 PM	(380)	3.399	3.495	3.447	0.068	0.048	1.97%
12:00 AM	(390)	3.754	3.822	3.788	0.048	0.034	1.27%
12:10 AM	(400)	3.053	2.908	2.981	0.103	0.073	3.44%
12:20 AM	(410)	2.118	2.26	2.189	0.100	0.071	4.59%
12:30 AM	(420)	0.992	0.934	0.963	0.041	0.029	4.26%
12:40 AM	(430)	0.889	0.84	0.865	0.035	0.025	4.01%
12:50 AM	(440)	0.499	0.458	0.479	0.029	0.021	6.06%
1:00 AM	(450)	0.337	0.346	0.342	0.006	0.004	1.86%
1:10 AM	(460)	0.271	0.261	0.266	0.007	0.005	2.66%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	0.209	0.176	0.193	0.023	0.017	12.12%
1:30 AM	(480)	0.164	0.176	0.170	0.008	0.006	4.99%
1:40 AM	(490)	0.337	0.251	0.294	0.061	0.043	20.68%
1:50 AM	(500)	0.3	0.198	0.249	0.072	0.051	28.97%
2:00 AM	(510)	0.187	0.187	0.187	0.000	0.000	0.00%
2:10 AM	(520)	0.127	0.114	0.121	0.009	0.007	7.63%
2:20 AM	(530)	0.164	0.164	0.164	0.000	0.000	0.00%
2:30 AM	(540)						
2:40 AM	(550)	0.595	0.564	0.580	0.022	0.016	3.78%
2:50 AM	(560)	0.71	0.748	0.729	0.027	0.019	3.69%
3:00 AM	(570)	0.808	0.801	0.805	0.005	0.004	0.62%
3:10 AM	(580)	0.771	0.793	0.782	0.016	0.011	1.99%
3:20 AM	(590)	0.957	0.979	0.968	0.016	0.011	1.61%
3:30 AM	(600)	1.843	1.865	1.854	0.016	0.011	0.84%
3:40 AM	(610)	2.179	2.014	2.097	0.117	0.083	5.57%
3:50 AM	(620)	1.156	1.02	1.088	0.096	0.068	8.84%
4:00 AM	(630)	0.868	0.86	0.864	0.006	0.004	0.65%
4:10 AM	(640)	0.831	0.823	0.827	0.006	0.004	0.68%
4:20 AM	(650)	1.285	1.408	1.347	0.087	0.062	6.46%
4:30 AM	(660)	0.509	0.342	0.426	0.118	0.084	27.75%
4:40 AM	(670)	0.778	0.763	0.771	0.011	0.008	1.38%
4:50 AM	(680)	0.451	0.462	0.457	0.008	0.006	1.70%
5:00 AM	(690)	0.284	0.304	0.294	0.014	0.010	4.81%
5:10 AM	(700)	0.183	0.233	0.208	0.035	0.025	17.00%
5:20 AM	(710)	0.154	0.164	0.159	0.007	0.005	4.45%
5:30 AM	(720)	0.125	0.135	0.130	0.007	0.005	5.44%
5:40 AM	(730)	0.203	0.203	0.203	0.000	0.000	0.00%
5:50 AM	(740)	0.294	0.294	0.294	0.000	0.000	0.00%
6:00 AM	(750)	0.784	0.739	0.762	0.032	0.023	4.18%

Note: Time points highlighted in grey are missing values. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L6. Raw Growth Hormone Data for GH-002: High-Intensity Interval Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	0.152	0.29	0.221	0.098	0.069	44.15%
5:40 PM	(10)	1.175	0.987	1.081	0.133	0.094	12.30%
5:50 PM	(20)	3.493	3.709	3.601	0.153	0.108	4.24%
6:00 PM	(30)	7.753	7.346	7.550	0.288	0.204	3.81%
6:10 PM	(40)	6.736	6.774	6.755	0.027	0.019	0.40%
6:20 PM	(50)						
6:30 PM	(60)						
6:40 PM	(70)	5.197	5.476	5.337	0.197	0.140	3.70%
6:50 PM	(80)	5.653	5.431	5.542	0.157	0.111	2.83%
7:00 PM	(90)	4.908	5.067	4.988	0.112	0.079	2.25%
7:10 PM	(100)	3.85	4.087	3.969	0.168	0.119	4.22%
7:20 PM	(110)	2.863	2.727	2.795	0.096	0.068	3.44%
7:30 PM	(120)	1.619	1.785	1.702	0.117	0.083	6.90%
7:40 PM	(130)	0.934	1.037	0.986	0.073	0.051	7.39%
7:50 PM	(140)	0.645	0.661	0.653	0.011	0.008	1.73%
8:00 PM	(150)	0.366	0.349	0.358	0.012	0.009	3.36%
8:10 PM	(160)	0.121	0.142	0.132	0.015	0.011	11.29%
8:20 PM	(170)	<0.000	0.076	0.076			
8:30 PM	(180)	0.024	<0.000	0.024			
8:40 PM	(190)	<0.000	<0.000				
8:50 PM	(200)	<0.000	0.076	0.076			
9:00 PM	(210)	0.051	<0.000	0.051			
9:10 PM	(220)	0.142	<0.000	0.142			
9:20 PM	(230)	<0.000	<0.000				
9:30 PM	(240)	<0.000	<0.000				
9:40 PM	(250)	<0.000	0.202	0.202			
9:50 PM	(260)	0.24	0.277	0.259	0.026	0.019	10.12%
10:00 PM	(270)	0.099	0.099	0.099	0.000	0.000	0.00%
10:10 PM	(280)	0.076	<0.000	0.076			
10:20 PM	(290)	<0.000	<0.000				
10:30 PM	(300)	<0.000	<0.000				
10:40 PM	(310)	<0.000	<0.000				
10:50 PM	(320)	<0.000	<0.000				
11:00 PM	(330)	<0.000	<0.000				
11:10 PM	(340)	<0.000	<0.000				
11:20 PM	(350)	<0.000	<0.000				
11:30 PM	(360)	<0.000	<0.000				
11:40 PM	(370)	<0.000	<0.000				
11:50 PM	(380)	<0.000	<0.000				
12:00 AM	(390)	<0.000	<0.000				
12:10 AM	(400)	<0.000	<0.000				
12:20 AM	(410)	<0.000	<0.000				
12:30 AM	(420)	0.41	<0.000	0.410			
12:40 AM	(430)	<0.000	0.242	0.242			
12:50 AM	(440)	<0.000	<0.000				
1:00 AM	(450)	0.039	0.039	0.039	0.000	0.000	0.00%
1:10 AM	(460)	0.076	<0.000	0.076			

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	0.194	0.194	0.194	0.000	0.000	0.00%
1:30 AM	(480)	0.019	0.039	0.029	0.014	0.010	48.77%
1:40 AM	(490)	0.019	<0.000	0.019			
1:50 AM	(500)	0.076	0.111	0.094	0.025	0.018	26.47%
2:00 AM	(510)	0.334	0.21	0.272	0.088	0.062	32.24%
2:10 AM	(520)	0.968	0.996	0.982	0.020	0.014	2.02%
2:20 AM	(530)	3.119	3.133	3.126	0.010	0.007	0.32%
2:30 AM	(540)	4.764	5.005	4.885	0.170	0.121	3.49%
2:40 AM	(550)	4.645	4.824	4.735	0.127	0.090	2.67%
2:50 AM	(560)	5.035	4.66	4.848	0.265	0.188	5.47%
3:00 AM	(570)	4.247	4.204	4.226	0.030	0.022	0.72%
3:10 AM	(580)	3.684	3.943	3.814	0.183	0.130	4.80%
3:20 AM	(590)	3.414	3.414	3.414	0.000	0.000	0.00%
3:30 AM	(600)	3.077	3.007	3.042	0.049	0.035	1.63%
3:40 AM	(610)	2.604	2.521	2.563	0.059	0.042	2.29%
3:50 AM	(620)	1.712	1.685	1.699	0.019	0.014	1.12%
4:00 AM	(630)	1.024	0.982	1.003	0.030	0.021	2.96%
4:10 AM	(640)	0.786	0.701	0.744	0.060	0.043	8.08%
4:20 AM	(650)	0.38	0.498	0.439	0.083	0.059	19.01%
4:30 AM	(660)	0.334	0.304	0.319	0.021	0.015	6.65%
4:40 AM	(670)	0.161	0.194	0.178	0.023	0.017	13.15%
4:50 AM	(680)	0.128	0.111	0.120	0.012	0.009	10.06%
5:00 AM	(690)	0.128	0.128	0.128	0.000	0.000	0.00%
5:10 AM	(700)	0.058	0.058	0.058	0.000	0.000	0.00%
5:20 AM	(710)	0.019	0.058	0.039	0.028	0.020	71.63%
5:30 AM	(720)	0.111	0.128	0.120	0.012	0.009	10.06%
5:40 AM	(730)	0.111	0.128	0.120	0.012	0.009	10.06%
5:50 AM	(740)	0.076	0.076	0.076	0.000	0.000	0.00%
6:00 AM	(750)	0.039	0.076	0.058	0.026	0.019	45.50%

Note: Time points highlighted in grey are missing values. Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L7. Raw Growth Hormone Data for GH-008: Control Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	0.272	0.262	0.267	0.007	0.005	2.65%
5:40 PM	(10)	0.164	0.123	0.144	0.029	0.020	20.20%
5:50 PM	(20)	0.093	0.072	0.083	0.015	0.011	18.00%
6:00 PM	(30)	0.072	0.072	0.072	0.000	0.000	0.00%
6:10 PM	(40)						
6:20 PM	(50)	0.072	0.093	0.083	0.015	0.011	18.00%
6:30 PM	(60)	0.359	< 0.000	0.359			
6:40 PM	(70)	0.083	0.093	0.088	0.007	0.005	8.04%
6:50 PM	(80)	0.083	0.093	0.088	0.007	0.005	8.04%
7:00 PM	(90)	0.154	0.113	0.134	0.029	0.021	21.72%
7:10 PM	(100)	0.134	0.144	0.139	0.007	0.005	5.09%
7:20 PM	(110)	0.193	0.193	0.193	0.000	0.000	0.00%
7:30 PM	(120)	0.113	0.144	0.129	0.022	0.016	17.06%
7:40 PM	(130)	0.093	0.083	0.088	0.007	0.005	8.04%
7:50 PM	(140)	0.083	0.072	0.078	0.008	0.006	10.04%
8:00 PM	(150)	0.04	0.04	0.040	0.000	0.000	0.00%
8:10 PM	(160)	0.029	0.051	0.040	0.016	0.011	38.89%
8:20 PM	(170)	0.093	0.051	0.072	0.030	0.021	41.25%
8:30 PM	(180)	0.252	0.223	0.238	0.021	0.015	8.63%
8:40 PM	(190)	0.426	0.445	0.436	0.013	0.010	3.08%
8:50 PM	(200)	0.311	0.311	0.311	0.000	0.000	0.00%
9:00 PM	(210)	0.262	0.213	0.238	0.035	0.025	14.59%
9:10 PM	(220)	0.272	0.272	0.272	0.000	0.000	0.00%
9:20 PM	(230)	0.378	0.272	0.325	0.075	0.053	23.06%
9:30 PM	(240)	0.636	0.712	0.674	0.054	0.038	7.97%
9:40 PM	(250)	1.998	2.059	2.029	0.043	0.031	2.13%
9:50 PM	(260)	2.733	2.636	2.685	0.069	0.049	2.56%
10:00 PM	(270)	3.151	3.445	3.298	0.208	0.147	6.30%
10:10 PM	(280)	2.657	2.918	2.788	0.185	0.131	6.62%
10:20 PM	(290)	1.967	1.998	1.983	0.022	0.016	1.11%
10:30 PM	(300)	2.266	2.245	2.256	0.015	0.011	0.66%
10:40 PM	(310)	2.018	2.039	2.029	0.015	0.011	0.73%
10:50 PM	(320)	1.957	2.162	2.060	0.145	0.103	7.04%
11:00 PM	(330)	1.388	1.615	1.502	0.161	0.114	10.69%
11:10 PM	(340)	1.835	2.049	1.942	0.151	0.107	7.79%
11:20 PM	(350)	2.962	3.151	3.057	0.134	0.094	4.37%
11:30 PM	(360)	5.719	6.041	5.880	0.228	0.161	3.87%
11:40 PM	(370)	4.592	5.077	4.835	0.343	0.243	7.09%
11:50 PM	(380)	2.878	3.236	3.057	0.253	0.179	8.28%
12:00 AM	(390)	2.004	1.927	1.966	0.054	0.039	2.77%
12:10 AM	(400)	1.141	1.124	1.133	0.012	0.008	1.06%
12:20 AM	(410)	0.756	0.799	0.778	0.030	0.022	3.91%
12:30 AM	(420)	0.456	0.439	0.448	0.012	0.009	2.69%
12:40 AM	(430)	0.362	0.311	0.337	0.036	0.026	10.72%
12:50 AM	(440)	0.277	0.302	0.290	0.018	0.013	6.11%
1:00 AM	(450)	0.259	0.259	0.259	0.000	0.000	0.00%
1:10 AM	(460)	0.19	0.268	0.229	0.055	0.039	24.08%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	0.216	0.19	0.203	0.018	0.013	9.06%
1:30 AM	(480)	0.147	0.147	0.147	0.000	0.000	0.00%
1:40 AM	(490)	0.129	0.147	0.138	0.013	0.009	9.22%
1:50 AM	(500)	0.319	0.345	0.332	0.018	0.013	5.54%
2:00 AM	(510)	0.704	0.653	0.679	0.036	0.026	5.32%
2:10 AM	(520)	1.965	1.927	1.946	0.027	0.019	1.38%
2:20 AM	(530)	4.877	4.682	4.780	0.138	0.097	2.88%
2:30 AM	(540)	6.736	6.288	6.512	0.317	0.224	4.86%
2:40 AM	(550)	11.871	12.589	12.230	0.508	0.359	4.15%
2:50 AM	(560)	9.003	9.656	9.330	0.462	0.327	4.95%
3:00 AM	(570)	6.767	7.47	7.119	0.497	0.352	6.98%
3:10 AM	(580)	3.854	3.878	3.866	0.017	0.012	0.44%
3:20 AM	(590)	2.309	2.111	2.210	0.140	0.099	6.34%
3:30 AM	(600)	1.586	1.624	1.605	0.027	0.019	1.67%
3:40 AM	(610)	1.009	0.982	0.996	0.019	0.014	1.92%
3:50 AM	(620)	0.704	0.687	0.696	0.012	0.008	1.73%
4:00 AM	(630)	0.482	0.465	0.474	0.012	0.008	2.54%
4:10 AM	(640)	0.345	0.379	0.362	0.024	0.017	6.64%
4:20 AM	(650)	0.259	0.285	0.272	0.018	0.013	6.76%
4:30 AM	(660)	0.19	0.208	0.199	0.013	0.009	6.40%
4:40 AM	(670)	0.19	0.164	0.177	0.018	0.013	10.39%
4:50 AM	(680)	0.147	0.173	0.160	0.018	0.013	11.49%
5:00 AM	(690)	0.173	0.138	0.156	0.025	0.018	15.92%
5:10 AM	(700)	0.121	0.085	0.103	0.025	0.018	24.71%
5:20 AM	(710)	0.094	0.138	0.116	0.031	0.022	26.82%
5:30 AM	(720)	0.112	0.112	0.112	0.000	0.000	0.00%
5:40 AM	(730)	0.173	0.129	0.151	0.031	0.022	20.60%
5:50 AM	(740)	0.07	0.06	0.065	0.007	0.005	10.88%
6:00 AM	(750)	0.08	0.109	0.095	0.021	0.015	21.70%

Note: Time points highlighted in grey are missing values. Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L8. Raw Growth Hormone Data for GH-008: Moderate-Intensity Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	2.964	2.86	2.912	0.074	0.052	2.53%
5:40 PM	(10)	7.416	7.842	7.629	0.301	0.213	3.95%
5:50 PM	(20)	11.35	12.866	12.108	1.072	0.758	8.85%
6:00 PM	(30)	10.85	11.181	11.016	0.234	0.166	2.12%
6:10 PM	(40)	6.196	6.255	6.226	0.042	0.030	0.67%
6:20 PM	(50)	3.175	3.389	3.282	0.151	0.107	4.61%
6:30 PM	(60)	1.936	1.839	1.888	0.069	0.049	3.63%
6:40 PM	(70)	1.274	1.223	1.249	0.036	0.026	2.89%
6:50 PM	(80)	0.741	0.751	0.746	0.007	0.005	0.95%
7:00 PM	(90)	0.437	0.456	0.447	0.013	0.010	3.01%
7:10 PM	(100)						
7:20 PM	(110)						
7:30 PM	(120)	0.138	0.147	0.143	0.006	0.004	4.47%
7:40 PM	(130)	0.109	0.118	0.114	0.006	0.005	5.61%
7:50 PM	(140)	0.109	0.118	0.114	0.006	0.005	5.61%
8:00 PM	(150)	0.06	0.07	0.065	0.007	0.005	10.88%
8:10 PM	(160)	0.041	0.021	0.031	0.014	0.010	45.62%
8:20 PM	(170)	0.012	0.031	0.022	0.013	0.010	62.49%
8:30 PM	(180)	0.051	0.041	0.046	0.007	0.005	15.37%
8:40 PM	(190)	0.089	0.128	0.109	0.028	0.020	25.42%
8:50 PM	(200)	0.176	0.186	0.181	0.007	0.005	3.91%
9:00 PM	(210)	0.244	0.253	0.249	0.006	0.005	2.56%
9:10 PM	(220)	0.35	0.369	0.360	0.013	0.010	3.74%
9:20 PM	(230)	0.35	0.359	0.355	0.006	0.005	1.80%
9:30 PM	(240)	0.583	0.534	0.559	0.035	0.025	6.20%
9:40 PM	(250)	2.241	<0.000	2.241			
9:50 PM	(260)	3.282	3.413	3.348	0.093	0.065	2.77%
10:00 PM	(270)	4.793	4.873	4.833	0.057	0.040	1.17%
10:10 PM	(280)	5.062	5.546	5.304	0.342	0.242	6.45%
10:20 PM	(290)	4.981	5.199	5.090	0.154	0.109	3.03%
10:30 PM	(300)	3.866	4.029	3.948	0.115	0.081	2.92%
10:40 PM	(310)	2.131	2.109	2.120	0.016	0.011	0.73%
10:50 PM	(320)	1.305	1.274	1.290	0.022	0.016	1.70%
11:00 PM	(330)	0.9	0.87	0.885	0.021	0.015	2.40%
11:10 PM	(340)	0.554	0.564	0.559	0.007	0.005	1.26%
11:20 PM	(350)	0.323	0.315	0.319	0.006	0.004	1.77%
11:30 PM	(360)	0.234	0.234	0.234	0.000	0.000	0.00%
11:40 PM	(370)	0.212	0.183	0.198	0.021	0.015	10.38%
11:50 PM	(380)	0.183	0.162	0.173	0.015	0.011	8.61%
12:00 AM	(390)	0.148	0.162	0.155	0.010	0.007	6.39%
12:10 AM	(400)	0.127	0.134	0.131	0.005	0.004	3.79%
12:20 AM	(410)	0.169	0.162	0.166	0.005	0.004	2.99%
12:30 AM	(420)	0.141	0.141	0.141	0.000	0.000	0.00%
12:40 AM	(430)	0.197	0.197	0.197	0.000	0.000	0.00%
12:50 AM	(440)	0.241	0.212	0.227	0.021	0.015	9.05%
1:00 AM	(450)	0.285	0.285	0.285	0.000	0.000	0.00%
1:10 AM	(460)						

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	2.575	2.717	2.646	0.100	0.071	3.79%
1:30 AM	(480)	6.12	6.014	6.067	0.075	0.053	1.24%
1:40 AM	(490)	9.576	10.014	9.795	0.310	0.219	3.16%
1:50 AM	(500)	10.528	10.362	10.445	0.117	0.083	1.12%
2:00 AM	(510)	10.014	10.612	10.313	0.423	0.299	4.10%
2:10 AM	(520)	9.265	8.737	9.001	0.373	0.264	4.15%
2:20 AM	(530)	8.443	8.644	8.544	0.142	0.101	1.66%
2:30 AM	(540)	7.771	7.91	7.841	0.098	0.070	1.25%
2:40 AM	(550)						
2:50 AM	(560)	4.558	4.705	4.632	0.104	0.074	2.24%
3:00 AM	(570)	2.607	2.575	2.591	0.023	0.016	0.87%
3:10 AM	(580)	1.719	1.867	1.793	0.105	0.074	5.84%
3:20 AM	(590)	1.164	1.228	1.196	0.045	0.032	3.78%
3:30 AM	(600)	0.882	0.797	0.840	0.060	0.043	7.16%
3:40 AM	(610)	0.501	0.501	0.501	0.000	0.000	0.00%
3:50 AM	(620)	0.353	0.361	0.357	0.006	0.004	1.58%
4:00 AM	(630)	0.234	0.226	0.230	0.006	0.004	2.46%
4:10 AM	(640)	0.241	0.226	0.234	0.011	0.007	4.54%
4:20 AM	(650)	0.19	0.183	0.187	0.005	0.004	2.65%
4:30 AM	(660)	0.155	0.141	0.148	0.010	0.007	6.69%
4:40 AM	(670)	0.066	0.113	0.090	0.033	0.024	37.13%
4:50 AM	(680)	0.106	0.12	0.113	0.010	0.007	8.76%
5:00 AM	(690)	0.12	0.12	0.120	0.000	0.000	0.00%
5:10 AM	(700)	0.176	0.141	0.159	0.025	0.018	15.61%
5:20 AM	(710)						
5:30 AM	(720)	0.05	0.041	0.046	0.006	0.005	13.99%
5:40 AM	(730)	0.004	0.014	0.009	0.007	0.005	78.57%
5:50 AM	(740)	0.004	0.014	0.009	0.007	0.005	78.57%
6:00 AM	(750)	0.023	0.032	0.028	0.006	0.005	23.14%

Note: Time points highlighted in grey are missing values. Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L9. Raw Growth Hormone Data for GH-008: High-Intensity Interval Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	6.413	6.736	6.575	0.228	0.162	3.47%
5:40 PM	(10)	4.34	4.168	4.254	0.122	0.086	2.86%
5:50 PM	(20)	4.443	4.676	4.560	0.165	0.117	3.61%
6:00 PM	(30)	9.891	10.498	10.195	0.429	0.304	4.21%
6:10 PM	(40)	11.375	10.676	11.026	0.494	0.350	4.48%
6:20 PM	(50)						
6:30 PM	(60)	7.49	7.136	7.313	0.250	0.177	3.42%
6:40 PM	(70)	4.282	4.49	4.386	0.147	0.104	3.35%
6:50 PM	(80)	2.879	2.91	2.895	0.022	0.016	0.76%
7:00 PM	(90)	1.752	1.666	1.709	0.061	0.043	3.56%
7:10 PM	(100)	1.048	1.048	1.048	0.000	0.000	0.00%
7:20 PM	(110)	0.675	0.738	0.707	0.045	0.032	6.31%
7:30 PM	(120)	0.415	0.468	0.442	0.037	0.027	8.49%
7:40 PM	(130)	0.326	0.281	0.304	0.032	0.023	10.48%
7:50 PM	(140)	0.202	0.219	0.211	0.012	0.008	5.71%
8:00 PM	(150)	0.139	0.148	0.144	0.006	0.004	4.43%
8:10 PM	(160)	0.104	0.113	0.109	0.006	0.005	5.87%
8:20 PM	(170)	0.086	0.086	0.086	0.000	0.000	0.00%
8:30 PM	(180)	0.086	0.059	0.073	0.019	0.014	26.33%
8:40 PM	(190)	0.059	0.05	0.055	0.006	0.005	11.68%
8:50 PM	(200)	0.113	0.113	0.113	0.000	0.000	0.00%
9:00 PM	(210)	0.344	0.353	0.349	0.006	0.005	1.83%
9:10 PM	(220)	0.675	0.684	0.680	0.006	0.005	0.94%
9:20 PM	(230)	1.233	1.223	1.228	0.007	0.005	0.58%
9:30 PM	(240)	2.29	2.33	2.310	0.028	0.020	1.22%
9:40 PM	(250)	3.109	3.067	3.088	0.030	0.021	0.96%
9:50 PM	(260)	5.955	6.123	6.039	0.119	0.084	1.97%
10:00 PM	(270)	3.843	4.067	3.955	0.158	0.112	4.00%
10:10 PM	(280)	2.41	2.521	2.466	0.078	0.055	3.18%
10:20 PM	(290)	1.205	1.223	1.214	0.013	0.009	1.05%
10:30 PM	(300)	0.765	0.765	0.765	0.000	0.000	0.00%
10:40 PM	(310)	0.477	0.522	0.500	0.032	0.023	6.37%
10:50 PM	(320)	0.379	0.353	0.366	0.018	0.013	5.02%
11:00 PM	(330)	0.195	0.195	0.195	0.000	0.000	0.00%
11:10 PM	(340)	0.124	0.124	0.124	0.000	0.000	0.00%
11:20 PM	(350)	0.124	0.098	0.111	0.018	0.013	16.56%
11:30 PM	(360)	0.071	0.089	0.080	0.013	0.009	15.91%
11:40 PM	(370)	0.062	0.053	0.058	0.006	0.005	11.07%
11:50 PM	(380)	0.035	0.026	0.031	0.006	0.005	20.87%
12:00 AM	(390)	0.062	0.035	0.049	0.019	0.014	39.36%
12:10 AM	(400)	0.053	0.035	0.044	0.013	0.009	28.93%
12:20 AM	(410)	0.062	0.044	0.053	0.013	0.009	24.01%
12:30 AM	(420)	0.053	0.062	0.058	0.006	0.005	11.07%
12:40 AM	(430)	0.089	0.089	0.089	0.000	0.000	0.00%
12:50 AM	(440)	0.089	0.089	0.089	0.000	0.000	0.00%
1:00 AM	(450)	0.062	0.116	0.089	0.038	0.027	42.90%
1:10 AM	(460)	0.026	0.026	0.026	0.000	0.000	0.00%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	0.017	0.035	0.026	0.013	0.009	48.95%
1:30 AM	(480)	0.044	0.044	0.044	0.000	0.000	0.00%
1:40 AM	(490)	0.151	0.16	0.156	0.006	0.005	4.09%
1:50 AM	(500)	0.221	0.204	0.213	0.012	0.009	5.66%
2:00 AM	(510)	0.212	0.177	0.195	0.025	0.018	12.72%
2:10 AM	(520)	0.186	0.177	0.182	0.006	0.005	3.51%
2:20 AM	(530)	0.151	0.151	0.151	0.000	0.000	0.00%
2:30 AM	(540)	0.133	0.16	0.147	0.019	0.014	13.03%
2:40 AM	(550)	0.274	0.3	0.287	0.018	0.013	6.41%
2:50 AM	(560)	0.475	0.466	0.471	0.006	0.004	1.35%
3:00 AM	(570)	1.299	1.391	1.345	0.065	0.046	4.84%
3:10 AM	(580)	1.891	1.929	1.910	0.027	0.019	1.41%
3:20 AM	(590)	2.162	2.123	2.143	0.028	0.019	1.29%
3:30 AM	(600)	2.601	2.662	2.632	0.043	0.031	1.64%
3:40 AM	(610)	4.053	4.167	4.110	0.081	0.057	1.96%
3:50 AM	(620)	5.812	5.682	5.747	0.092	0.065	1.60%
4:00 AM	(630)	4.696	4.318	4.507	0.267	0.189	5.93%
4:10 AM	(640)	3.333	3.398	3.366	0.046	0.033	1.37%
4:20 AM	(650)	2.46	2.37	2.415	0.064	0.045	2.64%
4:30 AM	(660)	1.615	1.606	1.611	0.006	0.004	0.40%
4:40 AM	(670)	1.116	1.026	1.071	0.064	0.045	5.94%
4:50 AM	(680)	0.713	0.695	0.704	0.013	0.009	1.81%
5:00 AM	(690)	0.449	0.457	0.453	0.006	0.004	1.25%
5:10 AM	(700)	0.219	0.229	0.224	0.007	0.005	3.16%
5:20 AM	(710)	0.131	0.151	0.141	0.014	0.010	10.03%
5:30 AM	(720)	0.101	0.131	0.116	0.021	0.015	18.29%
5:40 AM	(730)	0.06	0.05	0.055	0.007	0.005	12.86%
5:50 AM	(740)	0.05	<0.000	0.050			
6:00 AM	(750)	0.04	0.008	0.024	0.023	0.016	94.28%

Note: Time points highlighted in grey are missing values. Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L10. Raw Growth Hormone Data for GH-009: Control Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	<0.000	0.04	0.040			
5:40 PM	(10)	0.032	0.063	0.048	0.022	0.016	46.15%
5:50 PM	(20)	0.01	0.025	0.018	0.011	0.008	60.61%
6:00 PM	(30)	<0.000	<0.000				
6:10 PM	(40)	9.828	10.125	9.977	0.210	0.149	2.11%
6:20 PM	(50)	6.32	6.739	6.530	0.296	0.210	4.54%
6:30 PM	(60)	5.017	5.537	5.277	0.368	0.260	6.97%
6:40 PM	(70)	3.801	3.751	3.776	0.035	0.025	0.94%
6:50 PM	(80)	2.403	2.502	2.453	0.070	0.049	2.85%
7:00 PM	(90)	1.319	1.28	1.300	0.028	0.020	2.12%
7:10 PM	(100)	0.691	0.736	0.714	0.032	0.023	4.46%
7:20 PM	(110)	0.47	0.479	0.475	0.006	0.005	1.34%
7:30 PM	(120)	0.291	0.324	0.308	0.023	0.017	7.59%
7:40 PM	(130)	0.175	0.15	0.163	0.018	0.013	10.88%
7:50 PM	(140)	0.11	0.126	0.118	0.011	0.008	9.59%
8:00 PM	(150)	0.071	0.087	0.079	0.011	0.008	14.32%
8:10 PM	(160)	0.087	0.063	0.075	0.017	0.012	22.63%
8:20 PM	(170)	0.102	0.025	0.064	0.054	0.039	85.74%
8:30 PM	(180)	0.01	0.025	0.018	0.011	0.008	60.61%
8:40 PM	(190)	0.025	0.017	0.021	0.006	0.004	26.94%
8:50 PM	(200)	0.094	0.087	0.091	0.005	0.004	5.47%
9:00 PM	(210)	0.682	0.718	0.700	0.025	0.018	3.64%
9:10 PM	(220)	2.187	2.219	2.203	0.023	0.016	1.03%
9:20 PM	(230)	5.437	5.58	5.509	0.101	0.071	1.84%
9:30 PM	(240)	9.927	10.84	10.384	0.646	0.457	6.22%
9:40 PM	(250)	11.723	11.879	11.801	0.110	0.078	0.93%
9:50 PM	(260)	12.309	12.081	12.195	0.161	0.114	1.32%
10:00 PM	(270)	11.856	11.591	11.724	0.187	0.133	1.60%
10:10 PM	(280)	10.326	10.966	10.646	0.453	0.320	4.25%
10:20 PM	(290)	8.112	8.199	8.156	0.062	0.043	0.75%
10:30 PM	(300)	5.408	5.623	5.516	0.152	0.108	2.76%
10:40 PM	(310)	3.126	3.244	3.185	0.083	0.059	2.62%
10:50 PM	(320)	1.707	1.789	1.748	0.058	0.041	3.32%
11:00 PM	(330)	0.203	0.194	0.199	0.006	0.005	3.21%
11:10 PM	(340)	0.169	0.169	0.169	0.000	0.000	0.00%
11:20 PM	(350)	0.126	0.118	0.122	0.006	0.004	4.64%
11:30 PM	(360)	0.101	0.118	0.110	0.012	0.008	10.98%
11:40 PM	(370)	0.118	0.118	0.118	0.000	0.000	0.00%
11:50 PM	(380)	0.118	0.101	0.110	0.012	0.008	10.98%
12:00 AM	(390)	0.118	0.152	0.135	0.024	0.017	17.81%
12:10 AM	(400)	0.177	0.203	0.190	0.018	0.013	9.68%
12:20 AM	(410)	0.229	0.247	0.238	0.013	0.009	5.35%
12:30 AM	(420)	0.427	0.418	0.423	0.006	0.005	1.51%
12:40 AM	(430)	0.539	0.539	0.539	0.000	0.000	0.00%
12:50 AM	(440)	0.549	0.539	0.544	0.007	0.005	1.30%
1:00 AM	(450)	0.464	0.473	0.469	0.006	0.004	1.36%
1:10 AM	(460)	0.399	0.381	0.390	0.013	0.009	3.26%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	0.418	0.39	0.404	0.020	0.014	4.90%
1:30 AM	(480)	0.399	0.345	0.372	0.038	0.027	10.26%
1:40 AM	(490)	0.247	0.247	0.247	0.000	0.000	0.00%
1:50 AM	(500)	0.152	0.169	0.161	0.012	0.009	7.49%
2:00 AM	(510)	0.118	0.11	0.114	0.006	0.004	4.96%
2:10 AM	(520)	0.093	0.093	0.093	0.000	0.000	0.00%
2:20 AM	(530)	0.085	0.069	0.077	0.011	0.008	14.69%
2:30 AM	(540)	0.053	0.069	0.061	0.011	0.008	18.55%
2:40 AM	(550)	0.061	0.061	0.061	0.000	0.000	0.00%
2:50 AM	(560)	0.053	0.053	0.053	0.000	0.000	0.00%
3:00 AM	(570)	0.045	0.045	0.045	0.000	0.000	0.00%
3:10 AM	(580)	0.085	0.101	0.093	0.011	0.008	12.17%
3:20 AM	(590)	0.264	0.273	0.269	0.006	0.005	2.37%
3:30 AM	(600)	0.354	0.427	0.391	0.052	0.037	13.22%
3:40 AM	(610)	0.409	0.381	0.395	0.020	0.014	5.01%
3:50 AM	(620)	0.427	0.427	0.427	0.000	0.000	0.00%
4:00 AM	(630)	0.336	0.381	0.359	0.032	0.023	8.88%
4:10 AM	(640)	0.282	0.282	0.282	0.000	0.000	0.00%
4:20 AM	(650)	0.212	0.203	0.208	0.006	0.004	3.07%
4:30 AM	(660)	0.143	0.152	0.148	0.006	0.005	4.31%
4:40 AM	(670)	0.093	0.101	0.097	0.006	0.004	5.83%
4:50 AM	(680)	0.069	0.093	0.081	0.017	0.012	20.95%
5:00 AM	(690)	0.045	0.045	0.045	0.000	0.000	0.00%
5:10 AM	(700)	<0.000	<0.000				
5:20 AM	(710)	<0.000	<0.000				
5:30 AM	(720)	<0.000	<0.000				
5:40 AM	(730)	<0.000	<0.000				
5:50 AM	(740)	<0.000	<0.000				
6:00 AM	(750)	0.239	0.258	0.249	0.013	0.010	5.41%

Note: Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L11. Raw Growth Hormone Data for GH-009: Moderate-Intensity Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	6.525	6.495	6.510	0.021	0.015	0.33%
5:40 PM	(10)	11.538	11.382	11.460	0.110	0.078	0.96%
5:50 PM	(20)	11.925	11.81	11.868	0.081	0.058	0.69%
6:00 PM	(30)	10.38	8.553	9.467	1.292	0.914	13.65%
6:10 PM	(40)	5.404	5.789	5.597	0.272	0.193	4.86%
6:20 PM	(50)	3.514	3.805	3.660	0.206	0.146	5.62%
6:30 PM	(60)	2.112	2.112	2.112	0.000	0.000	0.00%
6:40 PM	(70)	1.273	1.273	1.273	0.000	0.000	0.00%
6:50 PM	(80)	0.735	0.697	0.716	0.027	0.019	3.75%
7:00 PM	(90)	0.362	0.38	0.371	0.013	0.009	3.43%
7:10 PM	(100)	0.268	0.268	0.268	0.000	0.000	0.00%
7:20 PM	(110)	0.211	0.192	0.202	0.013	0.009	6.67%
7:30 PM	(120)	0.115	0.182	0.149	0.047	0.034	31.90%
7:40 PM	(130)	0.076	0.086	0.081	0.007	0.005	8.73%
7:50 PM	(140)	0.076	0.066	0.071	0.007	0.005	9.96%
8:00 PM	(150)	0.035	0.056	0.046	0.015	0.011	32.64%
8:10 PM	(160)	0.046	0.056	0.051	0.007	0.005	13.86%
8:20 PM	(170)	0.035	0.046	0.041	0.008	0.006	19.21%
8:30 PM	(180)	0.035	0.046	0.041	0.008	0.006	19.21%
8:40 PM	(190)	0.035	0.035	0.035	0.000	0.000	0.00%
8:50 PM	(200)	0.025	0.046	0.036	0.015	0.011	41.83%
9:00 PM	(210)	0.02	<0.000	0.020			
9:10 PM	(220)	0.046	0.025	0.036	0.015	0.011	41.83%
9:20 PM	(230)	0.125	0.144	0.135	0.013	0.009	9.99%
9:30 PM	(240)	1.111	1.13	1.121	0.013	0.009	1.20%
9:40 PM	(250)	1.98	2.173	2.077	0.136	0.097	6.57%
9:50 PM	(260)	3.209	3.265	3.237	0.040	0.028	1.22%
10:00 PM	(270)	3.935	3.946	3.941	0.008	0.006	0.20%
10:10 PM	(280)	6.115	5.944	6.030	0.121	0.086	2.01%
10:20 PM	(290)	4.811	5.54	5.176	0.515	0.365	9.96%
10:30 PM	(300)	3.935	3.982	3.959	0.033	0.024	0.84%
10:40 PM	(310)	3.176	2.955	3.066	0.156	0.111	5.10%
10:50 PM	(320)	1.88	1.96	1.920	0.057	0.040	2.95%
11:00 PM	(330)	1.168	1.158	1.163	0.007	0.005	0.61%
11:10 PM	(340)	1.064	1.073	1.069	0.006	0.004	0.60%
11:20 PM	(350)	0.707	0.623	0.665	0.059	0.042	8.93%
11:30 PM	(360)	0.585	0.511	0.548	0.052	0.037	9.55%
11:40 PM	(370)	0.293	0.376	0.335	0.059	0.041	17.55%
11:50 PM	(380)	0.16	0.16	0.160	0.000	0.000	0.00%
12:00 AM	(390)	0.072	0.072	0.072	0.000	0.000	0.00%
12:10 AM	(400)	0.02	0.02	0.020	0.000	0.000	0.00%
12:20 AM	(410)	0.009	<0.000	0.009			
12:30 AM	(420)	<0.000	<0.000				
12:40 AM	(430)	<0.000	<0.000				
12:50 AM	(440)	<0.000	<0.000				
1:00 AM	(450)	<0.000	<0.000				
1:10 AM	(460)	<0.000	<0.000				

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	<0.000	<0.000				
1:30 AM	(480)	0.112	<0.000	0.112			
1:40 AM	(490)	<0.000	<0.000				
1:50 AM	(500)	<0.000	<0.000				
2:00 AM	(510)	<0.000	<0.000				
2:10 AM	(520)	<0.000	0.062	0.062			
2:20 AM	(530)	0.009	<0.000	0.009			
2:30 AM	(540)	0.122	0.092	0.107	0.021	0.015	19.83%
2:40 AM	(550)	0.072	0.009	0.041	0.045	0.032	109.99%
2:50 AM	(560)	0.02	0.009	0.015	0.008	0.006	53.64%
3:00 AM	(570)	0.052	0.052	0.052	0.000	0.000	0.00%
3:10 AM	(580)	1.108	0.98	1.044	0.091	0.064	8.67%
3:20 AM	(590)	2.053	2.159	2.106	0.075	0.053	3.56%
3:30 AM	(600)	2.13	2.236	2.183	0.075	0.053	3.43%
3:40 AM	(610)	4.987	5.13	5.059	0.101	0.071	2.00%
3:50 AM	(620)	4.216	4.59	4.403	0.264	0.187	6.01%
4:00 AM	(630)	3.929	3.81	3.870	0.084	0.059	2.17%
4:10 AM	(640)	2.789	2.819	2.804	0.021	0.015	0.76%
4:20 AM	(650)	2.072	2.178	2.125	0.075	0.053	3.53%
4:30 AM	(660)	1.385	1.636	1.511	0.177	0.126	11.75%
4:40 AM	(670)	0.998	1.008	1.003	0.007	0.005	0.70%
4:50 AM	(680)	0.597	0.615	0.606	0.013	0.009	2.10%
5:00 AM	(690)	0.311	0.311	0.311	0.000	0.000	0.00%
5:10 AM	(700)	0.122	0.131	0.127	0.006	0.005	5.03%
5:20 AM	(710)	0.082	0.062	0.072	0.014	0.010	19.64%
5:30 AM	(720)	0.02	<0.000	0.020			
5:40 AM	(730)	<0.000	<0.000				
5:50 AM	(740)	<0.000	0.003	0.003			
6:00 AM	(750)	<0.000	0.056	0.056			

Note: Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L12. Raw Growth Hormone Data for GH-009: High-Intensity Interval Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	12.66	13.949	13.305	0.911	0.645	6.85%
5:40 PM	(10)	18.01	19.014	18.512	0.710	0.502	3.84%
5:50 PM	(20)	26.929	27.699	27.314	0.544	0.385	1.99%
6:00 PM	(30)	22.728	23.12	22.924	0.277	0.196	1.21%
6:10 PM	(40)	21.122	21.302	21.212	0.127	0.090	0.60%
6:20 PM	(50)	13.827	13.421	13.624	0.287	0.203	2.11%
6:30 PM	(60)	9.2	9.792	9.496	0.419	0.296	4.41%
6:40 PM	(70)	4.796	4.877	4.837	0.057	0.040	1.18%
6:50 PM	(80)	3.634	3.524	3.579	0.078	0.055	2.17%
7:00 PM	(90)	2.116	2.246	2.181	0.092	0.065	4.21%
7:10 PM	(100)	1.3	1.392	1.346	0.065	0.046	4.83%
7:20 PM	(110)	0.663	0.846	0.755	0.129	0.091	17.15%
7:30 PM	(120)	0.493	0.531	0.512	0.027	0.019	5.25%
7:40 PM	(130)	0.382	0.391	0.387	0.006	0.005	1.65%
7:50 PM	(140)	0.253	0.208	0.231	0.032	0.023	13.80%
8:00 PM	(150)	0.118	0.127	0.123	0.006	0.005	5.20%
8:10 PM	(160)	0.136	0.091	0.114	0.032	0.023	28.04%
8:20 PM	(170)	0.064	0.082	0.073	0.013	0.009	17.44%
8:30 PM	(180)	0.056	0.047	0.052	0.006	0.005	12.36%
8:40 PM	(190)	0.02	0.047	0.034	0.019	0.014	56.99%
8:50 PM	(200)	0.029	0.012	0.021	0.012	0.009	58.64%
9:00 PM	(210)	0.012	0.012	0.012	0.000	0.000	0.00%
9:10 PM	(220)	0.02	<0.000	0.020			
9:20 PM	(230)	<0.000	0.012	0.012			
9:30 PM	(240)	0.003	0.02	0.012	0.012	0.009	104.53%
9:40 PM	(250)	0.012	0.02	0.016	0.006	0.004	35.36%
9:50 PM	(260)	0.073	0.082	0.078	0.006	0.005	8.21%
10:00 PM	(270)	0.118	0.163	0.141	0.032	0.022	22.65%
10:10 PM	(280)	0.235	0.262	0.249	0.019	0.014	7.68%
10:20 PM	(290)	0.475	0.475	0.475	0.000	0.000	0.00%
10:30 PM	(300)	0.692	0.701	0.697	0.006	0.005	0.91%
10:40 PM	(310)	0.74	0.826	0.783	0.061	0.043	7.77%
10:50 PM	(320)	0.963	1.031	0.997	0.048	0.034	4.82%
11:00 PM	(330)	0.943	0.982	0.963	0.028	0.020	2.87%
11:10 PM	(340)	1.331	1.341	1.336	0.007	0.005	0.53%
11:20 PM	(350)	1.434	1.478	1.456	0.031	0.022	2.14%
11:30 PM	(360)	1.467	1.478	1.473	0.008	0.005	0.53%
11:40 PM	(370)	1.205	1.27	1.238	0.046	0.033	3.71%
11:50 PM	(380)	1.216	1.27	1.243	0.038	0.027	3.07%
12:00 AM	(390)	0.872	0.882	0.877	0.007	0.005	0.81%
12:10 AM	(400)	0.812	0.882	0.847	0.049	0.035	5.84%
12:20 AM	(410)	0.913	0.964	0.939	0.036	0.026	3.84%
12:30 AM	(420)	0.762	0.862	0.812	0.071	0.050	8.71%
12:40 AM	(430)	0.812	0.862	0.837	0.035	0.025	4.22%
12:50 AM	(440)	1.068	1.068	1.068	0.000	0.000	0.00%
1:00 AM	(450)	1.613	1.534	1.574	0.056	0.040	3.55%
1:10 AM	(460)	2.139	2.199	2.169	0.042	0.030	1.96%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	1.523	1.567	1.545	0.031	0.022	2.01%
1:30 AM	(480)	0.792	0.792	0.792	0.000	0.000	0.00%
1:40 AM	(490)	0.464	0.427	0.446	0.026	0.019	5.87%
1:50 AM	(500)	0.265	0.291	0.278	0.018	0.013	6.61%
2:00 AM	(510)	0.179	0.17	0.175	0.006	0.004	3.65%
2:10 AM	(520)	0.113	0.121	0.117	0.006	0.004	4.83%
2:20 AM	(530)	0.089	0.081	0.085	0.006	0.004	6.66%
2:30 AM	(540)	0.065	0.065	0.065	0.000	0.000	0.00%
2:40 AM	(550)	0.121	0.113	0.117	0.006	0.004	4.83%
2:50 AM	(560)	0.23	0.23	0.230	0.000	0.000	0.00%
3:00 AM	(570)	0.417	0.417	0.417	0.000	0.000	0.00%
3:10 AM	(580)	1.216	1.227	1.222	0.008	0.006	0.64%
3:20 AM	(590)	1.635	1.556	1.596	0.056	0.040	3.50%
3:30 AM	(600)	1.047	1.11	1.079	0.045	0.032	4.13%
3:40 AM	(610)	0.703	0.664	0.684	0.028	0.020	4.03%
3:50 AM	(620)	0.445	0.445	0.445	0.000	0.000	0.00%
4:00 AM	(630)	0.3	0.291	0.296	0.006	0.005	2.15%
4:10 AM	(640)	0.23	0.23	0.230	0.000	0.000	0.00%
4:20 AM	(650)	0.196	0.187	0.192	0.006	0.005	3.32%
4:30 AM	(660)	0.145	0.154	0.150	0.006	0.005	4.26%
4:40 AM	(670)	0.097	0.113	0.105	0.011	0.008	10.77%
4:50 AM	(680)	0.073	0.089	0.081	0.011	0.008	13.97%
5:00 AM	(690)	0.073	0.073	0.073	0.000	0.000	0.00%
5:10 AM	(700)	0.073	0.05	0.062	0.016	0.012	26.44%
5:20 AM	(710)	0.042	0.058	0.050	0.011	0.008	22.63%
5:30 AM	(720)	<0.000	0.04	0.040			
5:40 AM	(730)	0.032	0.063	0.048	0.022	0.016	46.15%
5:50 AM	(740)	0.01	0.025	0.018	0.011	0.008	60.61%
6:00 AM	(750)	<0.000	<0.000				

Note: Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L13. Raw Growth Hormone Data for GH-010: Control Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	6.898	7.194	7.046	0.209	0.148	2.97%
5:40 PM	(10)	9.379	9.342	9.361	0.026	0.018	0.28%
5:50 PM	(20)	6.699	6.806	6.753	0.076	0.054	1.12%
6:00 PM	(30)	5.426	5.481	5.454	0.039	0.027	0.71%
6:10 PM	(40)	3.571	3.522	3.547	0.035	0.025	0.98%
6:20 PM	(50)	1.952	2.054	2.003	0.072	0.051	3.60%
6:30 PM	(60)	0.917	1.026	0.972	0.077	0.055	7.93%
6:40 PM	(70)	0.703	0.712	0.708	0.006	0.005	0.90%
6:50 PM	(80)	0.454	0.488	0.471	0.024	0.017	5.10%
7:00 PM	(90)	0.263	0.271	0.267	0.006	0.004	2.12%
7:10 PM	(100)	0.223	0.231	0.227	0.006	0.004	2.49%
7:20 PM	(110)	0.183	0.191	0.187	0.006	0.004	3.03%
7:30 PM	(120)	0.128	0.128	0.128	0.000	0.000	0.00%
7:40 PM	(130)	0.112	0.12	0.116	0.006	0.004	4.88%
7:50 PM	(140)	0.167	0.143	0.155	0.017	0.012	10.95%
8:00 PM	(150)	0.167	0.167	0.167	0.000	0.000	0.00%
8:10 PM	(160)	0.199	0.191	0.195	0.006	0.004	2.90%
8:20 PM	(170)	0.312	0.329	0.321	0.012	0.009	3.75%
8:30 PM	(180)	0.712	0.703	0.708	0.006	0.005	0.90%
8:40 PM	(190)	0.935	0.944	0.940	0.006	0.004	0.68%
8:50 PM	(200)	1.372	1.314	1.343	0.041	0.029	3.05%
9:00 PM	(210)	2.147	2.23	2.189	0.059	0.042	2.68%
9:10 PM	(220)	2.517	2.495	2.506	0.016	0.011	0.62%
9:20 PM	(230)	2.636	2.442	2.539	0.137	0.097	5.40%
9:30 PM	(240)	1.722	1.672	1.697	0.035	0.025	2.08%
9:40 PM	(250)	0.962	0.971	0.967	0.006	0.005	0.66%
9:50 PM	(260)	0.599	0.547	0.573	0.037	0.026	6.42%
10:00 PM	(270)	0.353	0.362	0.358	0.006	0.005	1.78%
10:10 PM	(280)	0.255	0.247	0.251	0.006	0.004	2.25%
10:20 PM	(290)	0.183	0.239	0.211	0.040	0.028	18.77%
10:30 PM	(300)	0.167	0.151	0.159	0.011	0.008	7.12%
10:40 PM	(310)	0.151	0.128	0.140	0.016	0.012	11.66%
10:50 PM	(320)	0.136	0.097	0.117	0.028	0.020	23.67%
11:00 PM	(330)	0.104	0.104	0.104	0.000	0.000	0.00%
11:10 PM	(340)	0.089	0.074	0.082	0.011	0.008	13.01%
11:20 PM	(350)	0.104	0.089	0.097	0.011	0.008	10.99%
11:30 PM	(360)	0.167	0.136	0.152	0.022	0.015	14.47%
11:40 PM	(370)	0.247	0.239	0.243	0.006	0.004	2.33%
11:50 PM	(380)	0.395	0.403	0.399	0.006	0.004	1.42%
12:00 AM	(390)	1.22	1.248	1.234	0.020	0.014	1.60%
12:10 AM	(400)	3.426	3.16	3.293	0.188	0.133	5.71%
12:20 AM	(410)	3.263	3.473	3.368	0.148	0.105	4.41%
12:30 AM	(420)	3.93	3.723	3.827	0.146	0.104	3.83%
12:40 AM	(430)	2.971	3.161	3.066	0.134	0.095	4.38%
12:50 AM	(440)	3.524	3.55	3.537	0.018	0.013	0.52%
1:00 AM	(450)	4.988	4.666	4.827	0.228	0.161	4.72%
1:10 AM	(460)	5.182	5.122	5.152	0.042	0.030	0.82%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	4.409	4.424	4.417	0.011	0.008	0.24%
1:30 AM	(480)	3.51	3.497	3.504	0.009	0.006	0.26%
1:40 AM	(490)	1.789	1.835	1.812	0.033	0.023	1.80%
1:50 AM	(500)	0.99	1.043	1.017	0.037	0.027	3.69%
2:00 AM	(510)	0.647	0.586	0.617	0.043	0.031	7.00%
2:10 AM	(520)	0.364	0.374	0.369	0.007	0.005	1.92%
2:20 AM	(530)	0.255	0.236	0.246	0.013	0.010	5.47%
2:30 AM	(540)	0.167	0.206	0.187	0.028	0.020	14.79%
2:40 AM	(550)	0.138	0.128	0.133	0.007	0.005	5.32%
2:50 AM	(560)	0.09	0.09	0.090	0.000	0.000	0.00%
3:00 AM	(570)	0.09	0.099	0.095	0.006	0.005	6.73%
3:10 AM	(580)	0.07	0.08	0.075	0.007	0.005	9.43%
3:20 AM	(590)	0.119	0.099	0.109	0.014	0.010	12.97%
3:30 AM	(600)	0.09	0.09	0.090	0.000	0.000	0.00%
3:40 AM	(610)	0.08	0.09	0.085	0.007	0.005	8.32%
3:50 AM	(620)	0.285	0.295	0.290	0.007	0.005	2.44%
4:00 AM	(630)	0.374	0.374	0.374	0.000	0.000	0.00%
4:10 AM	(640)	0.285	0.325	0.305	0.028	0.020	9.27%
4:20 AM	(650)	0.206	0.206	0.206	0.000	0.000	0.00%
4:30 AM	(660)	0.177	0.187	0.182	0.007	0.005	3.89%
4:40 AM	(670)	0.187	0.187	0.187	0.000	0.000	0.00%
4:50 AM	(680)	0.148	0.148	0.148	0.000	0.000	0.00%
5:00 AM	(690)	0.206	0.206	0.206	0.000	0.000	0.00%
5:10 AM	(700)						
5:20 AM	(710)						
5:30 AM	(720)						
5:40 AM	(730)						
5:50 AM	(740)						
6:00 AM	(750)						

Note: Time points highlighted in grey are missing values. Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L14. Raw Growth Hormone Data for GH-010: Moderate-Intensity Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	0.833	0.812	0.823	0.015	0.011	1.81%
5:40 PM	(10)	4.638	4.797	4.718	0.112	0.079	2.38%
5:50 PM	(20)	8.009	8.178	8.094	0.120	0.085	1.48%
6:00 PM	(30)	11.656	10.451	11.054	0.852	0.603	7.71%
6:10 PM	(40)	4.604	4.511	4.558	0.066	0.047	1.44%
6:20 PM	(50)	2.538	2.538	2.538	0.000	0.000	0.00%
6:30 PM	(60)	1.334	1.345	1.340	0.008	0.005	0.58%
6:40 PM	(70)	0.819	0.796	0.808	0.016	0.012	2.01%
6:50 PM	(80)	0.526	0.549	0.538	0.016	0.012	3.03%
7:00 PM	(90)	0.261	0.261	0.261	0.000	0.000	0.00%
7:10 PM	(100)	0.141	0.116	0.129	0.018	0.013	13.76%
7:20 PM	(110)	0.026	0.026	0.026	0.000	0.000	0.00%
7:30 PM	(120)	<0.000	<0.000				
7:40 PM	(130)	<0.000	0.026	0.026			
7:50 PM	(140)	<0.000	<0.000				
8:00 PM	(150)	<0.000	<0.000				
8:10 PM	(160)	<0.000	<0.000				
8:20 PM	(170)	<0.000	<0.000				
8:30 PM	(180)	<0.000	<0.000				
8:40 PM	(190)	<0.000	0.011	0.011			
8:50 PM	(200)	0.091	0.141	0.116	0.035	0.025	30.48%
9:00 PM	(210)	0.366	0.412	0.389	0.033	0.023	8.36%
9:10 PM	(220)	1.233	1.143	1.188	0.064	0.045	5.36%
9:20 PM	(230)	1.923	1.911	1.917	0.008	0.006	0.44%
9:30 PM	(240)	1.706	1.797	1.752	0.064	0.046	3.67%
9:40 PM	(250)	1.48	1.458	1.469	0.016	0.011	1.06%
9:50 PM	(260)	2.479	2.479	2.479	0.000	0.000	0.00%
10:00 PM	(270)	2.992	3.077	3.035	0.060	0.043	1.98%
10:10 PM	(280)	3.937	4.104	4.021	0.118	0.084	2.94%
10:20 PM	(290)	4.97	4.685	4.828	0.202	0.143	4.17%
10:30 PM	(300)	5.905	5.891	5.898	0.010	0.007	0.17%
10:40 PM	(310)	4.658	4.82	4.739	0.115	0.081	2.42%
10:50 PM	(320)	3.42	3.495	3.458	0.053	0.038	1.53%
11:00 PM	(330)	2.188	2.234	2.211	0.033	0.023	1.47%
11:10 PM	(340)	1.132	1.143	1.138	0.008	0.006	0.68%
11:20 PM	(350)	0.852	0.83	0.841	0.016	0.011	1.85%
11:30 PM	(360)	0.469	0.481	0.475	0.008	0.006	1.79%
11:40 PM	(370)	0.261	0.261	0.261	0.000	0.000	0.00%
11:50 PM	(380)	0.129	0.202	0.166	0.052	0.037	31.19%
12:00 AM	(390)	0.039	0.066	0.053	0.019	0.014	36.37%
12:10 AM	(400)	0.066	0.026	0.046	0.028	0.020	61.49%
12:20 AM	(410)	0.154	0.154	0.154	0.000	0.000	0.00%
12:30 AM	(420)	0.135	0.135	0.135	0.000	0.000	0.00%
12:40 AM	(430)	0.144	0.144	0.144	0.000	0.000	0.00%
12:50 AM	(440)	0.183	0.193	0.188	0.007	0.005	3.76%
1:00 AM	(450)	0.538	0.538	0.538	0.000	0.000	0.00%
1:10 AM	(460)	0.923	0.935	0.929	0.008	0.006	0.91%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	1.511	1.46	1.486	0.036	0.026	2.43%
1:30 AM	(480)	3.359	3.483	3.421	0.088	0.062	2.56%
1:40 AM	(490)	3.948	4.179	4.064	0.163	0.116	4.02%
1:50 AM	(500)	7.466	7.401	7.434	0.046	0.033	0.62%
2:00 AM	(510)	4.57	4.399	4.485	0.121	0.086	2.70%
2:10 AM	(520)	4.92	5.317	5.119	0.281	0.199	5.48%
2:20 AM	(530)	5.633	6.073	5.853	0.311	0.220	5.32%
2:30 AM	(540)	5.226	5.354	5.290	0.091	0.064	1.71%
2:40 AM	(550)	4.691	4.761	4.726	0.049	0.035	1.05%
2:50 AM	(560)	2.51	2.567	2.539	0.040	0.029	1.59%
3:00 AM	(570)	1.372	1.524	1.448	0.107	0.076	7.42%
3:10 AM	(580)	0.888	0.877	0.883	0.008	0.006	0.88%
3:20 AM	(590)	0.516	0.516	0.516	0.000	0.000	0.00%
3:30 AM	(600)	0.346	0.366	0.356	0.014	0.010	3.97%
3:40 AM	(610)	0.243	0.253	0.248	0.007	0.005	2.85%
3:50 AM	(620)	0.203	0.213	0.208	0.007	0.005	3.40%
4:00 AM	(630)	0.174	0.164	0.169	0.007	0.005	4.18%
4:10 AM	(640)	0.144	0.174	0.159	0.021	0.015	13.34%
4:20 AM	(650)	0.154	0.154	0.154	0.000	0.000	0.00%
4:30 AM	(660)	0.183	0.183	0.183	0.000	0.000	0.00%
4:40 AM	(670)	0.294	0.304	0.299	0.007	0.005	2.36%
4:50 AM	(680)	0.604	0.582	0.593	0.016	0.011	2.62%
5:00 AM	(690)	0.527	0.538	0.533	0.008	0.006	1.46%
5:10 AM	(700)						
5:20 AM	(710)						
5:30 AM	(720)						
5:40 AM	(730)						
5:50 AM	(740)						
6:00 AM	(750)						

Note: Time points highlighted in grey are missing values. Values in red are below the detectable limit of the assay and were assigned a value of 0.05 for deconvolution analysis purposes. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.

Table L15. Raw Growth Hormone Data for GH-010: High-Intensity Interval Trial

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
5:30 PM	(0)	2.772	2.814	2.793	0.030	0.021	1.06%
5:40 PM	(10)	31.466	28.447	29.957	2.135	1.510	7.13%
5:50 PM	(20)	40.607	39.173	39.890	1.014	0.717	2.54%
6:00 PM	(30)	31.084	32.815	31.950	1.224	0.865	3.83%
6:10 PM	(40)	24.56	24.688	24.624	0.091	0.064	0.37%
6:20 PM	(50)						
6:30 PM	(60)	12.553	13.693	13.123	0.806	0.570	6.14%
6:40 PM	(70)	9.138	9.498	9.318	0.255	0.180	2.73%
6:50 PM	(80)	7.257	7.769	7.513	0.362	0.256	4.82%
7:00 PM	(90)	4.927	5.1	5.014	0.122	0.087	2.44%
7:10 PM	(100)	3.469	3.503	3.486	0.024	0.017	0.69%
7:20 PM	(110)	2.415	2.617	2.516	0.143	0.101	5.68%
7:30 PM	(120)	2.065	2.141	2.103	0.054	0.038	2.56%
7:40 PM	(130)	1.38	1.388	1.384	0.006	0.004	0.41%
7:50 PM	(140)	0.892	0.938	0.915	0.033	0.023	3.55%
8:00 PM	(150)	0.658	0.673	0.666	0.011	0.008	1.59%
8:10 PM	(160)	0.543	0.578	0.561	0.025	0.018	4.42%
8:20 PM	(170)	0.351	0.357	0.354	0.004	0.003	1.20%
8:30 PM	(180)	0.272	0.253	0.263	0.013	0.010	5.12%
8:40 PM	(190)	0.173	0.179	0.176	0.004	0.003	2.41%
8:50 PM	(200)	0.149	0.155	0.152	0.004	0.003	2.79%
9:00 PM	(210)	0.108	0.114	0.111	0.004	0.003	3.82%
9:10 PM	(220)	0.091	0.103	0.097	0.008	0.006	8.75%
9:20 PM	(230)	0.086	0.086	0.086	0.000	0.000	0.00%
9:30 PM	(240)	0.091	0.091	0.091	0.000	0.000	0.00%
9:40 PM	(250)	0.08	0.091	0.086	0.008	0.006	9.10%
9:50 PM	(260)	0.103	0.091	0.097	0.008	0.006	8.75%
10:00 PM	(270)	0.091	0.091	0.091	0.000	0.000	0.00%
10:10 PM	(280)	0.125	0.103	0.114	0.016	0.011	13.65%
10:20 PM	(290)	0.097	0.091	0.094	0.004	0.003	4.51%
10:30 PM	(300)	0.131	0.12	0.126	0.008	0.006	6.20%
10:40 PM	(310)	0.091	0.114	0.103	0.016	0.011	15.87%
10:50 PM	(320)	0.125	0.143	0.134	0.013	0.009	9.50%
11:00 PM	(330)	0.636	0.651	0.644	0.011	0.008	1.65%
11:10 PM	(340)	0.954	0.923	0.939	0.022	0.016	2.34%
11:20 PM	(350)	1.329	1.321	1.325	0.006	0.004	0.43%
11:30 PM	(360)	1.397	1.346	1.372	0.036	0.026	2.63%
11:40 PM	(370)	2.169	2.237	2.203	0.048	0.034	2.18%
11:50 PM	(380)	2.375	2.329	2.352	0.033	0.023	1.38%
12:00 AM	(390)	1.929	1.952	1.941	0.016	0.012	0.84%
12:10 AM	(400)	1.452	1.588	1.520	0.096	0.068	6.33%
12:20 AM	(410)	1.225	1.225	1.225	0.000	0.000	0.00%
12:30 AM	(420)	0.858	0.892	0.875	0.024	0.017	2.75%
12:40 AM	(430)	0.598	0.634	0.616	0.025	0.018	4.13%
12:50 AM	(440)	0.452	0.477	0.465	0.018	0.013	3.81%
1:00 AM	(450)	0.477	0.464	0.471	0.009	0.006	1.95%
1:10 AM	(460)	0.658	0.634	0.646	0.017	0.012	2.63%

TIME	TIME POINT	GH (ng/mL)		MEAN	STDEV	SE	%CV
		(a)	(b)				
1:20 AM	(470)	0.799	0.741	0.770	0.041	0.029	5.33%
1:30 AM	(480)	1.384	1.35	1.367	0.024	0.017	1.76%
1:40 AM	(490)	2.318	2.364	2.341	0.033	0.023	1.39%
1:50 AM	(500)	1.554	1.566	1.560	0.008	0.006	0.54%
2:00 AM	(510)	1.019	1.054	1.037	0.025	0.018	2.39%
2:10 AM	(520)	0.514	0.477	0.496	0.026	0.019	5.28%
2:20 AM	(530)	0.3	0.326	0.313	0.018	0.013	5.87%
2:30 AM	(540)	0.234	0.22	0.227	0.010	0.007	4.36%
2:40 AM	(550)	0.377	0.339	0.358	0.027	0.019	7.51%
2:50 AM	(560)	1.781	1.838	1.810	0.040	0.029	2.23%
3:00 AM	(570)	6.898	7.432	7.165	0.378	0.267	5.27%
3:10 AM	(580)	13.259	13.507	13.383	0.175	0.124	1.31%
3:20 AM	(590)	12.261	12.516	12.389	0.180	0.128	1.46%
3:30 AM	(600)	11.9	12.775	12.338	0.619	0.438	5.01%
3:40 AM	(610)	12.147	13.161	12.654	0.717	0.507	5.67%
3:50 AM	(620)	11.634	11.877	11.756	0.172	0.122	1.46%
4:00 AM	(630)	9.584	10.201	9.893	0.436	0.309	4.41%
4:10 AM	(640)	7.464	8.039	7.752	0.407	0.288	5.25%
4:20 AM	(650)	5.691	6.106	5.899	0.293	0.208	4.97%
4:30 AM	(660)	4.777	4.79	4.784	0.009	0.006	0.19%
4:40 AM	(670)	3.053	2.947	3.000	0.075	0.053	2.50%
4:50 AM	(680)						
5:00 AM	(690)						
5:10 AM	(700)						
5:20 AM	(710)						
5:30 AM	(720)						
5:40 AM	(730)						
5:50 AM	(740)						
6:00 AM	(750)						

Note: Time points highlighted in grey are missing values. Values for (a) and (b) are individual GH values from each time point measured in duplicate. MEAN = average of (a) and (b) value; STDEV = standard deviation of the mean; SE = standard error of the mean; %CV = coefficient of variation between (a) and (b) value.