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EFFECT OF BODY COMPOSITION ON GROWTH HORMONE RESPONSE TO EXERCISE IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Growth hormone (GH) is one of several hormones known to decrease with age. Low GH levels may be linked with various disease conditions because of its stimulatory role in muscle and bone maintenance, and lipolysis. Growth hormone is associated with insulin, glucose, and estrogen levels, and GH is greatly reduced following menopause. It has been established that obesity is inversely related to low GH levels and positively associated with insulin resistance and dyslipidemia. Exercise is a potent stimulator of GH secretion and the GH response to exercise is attenuated with obesity. The purpose of this study was to evaluate the effect of body composition on the GH response to exercise in postmenopausal women and how the response was related to insulin, glucose, and estradiol concentrations. To do this, 9 obese and 10 non-obese apparently healthy postmenopausal women (mean age ± SE; 56.3 ± 0.9 y) engaged in 30 minutes of treadmill exercise at 70% of their predetermined $\dot{V}O_{2max}$. Blood samples were collected periodically during pre-exercise, exercise, and recovery periods for the determination of GH, insulin, glucose, glycerol, and estradiol concentrations. The results of this study indicate that a 30-minute bout of treadmill exercise at 70% of $\dot{V}O_{2max}$ is sufficient to elicit a significant elevation of serum GH concentrations in untrained obese and non-obese postmenopausal women and that the GH response to exercise is negatively related to body weight and body fat. However, the large variance in the GH response made it impossible to demonstrate statistically significant group differences.

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NOMENCLATURE

y	year
h	hour
min	minute
L	liter
cm	centimeter
g	gram
kg	kilogram
m	milli (10^{-3})
μ	micro (10^{-6})
n	nano (10^{-9})
p	pico (10^{-12})
$\mu IU \cdot mL^{-1}$	micro-International Units per milliliter
$ng \cdot mL^{-1}$	nanogram per milliliter
$pg \cdot mL^{-1}$	picogram per milliliter
$L \cdot min^{-1}$	liter per minute
$mL \cdot kg^{-1} \cdot min^{-1}$	milliliter per kilogram body mass per minute
$mL \cdot kg\ FFM^{-1} \cdot min^{-1}$	milliliter per kilogram fat free mass per minute
$mmol \cdot L^{-1}$	millimole per liter
$kg \cdot m^{-2}$	kilogram per meter squared
kcal	kilocalorie
CPM	counts per minute
%CV	percent coefficient of variance

LIST OF ABREVEATIONS

GH	Growth Hormone
GHRH	Growth Hormone Releasing Hormone
GHD	Growth Hormone Deficiency
GHBP	Growth Hormone Binding Protein
rhGH	Recombinant Human Growth Hormone
GH_{Peak}	Absolute Peak Growth Hormone Concentration
$PEAK_{Corrected}$	Peak Growth Hormone Concentration Corrected for Control Concentration
AUC	Area-Under-the-Curve
AUC_{Total}	Total Area-Under-the-Curve
$AUC_{Baseline}$	Area-Under-the-Curve Corrected for Baseline Concentration
$AUC_{Corrected}$	Area-Under-the-Curve Corrected for Control Trial
nOb	Non-Obese
Ob	Obese
BMI	Body Mass Index
%BF	Percent Body Fat
FFM	Fat Free Mass
FM	Fat Mass
LBM	Lean Body Mass
BMC	Bone Mineral Content
BMD	Bone Mineral Density
RIA	Radioimmunoassay
$\dot{V}O_2$	Oxygen Consumption
$\dot{V}O_{2max}$	Maximal Oxygen Consumption
HR_{max}	Maximal Heart Rate
RPE	Rating of Perceived Exertion
P	Pre-Exercise Period
E	Exercise Period
R	Recovery Period
LT	Lactate Threshold
E_2	Estradiol
IGF-1	Insulin-like Growth Factor-1
FFA	Free Fatty Acid
LPL	Lipoprotein Lipase
HRT	Hormone Replacement Therapy
TSH	Thyrotropin
PRL	Prolactin
T_3	Triiodothyronine
T_4	Thyroxine

ACTH	Adrenocorticotropin
DHEA	Dehydroepinandrosterone
LDL	Low-Density Lipoprotein
HDL	High-Density Lipoprotein
ESPA	Sodium n-ethyl-N-(3-sulfopropyl)m-anisidine
4-AAP	4-aminoantipyrine Dye
ATP	Adenosine Triphosphate
G-1-P	Glycerol-1-Phosphate
ADP	Adenosine-5'-Diphosphate
GK	Glycerol Kinase
GPO	Glycerol Phosphate Oxidase
DAP	Dihydroxyacetone Phosphate
H_2O_2	Hydrogen Peroxide

CHAPTER I

INTRODUCTION

Hormonal changes in women following menopause have been well documented. These age associated hormonal changes increase a woman's risk of developing osteoporosis (27, 149), cardiovascular disease (94), obesity (3, 25, 32, 53, 132), dyslipidemia (130), and loss of lean body mass (LBM) (138, 139). Growth hormone (GH), one of several hormones known to decrease with age, is associated with estrogen levels, and is greatly reduced following menopause. This fall in GH may be linked to various disease conditions because of its role in stimulating the synthesis and maintenance of muscle and bone, as well as stimulating lipolysis (93).

Low levels of GH are associated with altered body composition (101) and a decrease in bone mineral density (BMD), which could lead to osteoporosis (22-24, 128). This is evident in individuals who are growth hormone deficient (GHD) as a result of hypothalamic or pituitary dysfunction. Characteristically, GHD people have higher relative levels of fat mass (FM) and lower relative levels of LBM compared to individuals with normal GH levels (75, 94, 145). People who are obese also have lower GH levels (77, 133, 134) and higher insulin levels (110) than people who are not obese. High levels of insulin and low levels of GH are associated with problems in the body's ability to store and utilize substrates and a reduced availability of fat for use as energy.

Following menopause, ovarian estrogen production is greatly reduced and the production of pituitary gonadotropins is altered. Ho and colleagues (66) reported that the 24-hour GH secretion pattern was significantly altered in older compared to younger men and women and that women experience a greater age-related decline in GH secretion compared to men. It was also reported that estradiol (E_2) is highly correlated with total and pulsatile GH release (66). Elevated E_2 concentrations have also been associated with increased GH concentrations in eumenorrheic (67), postmenopausal (84), and obese (134) women.

The greatly reduced GH levels following menopause (65) may be a contributing factor to the more rapid loss of muscle and bone density and gain in body fat seen in postmenopausal women. The low GH levels associated with menopause and obesity suggests that postmenopausal women are at increased risk of developing degenerative diseases, and postmenopausal women who are also obese may be at even greater risk.

Acute bouts of exercise have been shown to increase GH levels in young healthy males (20, 44, 82, 85, 87, 88, 118, 146) and females (11, 20, 67, 77, 82, 83, 86, 88, 91, 142, 144, 146), and older males (87, 108, 122) and females (14, 84, 100). The elevated GH levels during exercise are associated with increased sympathetic nervous system activity (29, 125, 140). The potent stimulatory effect of exercise on GH secretion is attenuated following menopause (84) and in obese people (77). This study will examine how GH levels change in response to exercise in postmenopausal women; how this change is affected by differences in body fat; and

investigate the relation between altered hormone levels (e.g., low GH levels, elevated insulin levels), and energy utilization.

Although exercise-induced GH release has been observed in older women, the hormonal response to exercise has not been well-defined in postmenopausal women. A study comparing sedentary and trained postmenopausal women found higher resting GH and insulin-like growth factor-1 (IGF-1) levels, and greater peak GH levels following graded maximal exercise in the trained group (100). Kraemer et al. (84) reported an enhanced GH response to exercise in postmenopausal women on hormone replacement therapy (HRT) compared to a control group. The sparse available data on older women and the effect of exercise on hormone secretion patterns suggests a strong need for research in this area.

There are a number of studies in the literature demonstrating that obese individuals have lower endogenous levels of growth hormone. Age and %BF are negatively related to 24-hour basal and pulsatile GH secretion, GH secretory burst mass, and GH half-life (134). Studies (3, 25, 32, 53) in which individuals were administered growth hormone releasing hormone (GHRH), the primary stimulator of GH release, found that GH levels increase to a lesser degree in obese subjects than in non-obese controls. This suggests that the blunted GH response seen with obesity may be related to enhanced somatostatinergic tone resulting from diminished cholinergic control of GH release (3, 25, 32, 53). Alterations in the somatotrophic axis associated with obesity are also evident in the GH response to exercise.

Kanaley and colleagues (77) evaluated the GH response to 30-minutes of treadmill exercise in non-obese and obese women (~34 years of age). Acute exercise stimulated a significantly increased serum GH concentration above basal levels in both groups but the response was significantly attenuated in the obese group. The non-obese groups also had significantly higher 6-hour integrated GH concentrations which were attributable to a greater 6-hour GH production rate and a greater mass of GH secreted per pulse (77). Similar findings have been reported for young obese and age-, and gender-matched control subjects for 24-hour spontaneous GH release profiles (109). Additionally, GH responses to hypoglycemia, basal IGF-1 levels, and insulin levels were altered in the obese group (109). However, the obesity-related defects in the somatotrophic axis may be reversible. Two studies reported that following large decreases in body weight, the 24-hour spontaneous GH release profiles, GH responses to hypoglycemia, basal IGF-1 levels, and insulin levels were normalized (109, 110).

The majority of GH's catabolic effects are mediated through the stimulation of IGF-1. Within adipose tissue, IGF-1 mimics the effects of insulin, promoting glucose oxidation and the synthesis of lipids and glycogen (15, 93). In high concentrations, insulin can activate IGF-1 receptors; so high insulin levels may stimulate anabolism of adipose tissues, despite insulin resistance, by activation of the IGF-1 receptors. The direct catabolic effects of GH on adipose tissue, causing the mobilization of FFA, may not be sufficient to counteract the anabolic effects of insulin stimulation of the IGF-1 receptors in individuals with low GH and elevated

insulin levels such as seen with obesity. Although this study was not designed to focus on the insulin/IGF-1 receptor interaction, this project is an important preliminary step providing the foundation for future research related to a hormonal mechanism for altered body composition following menopause.

Hypotheses

It has been shown that GH levels are lower in women following menopause, that obese women have lower GH levels and elevated insulin levels compared to non-obese women, and that obesity is associated with the attenuation of the GH response to exercise. Therefore, it was hypothesized that: a) obese postmenopausal women have lower basal GH levels, lower exercise-induced peak GH levels, and lower GH area-under-the-curve (AUC) compared to non-obese postmenopausal women; b) the exercise-induced GH levels are positively correlated with E_2 and negatively correlated with body fat, insulin, and glucose levels; and c) the altered GH concentration of obese postmenopausal women is associated with differences in substrate utilization during exercise and recovery compared to non-obese postmenopausal women.

CHAPTER II

REVIEW OF THE LITERATURE

Growth Hormone

Growth Hormone Synthesis and Regulation

Growth hormone is a single-chain polypeptide containing 191 amino acids, two disulfide bridges, and has a molecular weight of approximately 22,000 (50). Growth hormone synthesis occurs in the somatotrophic cells of the anterior pituitary gland and is stored in secretory granules that release GH into the blood stream through exocytosis. The primarily stimulus for GH release is through growth hormone releasing hormone (GHRH). Growth hormone releasing hormone stimulation of GH secretion is thought to be highly specific since no other anterior pituitary hormones are know to be released following intravenous injection of GHRH (93). Peak serum GH levels are reached 15-30 minutes following GHRH infusion and return to baseline levels within 2 hours (93). The primary inhibitor of GH release is the hypothalamic peptide somatostatin (19, 149), which blocks GHRH. Somatostatin is also a potent inhibitor of thyrotropin (TSH) release by the pituitary gland and the secretion of insulin and glucagon by the pancreas.

Growth hormone is released in pulses in response to pulsatile releases of GHRH from neurons of the hypothalamus into the hypophyseal-portal blood (113, 149). There is an ultradian pattern of GH release (128); the greatest concentrations are seen during slow wave (stage III-IV) sleep (17, 50, 149) when somatostatin

secretion is the lowest. Additionally, GH secretion during sleep may be adversely affected by moderate intensity daytime physical exercise (81), although other investigators have reported no effect (78, 98) or an attenuated effect (58). The pattern of GH secretion varies over the life span with the highest levels occurring during puberty and gradually declining through senescence. The lower GH levels seen with increased age are associated with decreases in pulse amplitude (66) and pulse frequency (45, 149). The decreases in GH secretion associated with age are thought to be a result of deficiencies within the hypothalamus, which affect the transcriptional regulation of GHRH and somatostatin (149). Evidence of altered somatostatinergic tone is supported by research in humans who showed increased GH secretion when administered a somatostatin inhibiting agent (52, 105, 149). Table 1(50) shows a number of variables that influence the secretion of GH, but ultimately, each of these factors act to stimulate or inhibit GHRH or somatostatin.

For more than thirty years researches have proposed that sex hormones influence GH regulation (46, 126, 152). While there are several sex hormones, of interest to this review is the relationship of the estrogen, estradiol (E_2), on GH secretion. In an early study of exercise-induced GH release, fasted men and women between 20 and 80 years of age were found to have similar resting GH concentrations (46). Following walking, women had an approximately 6-fold increase in GH levels while the GH concentration in men remained the same (46). This prompted the investigators to administer estrogen to four men over five days; they found that the estrogen treated men had a similar GH response to walking as

Table 1. Regulation of growth hormone secretion.

Stimulation	Inhibition
Decrease plasma glucose	Somatostatin
Decrease plasma free fatty acid	Increased plasma glucose
Increase plasma amino acid	Increased plasma free fatty acid
Fasting	Somatomedins
Prolonged caloric deprivation	Growth hormone
Stage IV sleep	Beta-Adrenergic agonists
Exercise	Cortisol
Stress	Senescence
Puberty	Obesity
Estrogens	Pregnancy
Androgens	
Dopamine	
Acetylcholine	
Serotonin	
Alpha-Adrenergic agonists	
Gamma-Amino butyric acid	
Enkephalins	

Source: Genuth, S.M. The Endocrine System. In: Physiology. R.M. Berne and M.N. Levy (Eds) St. Louis, MO: Mosby, 1993, p. 916.

women (46). The hypothesized mechanism for the effect of estrogen on GH release is by direct sensitization of the pituitary to GHRH and/or by indirectly affecting the pituitary by decreasing somatomedin-C concentrations (37, 136).

Growth Hormone Action

Growth hormone, which acts directly on the liver, muscle, and adipose tissues, stimulates a number of profound anabolic effects. Within the liver, GH stimulates RNA and protein synthesis, increases gluconeogenesis, and upregulates the production of somatomedins or insulin-like growth factors (IGF) (15, 50). The somatomedin that appears to be most closely related to GH is somatomedin-C (IGF-1). Growth hormone and IGF-1 increase amino acid uptake and increase protein

synthesis within muscle, leading to an increase in lean body mass (LBM) (50, 93). Adipose tissue is directly affected by GH, which stimulates lipolysis (120) and may lead to a decrease in adiposity (50).

Insulin-like growth factor-1 is released from the liver and acts on a number of organs and connective tissues by stimulating the synthesis of RNA, DNA, and protein. This leads to the proliferation of cells, a hypertrophic and hyperplasic effect on tissues, and increases the size and function of organs. Also, IGF-1 acts on chondrocytes to stimulate the incorporation of sulfate into proteoglycans and bone, leading to increased linear growth through puberty and the increase or maintenance of bone mineral density (BMD) and bone mineral content (BMC) throughout life (15, 50). The synthesis of IGF-1 is primarily mediated by GH but can also be affected by insulin, fasting, nutritional status (15, 93), and sex hormones (148). Structurally, IGF-1 and the IGF-1 receptor are very similar to insulin and the insulin receptor, respectively. During conditions of positive energy balance, IGF-1 mimics the actions of insulin within adipose tissue (15, 93). It is thought that IGF-1 may be an autocrine hormone since it is produced in a variety of cell types, which also express the IGF-1 receptor (15, 93).

Substrate Utilization

Homeostasis of plasma glucose levels is of vital importance to the proper function of the physiologic systems. Elevated blood glucose levels stimulate the secretion of insulin, which increases the uptake of glucose and amino acids by the

tissues for immediate utilization as a metabolic substrate, protein synthesis, or storage in the form of glycogen. Insulin stimulates glycolysis and inhibits lipolysis; this causes a decrease in plasma free fatty acids (FFA) levels and suppressed fat utilization. Within the adipose tissue, IGF-1 mimics the effects of insulin, and promotes glucose oxidation and the synthesis of lipids and glycogen (15, 93). In high concentrations, insulin can activate IGF-1 receptors; so high insulin levels may stimulate anabolism in adipose tissues by activation of IGF-1 receptors. Welbourne et al. (137) reviewed the role of GH in substrate utilization. Low plasma glucose levels stimulate the release of GH and glucagon and low glucose concentrations may be directly associated with low plasma insulin levels. Growth hormone release causes the mobilization of FFA from adipose stores (36) and gluconeogenesis by the liver. During fasting conditions, when plasma insulin concentrations are low and plasma GH is elevated, IGF-1 secretion is not stimulated since anabolic processes would be disadvantageous (15). Glucagon targets the liver and stimulates gluconeogenesis and glycogenolysis to return plasma glucose to homeostatic levels. Catecholamines have a part in catabolic energy metabolism by inhibiting insulin and stimulating substrate mobilization. Epinephrine stimulates glycogenolysis in the liver and active muscle and lipolysis in active muscle and adipose tissues. Norepinephrine is a potent stimulator of lipolysis within adipose tissue. Cortisol, a glucocorticoid, stimulates proteolysis and the mobilization of amino acids to the liver for gluconeogenesis. Cortisol supports the gluconeogenic actions of glucagon and GH, increases the mobilization of lipids, and is an insulin antagonist by

inhibiting the uptake and storage of glucose (95). Within adipose tissue, there is an intricate relationship between cortisol, insulin, and GH on lipoprotein lipase (LPL). Lipoprotein lipase is the major regulator of triglyceride absorption in adipose tissue. When GH levels are low, cortisol interacts with insulin to activate LPL and facilitate triglyceride storage. In the presence of GH, cortisol and GH interact to functionally inhibit LPL and stimulate lipolysis and FFA mobilization (10, 103, 104). It is apparent that there are a number of factors that influence metabolism and substrate use.

Growth Hormone Levels and Exercise

Exercise is a potent stimulator of hormone release. The release of GH is stimulated by endurance (20, 49, 67, 79, 82, 84, 122, 142, 146) and resistance (83, 85-88) exercise. It appears that cholinergic pathways play an important role in mediating the exercise-induced rise in GH concentration (105, 125, 140). It has also been suggested that exercise duration (48, 56), intensity (48, 56, 107, 142), fitness level (20, 79, 82, 142), training (80, 87, 88, 122, 142, 153), testing protocol (57, 86), gender (20, 65, 82, 88, 146), and age (65, 87, 88, 122, 153) may all influence the GH response to exercise. This study focuses on the acute effects of endurance exercise, therefore this review addresses only those studies with a similar focus.

Effect of Exercise Duration and Intensity

It has been proposed that exercise duration and intensity may have a vital role in GH secretion (107) and that this role may be related to circulating lactate levels (28, 29, 44, 56). Felsing and co-workers (44) reported that a duration of at least 10-minutes of high intensity (~70% $\dot{V}O_{2max}$) ergometer cycling was necessary before a significant increase in circulating GH was seen. However, Gordon et al. (56) reported a significant increase in GH concentrations following supramaximal exercise of only a 90-second duration. Some studies have reported increases in GH concentration following a single bout of resistance exercise (85, 87, 102), but these study protocols contained multiple exercises during a given time interval such that the duration of exercise was greater than 20 minutes. A study of acute high-intensity anaerobic exercise (90 s) reported a higher, post-exercise, peak serum GH response during a non-treatment trial compared to a trial where subjects were treated with $NaCO_3$; there was a correlation ($r=0.59$, $P=0.006$) between GH and plasma H^+ concentration (56). Elevated plasma GH levels have been observed in conditions of diabetes (51) and renal failure (117) supporting the idea that H^+ concentrations may influence GH in conditions of metabolic acidosis. In contrast to this, other studies have found GH levels to increase at exercise intensities below the lactate threshold (LT) (107) and others have shown no increase in GH secretion following the infusion of lactate (92). Sympathetic nervous system activity is highly correlated with exercise intensity and has known influences on GH release (29, 125, 140). In light of these studies, it is unlikely that lactate independently influences the release

of GH, although elevated lactate levels may serve as an indicator of exercise intensity sufficient to elevate GH levels. There may also be differences in the GH response to exercise between aerobic and anaerobic activities, which may be a possible explanation for discrepancies in some of the above studies.

Throughout the literature, a wide range of exercise intensities and durations has been used in studies of GH. Pritzlaff et al. (107) reported a well-controlled study on pulsatile GH release in young men in response to five exercise intensities. Study participants performed repeated trials at intensities below, equal to, and above LT that corresponded to a range of relative $\dot{V}O_{2peak}$ values between 26 and 90%. They found higher mean serum GH concentrations with each incremental increase in exercise intensity and that increased exercise intensity was associated with GH secretory pulses of shorter duration but of greater amplitude, resulting in an overall increase in the integrated growth hormone concentration (107). This was the first study of its kind and had a highly focused subject population. While the intensity of exercise clearly has an important role in the GH response to exercise, the duration of exercise may be less of a factor.

Effect of Fitness Level

Bunt et al. (20) investigated the GH response to exercise in young male and female runners compared to active, untrained controls during 60-minutes of continuous moderate intensity (60% $\dot{V}O_{2max}$) treadmill exercise. In this study, runners tended to have higher peak and absolute increases in GH levels compared to

controls. This suggested an increased GH response to exercise with higher fitness, levels but statistical significance was only reached in males. Another study reported higher resting GH concentrations in older males (mean age ~59 y) running more than 40 miles per week compared to age-matched controls (69). The endurance-trained men also had higher peak GH concentrations during 20 minutes of cycle ergometry at 40% $\dot{V}O_{2peak}$ compared to sedentary controls (69). These results contradict other studies (59, 86) that report no change or a decreased GH response to exercise in trained compared to untrained individuals. Higher GH concentrations were reported in untrained men compared to trained men during cycle ergometry at 100% $\dot{V}O_{2max}$, but this training effect was not seen in these subjects at 75% $\dot{V}O_{2max}$ (59). Kraemer and colleagues (82), reported no effect of training on the exercise-induced response of GH, prolactin (PRL), triiodothyronine (T_3), or thyroxine (T_4) in men and women performing 30-minutes of treadmill running at 80% maximal heart rate (HR_{max}). Interestingly, in this study, only GH concentration increased during exercise and no change was seen from pre-exercise levels in PRL, T_3, or T_4. The effect of fitness on the GH response to exercise is not clear.

Effect of Training

Several studies have been conducted investigating the effects of training on hormone concentrations. A 1-year study investigating the training effects of endurance running at LT or above LT in untrained, eumenorrheic women (18-40 years) on the 24-hour GH pulse profile (142). The efficacy of the training program

was represented by significant increases in $\dot{V}O_{2max}$, $\dot{V}O_2$ at LT, and $\dot{V}O_2$ at fixed blood lactate concentrations of 2.0, 2.5, and 4.0 mmol·L^{-1} in both groups, with greater increases seen in those training above LT. No significant changes were seen for fitness or blood parameters in the inactive control group over the 1-year trial. The somatotrophic secretory profile of the above LT group showed significant differences in integrated 24-hour GH concentration, maximal GH peak height, incremental GH peak amplitude, GH peak area, and nadir GH concentration between pre- and post-training values and compared to the other groups following training. There were no significant differences among groups before training or between the "at LT" and control groups after training (142). This study demonstrated a training adaptation of the somatotrophic axis following 1-year of endurance run training and suggested that it is necessary to train above the LT to produce these changes.

In another study, 6 untrained males demonstrated that after 3 and 6 weeks of endurance training, sufficient to increase $\dot{V}O_{2peak}$ and LT, the integrated GH concentration following 20 minutes of constant load endurance exercise and 45 minutes of recovery was significantly reduced compared to pre-training values (143). Subjects in this study were tested at the same absolute exercise power output for each of the constant load tests. As fitness increased with training, the same absolute intensity became a lower relative intensity suggesting that relative intensity is an important factor in determining the magnitude of the GH response.

Bonifazi and colleagues (12) evaluated the GH and adrenocorticotropin (ACTH) response to acute exercise of in elite male swimmers (17-23 years) at three time points over the course of a season. The intensity of each testing exercise protocol was determined as the velocity that produced a 4-mmol blood lactate level. A significant improvement in aerobic capacity was seen at the end of the season. These investigators reported that training enhanced the GH response to exercise while the ACTH response was attenuated.

In another examination of the training effect on hormones, older men and women were randomly assigned to participate in moderate intensity endurance training either three or five days per week or engage in a stretching program three days per week for six months (135). The two endurance-trained groups showed significant increases in $\dot{V}O_{2max}$ and fat free mass (FFM), and significant decreases in body weight, %BF and FM. No significant changes were seen in the stretching/flexibility group following training. A within-subjects comparison revealed that six months of endurance training failed to produce a significant change in basal IGF-1 levels despite significant changes in body composition and aerobic fitness (135). Significant changes in plasma IGF-1 and GH-binding protein (GHBP) have been reported with two weeks of intense endurance training in young, non-obese men compared to age matched controls (114). Growth hormone binding protein represents the extracellular domain of the GH-receptor and is a measure of cellular GH-receptor concentrations. Since there does not appear to be an immediate response to exercise for IGF-1 and GHBP, only basal measurements

were assessed in these two studies.

The literature seems to show that a training effect exists for the GH response to endurance exercise (12, 114, 135, 142, 143). Special consideration should be given to mode, intensity, and duration when assessing GH levels for basal or acute response to exercise conditions. The implications of the endurance training studies are that aerobic training of high enough intensity improves the somatotrophic secretory profile by increasing basal and exercise-induced GH levels, increasing pituitary sensitivity to GHRH, and possibly by increasing serum IGF-1 levels. If this is the case, then these positive alterations to the somatotrophic axis may support the use of endurance exercise training to minimize the degenerative changes associated with aging.

Effect of Gender

Growth hormone secretory patterns show distinct gender differences. Resting GH levels have been shown to be higher in females compared to males (65, 132, 146). Women, compared to age-matched men, demonstrate a daily GH secretion rate that is 1.5- to 2.5-fold higher and is accounted for by a mass of GH per burst (pulse amplitude) that is as much as 2-fold greater (132, 146). There does not seem to be a gender difference in GH secretory pulse number, half-duration of the GH pulse, or half-life of GH (65, 131, 146). Bunt et al. (20) suggested that the higher resting GH levels in females compared to males may be attributable to increased levels of E_2 in women. While basal levels of GH and 24-hour GH

secretion rates are higher in females compared to males, peak GH levels in response to acute exercise are similar between genders (20). This means that the magnitude of change in GH concentrations in response to exercise is greater in men compared to women. It has been suggested that the gender difference in the magnitude of GH response above resting concentrations is associated with the higher testosterone levels in men compared to women (88). In a study by Kraemer et al. (82) comparing trained and untrained men and women, no significant gender or training effect was seen in the exercise response of GH, PRL, T_3, or T_4, although a majority of the literature suggests gender differences do exist. It is apparent from the limited number of available studies that more research into gender differences is necessary.

The influence of sex steroid hormones on the GH secretion throughout the menstrual cycle is unclear. Some researchers have reported 2-fold variations in basal GH concentrations across the menstrual cycle (43, 152). A study of the GH response to exercise in amenorrheic and eumenorrhic female athletes reported that GH levels were not affected by menstrual phase or menstrual status independent of large variations in sex steroid hormones (76). The sex steroid hormone, E_2, has been correlated with GH levels (43, 66, 67, 134, 152) but its effects on GH secretion across the menstrual cycle has yet to be fully delineated.

Effect of Age

Aging is associated with a number of characteristics including decreased muscle mass, increased adiposity, reduced muscle strength, decreased bone density,

and alterations in the GH/IGF-1 axis. The lower GH levels seen with increased age are associated with decreases in pulse amplitude (66) and perhaps pulse frequency (45, 149). The decrease in GH secretion associated with age is thought to be a result of deficiencies within the hypothalamus affecting the transcriptional regulation of GHRH and somatostatin (149) resulting in increased somatostatinergic tone. Research in humans showed increased GH secretion when administered a somatostatin-inhibiting agent (52, 105, 149). A comparison between younger and older men and women showed that the 24-hour GH secretion pattern was significantly altered in older people (66). Integrated GH secretion, pulse duration, and pulse amplitude were significantly decreased with age. There was no significant difference in pulse frequency for age, although a significantly greater number of the pulses were large pulses in the younger group, whereas a majority of the pulses were small pulses in the older group. Additionally, the gender differences previously described were evident in the younger subjects but there was no significant difference seen between the older males and females, suggesting women experience a greater age-related decline in GH secretion. The authors concluded that both gender and age have independent and interrelated effects on total and pulsatile GH secretion and that E_2 is highly correlated with total and pulsatile GH release (66). The discrepancy with previous work indicating a reduction in pulse frequency with age (45) may be explained by a shortcoming in the sensitivity of the available technology to detect small pulses. The somatotrophic axis was also shown to be attenuated in eumenorrheic middle-aged (42-46 years)

women compared to younger women (148). Measurements taken during the follicular phase of the menstrual cycle found middle-aged women to have decreased integrated GH and serum IGF-1 levels despite E_2 levels nearly 2-fold higher than the younger controls (148). The authors suggest that the stimulatory effects of elevated E_2 were insufficient to override age-related decrements of the somatotrophic axis but did not propose an explanation.

It has been hypothesized that declines in levels of GH, IGF-1, testosterone, estrogen, and dehydroepinandrosterone (DHEA) are responsible for the age associated reductions in protein synthesis, muscle strength, and muscle mass (5). In an attempt to alter the GH/IGF-1 axis, Vitiello and associates (135) had older (~68 years) men and women undergo six months of endurance training. They found significant improvements in cardiovascular and body composition parameters yet reported no change in basal IGF-1 levels. In contrast to the hypothesis that age-associated degenerative conditions are related to hormonal factors, a cross-sectional study of 245 elderly women (70-90 years) and found no relationship between IGF-1 and strength of the knee extensor muscles despite marked decreases in strength and circulating IGF-1 concentrations (14). Since GH regulates IGF-1 synthesis, and IGF-1 levels are more stable throughout the day, IGF-1 and IGF-binding protein-3 concentrations are often used as markers of GH secretion in studies were single blood samples are collected (62). Another cross-sectional study of 11 older (50-78 years) marathon runners and age-matched sedentary controls (52-73 years) found no differences in the acute response of GH, IGF-1, or insulin levels to endurance

exercise, but the runners had higher levels of IGF-binding proteins (38). The authors concluded that the age-associated decline in GH and IGF-1 may not be preventable with intense endurance training (38), but this does not preclude the beneficial effects associated with regular physical activity. Similar to the male marathon runners, Cooper et al. (31) reported that chronic exercise did not prevent the age-associated decrement in the GH/IGF-1 axis in older (60-70 years) endurance trained men. While long-term training does not seem to attenuate the effects of age on the GH/IGF-1 axis, GHRH has been shown to cause a greater GH response in endurance trained older men. Ambrosio and coworkers (2) examined 25 runners and 24 controls (50-60 years) and demonstrated that resting GH release was significantly higher following injection of GHRH in the runners. Interestingly, a subset of these groups showed that the administration of arginine, an inhibitor of somatostatin, did not facilitate a greater GH response to GHRH than GHRH alone in the runners, but arginine significantly increased the GH response to GHRH in the controls (2). This study suggests that chronic endurance training may enhance the sensitivity of the GH/IGF-1 axis to GHRH and may minimize the age-associated decreases in somatotrophic function by manipulating somatostatinergic tone. From the literature, it appears that there are somatotrophic changes that occur as a natural part of the aging process. Although these changes cannot be stopped, it seems that the negative effects can be minimized through chronic physical activity.

Effect of Menopause

Menopause is the natural decline of cyclic hormonal production and function resulting in the stopping of menses. It typically occurs between 45 and 60 years of age but may happen earlier in life as a result of illness or the surgical removal of the uterus and/or both ovaries. As the production of ovarian estrogen and pituitary gonadotropins decreases, ovulation and menstruation become less frequent and eventually stop (54). A woman's risk for developing degenerative conditions like osteoporosis, cardiovascular disease, obesity, and decreased LBM, are greatly increased following menopause and may be related to low estrogen levels. The study by Ho and coworkers (66) on the effects of age and gender on 24-hour GH secretion found that women experienced a greater alteration of the somatotrophic axis with age compared to men and found that GH secretion was highly correlated with E_2 levels. The importance of E_2 on circulating GH was demonstrated in eumenorrheic women (age 19-48 years) during the follicular phase of the menstrual cycle when E_2 levels are low and during the periovulatory phase when E_2 levels are high (67). Resting GH and GH response to 10-min of high intensity exercise were significantly higher during the periovulatory phase compared to the follicular phase. Additionally, treating postmenopausal women with estrogen therapy significantly increased resting plasma GH concentrations and 22-hour GH secretion and, interestingly, the change in GH levels was inversely related to body fat (37). In a separate study, basal and exercise-induced GH levels were shown to be significantly

higher in lean postmenopausal women on hormone replacement therapy (HRT) compared to similar women not receiving HRT (84). Studies reporting decreased bone mineral density with age have found estrogen replacement therapy to be effective for prevention of bone loss during the early years following menopause (23, 24). Additionally, deficiencies in the GH/IGF-1 axis associated with aging were found to be related to osteoporotic alterations in body composition in women over 70 years of age (13) and a study of 112 Guatemalan women, age 20 to 87 years, found age to be positively related to body fat and inversely related to LBM and IGF-1 levels (106).

A study of 18 sedentary and 15 endurance trained postmenopausal women (mean age ~62 years) found that endurance trained women had higher resting GH and IGF-1 levels, and greater peak GH levels following a graded $\dot{V}O_{2max}$ test (100). Similar values were seen for E_2 and testosterone between the sedentary and endurance trained groups. This study suggests there is a training effect on the GH response to a maximal exercise test in postmenopausal women. However, this conclusion is questionable because the test was not a constant load protocol and the duration of the test was longer and the final absolute intensity level was higher for the trained group. A review of the literature found very few studies investigating the exercise-induced hormonal responses of older women with even fewer focusing on obesity. There is an apparent need for well-designed studies investigating aging and body composition in women.

Effect of Obesity

A persuasive amount of evidence exists showing that obese individuals have lower endogenous levels of GH, although there is little data on obese older women. The exact mechanism for the reduced GH secretion with obesity is not fully understood. It may be related to elevated insulin levels, commonly associated with obesity, or altered IGF-1 concentration (61). It has also been postulated that enhanced somatostatin activity is responsible for the diminished GH levels seen with obesity (3, 25, 32, 53, 147). Basal GH concentration and pulsatile GH secretion, ascertained in 24 healthy males (18-64 years) with a wide range of %BF (12-46%), revealed that 24-hour GH secretion was negatively related to age and %BF with a 140-fold range in GH secretion within the cohort (134). Stepwise multivariate analysis unveiled that 38% of the total variability was attributable to %BF and 50% of the total variability to the interaction between %BF and testosterone. A significant negative relationship was also seen between %BF and GH secretory burst mass and GH half-life, although GH half-life was positively associated with E_2 levels (134). When administered GHRH, GH levels increase to a lesser degree in obese subjects than in non-obese controls (3, 25, 32, 53). Following the administration of a cholinergic enhancing agent, pyridostigmine (25, 32, 53) or fenfluramine (3), basal and GHRH-induced GH levels were significantly higher in obese and control groups compared to pre-treatment levels; the obese groups demonstrate a blunted GH response compared to controls. These studies suggest

that the blunted GH response seen with obesity appears to be related to enhanced somatostatinergic tone resulting from diminished cholinergic control of GH release (3, 25, 32, 53).

Kanaley and colleagues (77) evaluated the GH response to 30-minutes of treadmill exercise in 8 non-obese and 23 obese women (~34 years) at 70% $\dot{V}O_{2peak}$. The mean serum GH concentration was significantly increased above basal levels in both groups in response to acute exercise but the response was significantly attenuated in the obese group. The non-obese group also had significantly higher 6-hour integrated GH concentrations which were attributable to a greater 6-hour GH production rate and a greater mass of GH secreted per pulse (77). A subset of the obese group (N=10) participated in 16-weeks of aerobic exercise training, 20-40 minutes per day, 3-days per week, at 65-80% HR_{max}. $\dot{V}O_{2peak}$ increased significantly following training, but no differences were seen in body weight, LBM, or FM, and exercise training did not produce significant changes in 6-hour integrated GH concentrations (77). The major finding of this study was that obese women have an attenuated GH response to exercise compared to non-obese women. The lack of a training effect seen in these obese women contradicts the findings of previously described training studies in normal weight individuals (142, 143). Since the somatotrophic axis is influenced by adiposity and there was no change in body weight or FM following training in the obese women, it is possible that any beneficial effects of training were masked by the high levels of body fat.

A comparison of 18 obese and age-, and gender-matched control subjects

(mean age 26 ± 1 years) confirmed that obese subjects had significantly lower 24-hour spontaneous GH release profiles, GH responses to hypoglycemia, basal IGF-1 levels, and significantly elevated insulin levels compared to non-obese subjects (109). A significant inverse relationship was found in obese subjects between %BF and 24-hour spontaneous GH secretion and serum IGF-1 levels. The effects of massive weight loss (mean loss of 30.3 ± 4.6 kg) was studied in 9 of these obese subjects (109). Following weight reduction, normalization was seen in 24-hour spontaneous GH release profiles, GH responses to hypoglycemia, basal IGF-1 levels, and insulin concentrations. The authors concluded that obesity-related defects in 24-hour spontaneous GH release profiles, and basal IGF-1 levels are reversible, and that the defects are transient rather than a persistent preexisting disorder (109). This same group reported another study, measuring the same variables. Following a prolonged fast (96-hour), significant differences were seen between obese and non-obese subjects; following weight loss, the prolonged fast showed similar responses between groups (110). This provides additional evidence that the obesity-related defects in the somatotrophic axis are a transient condition.

There is sufficient evidence to conclude that GH secretion patterns are altered with obesity and that these changes are similar to those seen with increased age. It seems reasonable to hypothesize that the combination of age and obesity could potentiate the negative influences of each condition on the somatotrophic axis. To this point, there have been no studies investigating exercise-induced hormonal secretion patterns in obese postmenopausal women.

Hormone Therapy

Growth Hormone Therapy

Growth hormone deficiency in adults is characterized by increased body fat, decreased LBM, decreased BMD, hyperlipidemia, and reduced exercise capacity (101). These characteristics are similar to those seen with aging and obesity. Several studies have demonstrated that treatment with recombinant human GH (rhGH) increases LBM and decreases adiposity (4, 7, 72, 101, 116, 145). Newman and Kleinberg (101) reviewed nine placebo-control studies of rhGH therapy in GHD adults with treatment between 6- and 18-months in duration. Combined, the LBM increases ranged from 2.2 to 6.3 kg and %BF decreases ranged from 1.7 to 6.5%. An extension of a 16-month study (73) continued to follow GHD adults over 3 years of GH-replacement therapy, which produced near normalization of body composition and physical performance compared to age- and gender-matched controls (75). Attanasio et al. (4) found significant improvements in body composition values following 18-months of rhGH treatment in GHD adults; this was associated with decreased levels of low density lipoprotein (LDL) cholesterol and increased high density lipoprotein (HDL) cholesterol levels. This is supported by long-term studies demonstrating beneficial changes in blood lipid profiles of GHD adults following GH-replacement therapy (9, 129). Several studies have also shown improvements in $\dot{V}O_{2max}$ and exercise capacity following rhGH therapy in GHD adults (4, 9, 35, 74, 99, 129, 145), which may be attributable to increased

plasma volume and total blood volume (26).

Because of the anabolic effects of GH on skeletal muscle, some non-GHD individuals have attempted to gain a physiological advantage through pharmacologically elevated levels of GH. The majority of the literature investigating the use of GH as an ergogenic aid have involved resistance exercise. Of the available studies, it appears GH supplementation does not potentiate the effects of resistance training alone within healthy individuals of any age (34, 39, 124, 150, 151, 153). There are significant side effects of high GH levels including: hypertension, cardiomyopathy, respiratory disease, diabetes, abnormal lipid metabolism, osteoarthritis, increased risk of colorectal and breast cancers, and acromegaly (71). At present, it appears that GH therapy above physiologic concentrations is unsafe and should not be implemented. For individuals with a GH deficiency, it appears medically advisable to return GH levels to within physiologic levels.

Hormone Replacement Therapy

The two most common forms of hormone therapy for postmenopausal women are estrogen replacement therapy and hormone replacement therapy (HRT). Estrogen replacement therapy refers to estrogen supplementation without progesterone and is typically given to women who have had their uterus surgically removed. Hormone replacement therapy is generally administered to women whose uterus is still intact because estrogen alone may cause excessive growth of the

uterine lining, vaginal dryness, breast tenderness, and may increase risk of developing uterine cancer. Progesterone helps to alleviate the side effects of estrogen alone. Two regimens for HRT are: 1) continuous combined treatment in which both estrogen and progesterone are taken daily; and 2) cyclic treatment, in which estrogen alone is taken daily for 10-15 days, estrogen and progesterone are taken the next 3-5 days, and then no hormones are taken for the remainder of the cycle. Perimenopausal women are often placed on HRT in order to help control "hot flashes" and other common symptoms associated with menopause. A number of studies have investigated effects of HRT on several degenerative conditions. Estrogen decreases the activation of bone turnover which inhibits bone resorption (22) and estrogen replacement therapy has been shown to be effective for preventing bone loss during the first few years following menopause (23, 24). The strong relationship of serum E_2 levels to GH and IGF-1 suggests that an elevation of E_2 concentrations may enhance GH secretion and IGF-1 production. This, in turn, may promote tissue maintenance or growth and minimize age-associated decrements in function. Ho et al. (66) proposed that "since GH stimulates protein synthesis and has indirect and direct effects on bone metabolism, it is possible that reduced GH secretion secondary to estrogen deficiency represents one mechanism through which bone loss occurs after the menopause."

The effects of HRT on GH secretion patterns was reported for 23 healthy postmenopausal (39-56 years) and 25 eumenorrheic (20-37 years) women (63). Pre-treatment GH and IGF-1 values were significantly lower in the postmenopausal

group compared to the eumenorrheic control group. Following 10-months of HRT, 24-hour GH and IGF-1 concentrations were significantly increased over pre-treatment values. Despite the elevation of GH and IGF-1 levels following the 10-month treatment, HRT failed to normalize GH and IGF-1 concentrations in the postmenopausal group compared to the young eumenorrheic controls (63).

The effect of HRT on the GH response to endurance exercise was studied in lean (BMI ~25 $kg \cdot m^{-2}$) postmenopausal women (84). Eight postmenopausal women taking HRT and nine postmenopausal women not taking HRT performed 30 minutes of treadmill exercise at 80% $\dot{V}O_{2max}$. Resting concentrations of GH, PRL, and E_2 were significantly higher in the HRT group compared to the non-HRT group. Growth hormone and PRL concentrations increased above control values in both groups, but the GH response to exercise and GH AUC were significantly greater in the HRT group compared to the non-HRT group (84). The authors suggested that the effects of HRT and exercise might enhance the health benefits of HRT or exercise alone.

Summary Statement

Growth hormone is involved in the regulation of a number of physiologic processes and low GH concentrations are adversely related to several pathologic conditions. It is clear that GH levels are influenced by a number of factors, and age, body fat, glucoregulation, and estrogen are linked with decreased GH secretion through senescence. Despite a multitude of studies investigating GH, little is known

about the GH response in older women. To this point, no investigation has focused on the impact of body composition on the exercise-induced GH response in postmenopausal women.

CHAPTER III

METHODS AND PROCEDURES

Subjects

Nineteen apparently healthy, untrained, postmenopausal women between 50 and 65 years of age were recruited from Knoxville, TN and the surrounding community by flyers, word of mouth, newspaper advertisements, and public service announcements. Each participant was placed into one of two groups based on body composition: a) non-obese (nOb) (BMI <27 $kg \cdot m^{-2}$); or b) obese (Ob) (BMI >30 $kg \cdot m^{-2}$). Criteria for participation in this study included: a) being postmenopausal; b) being able to complete all test protocols; c) being free of chronic illnesses; d) not taking any medications known to alter endogenous hormone concentrations; e) being a non-smoker; and f) being weight stable. Level of training was assessed by physical activity questionnaire and "untrained" was defined as the absence of regular exercise (<1-day per week) during the previous six months. Postmenopausal status was defined as the absence of menses for a period of at least one year through natural menopause or the surgical removal of both ovaries and uterus. Health status and physical activity level was determined through self-report by health status questionnaire. Disqualifying chronic illnesses included coronary heart disease, diabetes mellitus, malignancy, anemia, renal disorders, liver or gallbladder disease, pituitary disease, and uncontrolled hypertension. Exclusionary medications included β-blockers, glucocorticoids, drugs known to alter hormone concentrations,

or other hormonal or hormone-mimetic medications. A condition of stable body weight was defined as no change (± 2 kg) in body weight and the absence of a special diet and/or program for the purposes of weight reduction during the previous three months.

Prior to any testing, subjects were provided a written informed consent form, which specified all information necessary to ensure their knowledgeable consent (Appendix A). Subjects were given an opportunity to carefully read the document and ask a research team member questions to clarify any unclear information before signing the consent form. The informed consent form was approved by the Institutional Review Boards of The University of Tennessee-Knoxville (IRB #5812B) and The University of Tennessee Graduate School of Medicine in Knoxville (IRB #1077).

Nutritional Status

Each subject was asked to complete a dietary record for the three days immediately preceding each of the two experimental testing sessions for intra-individual and inter-group comparisons. The self-reported diet was analyzed for total caloric intake; percentage of calories from fat, carbohydrate and protein; and percentage of fat from saturated and unsaturated sources, using the computer program Nutritionist Five (First DataBank, San Bruno, CA).

Experimental Protocols

Each study participant was asked to report to the Cardiovascular and Pulmonary Rehabilitation Center, The University of Tennessee Medical Center (UTMC), on one occasion for a symptom-limited exercise capacity test ($\dot{V}O_{2max}$) and to the Applied Physiology Laboratory (RM 310, HPER Bldg.) on two separate occasions for experimental testing. Testing sessions were separated by at least one week. During the first session, participants gave their signed consent to participate and completed a health status questionnaire. Measurements of height and weight were made, followed by the $\dot{V}O_{2max}$ test. The second and third testing sessions were in the Applied Physiology Laboratory and consisted of a prolonged exercise trial and a control trial. These trials were counterbalanced to control for a test order effect. Additional descriptive characteristics such as height, weight, percentage of body fat, and girth measurements were obtained at the end of the control trial testing session.

Descriptive Characteristics

Participants were measured for height (cm) using a stadiometer (SECA Corp., Columbia, MD) and weight (kg) with a calibrated standard physician's scale (Health-O-Meter, Inc., Bridgeview, IL). Girth measurements were made at the waist and hips (waist-to-hip ratio) with a plastic tape measure fitted with a tension handle. Percentage of body fat was determined by whole body plethysmography (Bob Pod®, Life Measurement Instruments, Concord, CA) (55, 96). Health status

and level of training were assessed by self-report on a health status questionnaire (Appendix B).

Maximal Oxygen Consumption Test

The $\dot{V}O_{2max}$ test (Appendix C) was performed on a motorized treadmill. The initial speed of the treadmill was 54 $m \cdot min^{-1}$ (2 mph) and grade was 0%. Speed was increased by 13 $m \cdot min^{-1}$ (0.5 mph) each minute up to 94 $m \cdot min^{-1}$ (3.5 mph) then grade was increased 2% each minute until volitional exhaustion. Expired gases were collected through a large 2-way valve (Hans-Rudolph, Inc., Kansas City, MO) with a mouthpiece and accompanying nose clips. Expired gases were measured using the Quinton Q-Plex I Metabolic Measurement System (Quinton, Bothell, WA). The system uses a heated pneumatach (~37° C) for the measurement of ventilation, a zirconia oxide high temperature furnace oxygen analyzer and an infrared carbon dioxide analyzer for the measurement of gas fractions. This system has been previously shown to be valid (30). Gas analyzers were calibrated using a 3-point calibration method against gases of known concentration determined by the Scholander technique (119). A maximal effort was determined using the primary criterion of a plateau in $\dot{V}O_2$ with increased workload or two of the three secondary criteria of reaching age-predicted maximal heart rate (220-age); respiratory exchange ratio >1.15 (68); or a rating of perceived exertion (RPE) ≥17 (6 to 20-point Borg Scale (16)). Electrocardiographic activity (Quinton Q-4000, Quinton, Bothell, WA) and blood pressure (Baumanometer, W.A. Baum Co. Inc., Copiague,

NY) were monitored throughout the test. The test was terminated as outlined by the American College of Sports Medicine's Guidelines for Exercise Testing (1), and a physician was present during testing.

Prolonged Exercise Trial

Each participant reported to the laboratory at 0700 hours, at least 8-hours post-prandial (subjects were free to consume water) and having refrained from all moderate or higher intensity activity and alcohol consumption during the previous 48 hours. Previous studies investigating the 24-hour GH secretion pattern have shown the basal concentration to be lowest in the morning (~0800 h) (60, 81, 128), when GH concentrations are declining following the overnight peak stimulated by phase III-IV sleep, and when cortisol levels tend to be elevated. On the morning of the test, participants were asked to refrain from all activity above what was necessary to reach the laboratory. A detailed outline of the prolonged exercise trial procedures is outlined in Appendix D. The participant was asked to rest, awake, in a reclined position for a period of 30 minutes during which time an indwelling catheter was inserted into a forearm vein for the collection of blood samples. Patency was maintained with a saline solution of physiologic concentration. Following the 30-minute rest period, the test trial was initiated. Expired gases were collected continuously over the duration of each trial for the determination of resting metabolic rate, substrate utilization, and intensity level during exercise, using the TrueMax 2400 Metabolic Measurement System (Consentius Technologies, Sandy,

UT). The system uses a Hans Rudolph 3813 heated pneumotach (Hans-Rudolph Inc., Kansas City, MO) for the measurement of ventilation, a paramagnetic oxygen analyzer and an infrared, single beam, single wave-length carbon dioxide analyzer for the measurement of gas fractions. This system has been previously shown to be valid (6). Expired gases were collected through a large 2-way non-rebreathing valve (Hans-Rudolph, Inc., Kansas City, MO) attached to a sealed facemask (Hans-Rudolph, Inc., Kansas City, MO) at rest and through a mouthpiece with an accompanying nose clip during exercise. The participant remained in the reclined position during the 30-minute pre-exercise period (P). The participant was then asked to walk on a treadmill at a heart-rate <110 beats per minute for 5 minutes as a warm-up period before the initiation of the experimental exercise intensity. Following the warm-up interval, the speed and grade of the treadmill was increased to elicit an intensity equal to 70% of the previously determined $\dot{V}O_{2max}$. Speed and grade were modified throughout the 30-minute exercise period (E) to maintain the prescribed intensity. Speed did not exceed 3.5 mph; therefore, all exercise was walking. Previous investigators have shown that obese women (mean BMI = ~33) were able to maintain a relative exercise intensity equal to 70% $\dot{V}O_{2peak}$ for 30 minutes of continuous treadmill work (77) and 60-65% $\dot{V}O_{2peak}$ for 1-hour of cycle ergometry (42), and that postmenopausal women were able to maintain a relative intensity of 80% $\dot{V}O_{2peak}$ for 30 minutes of treadmill exercise (84). Following exercise, the participant was asked to return to the reclined position for 90 minutes of recovery (R). Several studies in humans have shown that maximal serum GH

concentrations are attained at or near the end of a 30-minute aerobic exercise bout and that GH levels returned to baseline within 90 minutes of the cessation of exercise (89, 112, 123, 125, 146). A 5-mL blood sample was collected 30 and 10 minutes before exercise (P30 and P10, respectively); at 10 and 20 minutes of exercise (E10 and E20, respectively); at the cessation of exercise (R0); and at 10, 30, 60, and 90 minutes of recovery (R10, R30, R60, and R90, respectively) for determination of GH, insulin, glucose, lactate, glycerol, E_2, and hematocrit concentrations. Growth hormone, insulin, and glucose levels were used to establish a response curve and determine the integrated area under the curve (AUC). Estradiol was measured at rest (P10) in order to establish baseline values. Hematocrit values were measured to assess the hemoconcentration effect of serial blood sampling and exercise (40), although adjustments to the plasma volume were not made. Lactate values were used to monitor exercise intensity.

Control Trial

All trials were separated by at least one week. Procedures for the control trial were the same as for the prolonged exercise trial except that the subject was asked to remain at rest in the reclined position during the entire period. Each trial began at the same time to insure that blood samples were collected at the same diurnal points of each trial. The trials were counterbalanced with ten of the total subjects performing the prolonged exercise trial first (5 Ob and 5 nOb) and nine

performing the control trial first (4 Ob and 5 nOb) in order to control for a possible ordering effect.

Blood Analysis

The 5-ml blood sample was divided between two microcapillary tubes for the determination of hematocrit level, a single microcentrifuge blood collection tube with an antiglycolytic (sodium fluoride) and a cell-lysing (cetrimonium bromide) agent for determination of glucose and lactate levels, and a single blank serum vacutainer tube. The serum tube was allowed to clot (30-60 minutes) before it was centrifuged for 20 minutes. The serum aliquots were separated into four cryule vials (Wheaton, Millville, NJ) and frozen at -50° C for later analysis of GH, insulin, E_2, and glycerol. All samples for an individual subject were run in duplicate and within the same assay.

The reliability of each pipettor used during a assay procedure was assessed. The amount of fluid dispensed by the pipettor was weighed after each of 20 repeated samples of deionized water. When appropriate, the pipette tip was changed between each measurement. The co-efficient of variation (%CV) was 0.25% for the 1 mL repeater; 0.26 and 0.41% for two 100 µL pipettors; and 3.1% for a 10 µL pipettor.

Assay Procedures

Serum GH was assessed by double antibody ^{125}I radioimmunoassay (RIA) (ICN Pharmaceuticals, Inc., Costa Mesa, CA). The principle of the double antibody

method is that the labeled and unlabeled hormones compete for a binding site on a specific antibody. Following an incubation period, a second antibody is added which reacts with the hormone-antibody complex forming a precipitant pellet. The amount of hormone in the sample is inversely proportional to the amount of radioactivity measured in a gamma counter. The GH RIA is reported to have a percent cross-reaction with hGH of 100.00% and a sensitivity of 0.31 $ng \cdot mL^{-1}$. The intra-assay %CV was 9.9, 5.5, and 3.9% for low, medium, and high GH standards, respectively and the inter-assay %CV was 11.9, 8.0, and 5.3% for low, medium, and high standards, respectively. The specific procedures of the GH assay are explained in Appendix E.

A ^{125}I RIA using the coated tube method was used for the assessment of serum insulin (ICN Pharmaceuticals, Inc., Costa Mesa, CA) and total serum estradiol (Diagnostics Products Corp., Los Angeles, CA) concentrations. The principle for this method is that the hormone binds with an antibody covalently bound to the inner surface of a polypropylene tube, forming an antibody-bound hormone complex. Following an incubation period, the unbound hormone is decanted and the remaining antibody-bound hormone is counted in a gamma counter. The insulin RIA is reported to have a percent cross-reaction with human insulin of 100.00% and sensitivity of 1.5 $\mu IU \cdot mL^{-1}$. The intra-assay %CV was 10.9 and 6.4% for low and high standards, respectively and the inter-assay %CV was 12.3 and 7.9% for low and high standards, respectively. A complete description of the insulin assay procedure is provided in Appendix F. The E_2 immunoassay is

reported to have a detection limit of 8 $pg \cdot mL^{-1}$ and the antiserum has a non-detectable percent cross-reaction with estradiol-17β. The intra-assay %CV was 5.5, 2.1, and 3.7% for low, medium, and high standards, respectively. All E_2 samples were run within the same test, therefore the inter-assay %CV was not determined. The specific procedures of the E_2 assay are provided in Appendix G.

The radioactivity of the RIA's was assessed for a counting duration of 1-minute using a LBK-Wallac 1272 Clinigamma Gamma Counter (LBK-Wallac, Wallac Oy, Finland). The gamma counter was calibrated for ^{125}I and corrected for background radiation prior to the counting of assays. Raw counts for the 1-minute count period were corrected for the actual time of counting to provide corrected counts pre minute (CPM). The concentration of the samples was determined using the spline function data reduction method (111). A series of calibration standards was used to establish a standard curve for each assay. Each sample was measured in duplicate and the algorithm developed from the spline function was used to calculate the concentration of each tube. The mean of the duplicate tests was reported as the serum concentration for the sample.

Glycerol concentrations in the serum were determined by a modified procedure of the enzymatic method originally described by McGowan (97) and were measured at 540 nm (Sigma Diagnostics, St. Louis, MO). In this assay glycerol is phosphorylated by adenosine triphosphate (ATP) forming glycerol-1-phosphate (G-1-P) and adenosine-5'-diphosphate (ADP) in the reaction catalyzed by glycerol kinase (GK). G-1-P is then oxidized by glycerol phosphate oxidase (GPO)

to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). A quinoneimine dye is produced by the peroxidase catalyzed coupling of 4-aminoantipyrine (4-AAP) and sodium n-ethyl-N-(3-sulfopropyl)m-anisidine (ESPA) with H_2O_2 which shows an absorbance maximum at 540 nm. The increase in absorbance at 540 nm is directly proportional to the glycerol concentration of the sample. Absorbance was measured using the Spectronic Genesys 8 (Spectronic Instruments, Rochester, NY). The glycerol assay had a measured intra-assay %CV of 2.5 and 2.0% for low and high standards, respectively and an inter-assay %CV of 4.9 and 2.3% for low and high standards, respectively. The sensitivity of the glycerol assay is reported to be 0.0013 absorbance change at 1 $mg \cdot dL^{-1}$. Serum glycerol concentrations were reported as the mean of the duplicate test for each sample. Appendix H contains the specific procedures of the glycerol assay.

Whole blood glucose and lactate concentrations were measured using a YSI-2300 STAT Plus automated blood analysis machine (Yellow Springs Instrumentation Company, Inc. Yellow Springs, OH). This system utilizes a platinum anode probe covered by a three layer immobilized enzyme membrane. When the whole blood sample is exposed to the substrate specific membrane, it is rapidly oxidized producing H_2O_2, which is further oxidized producing electrons. A dynamic equilibrium is achieved when the rate of H_2O_2 production and the rate at which H_2O_2 leaves the immobilized enzyme layer are constant and is indicated by a steady state response. The electron flow is linearly proportional to the steady state H_2O_2 concentration and, therefore, to the concentration of the substrate. The system

is designed to measure whole blood glucose and L-lactate with glucose linearity to 50.0 mmol·L^{-1} and L-lactate linearity to 30.0 mmol·L^{-1}. The glucose values measured by the YSI analyzer had an intra-assay %CV of 5.5 and 5.5% for low (11.1 mmol·L^{-1}) and high standards (27.8 mmol·L^{-1}), respectively; and an inter-assay %CV of 5.9 and 5.7% for low and high standards, respectively. The L-lactate values measured by the YSI analyzer had an intra-assay %CV of 1.6 and 1.7% for low (5.0 mmol·L^{-1}) and high standards (15.0 mmol·L^{-1}), respectively; and an inter-assay %CV of 1.4 and 1.6% for low and high standards, respectively.

Statistical Analysis

Statistical analysis was completed using the statistics software package SPSS (SPSS Inc., Chicago, IL). Significance was set at an alpha level of 0.05. Repeated measures analysis of variance and Student's *t*-tests were used for data comparisons. Unpaired *t*-tests were used for descriptive comparisons between groups and paired *t*-tests were used to compare descriptive data between trials.

Concentrations

The blood variable concentration was determined for all time points of each trial. A group x trial x time repeated measures ANOVA was used to examine differences in the GH, insulin, glucose, and glycerol concentrations during the prolonged exercise trial and control trial. Areas of significance were further tested using estimated marginal means and Student's *t*-tests with Bonferroni's adjustments.

Baseline concentrations were established as the mean concentration of the pre-exercise period (P30-P10) for each trial, and resting concentrations were calculated as the mean of all time points during the control trial. The peak GH (GH_{Peak}) concentration of the exercise trial was defined as the highest GH value observed at any time point. The GH_{Peak} concentration of the control trial was the GH concentration at the time point corresponding to the GH_{Peak} concentration of the prolonged exercise trial. For example, a GH_{Peak} concentration during the prolonged exercise trial at R0 would have the GH concentration at the R0 time point of the control trial reported as the peak. Comparisons for baseline, resting and peak concentrations were made using Student's *t*-tests.

Area-Under-the-Curve

Area-under-the-curve (AUC) was calculated in three ways using the trapezoidal method. a) Total Area-Under-the-Curve (AUC_{Total}) was calculated as the sum of the area for all time points of each trial. b) Baseline adjusted AUC ($AUC_{Baseline}$) was calculated as the sum of the area adjusted for baseline concentrations over the exercise and recovery time periods of each trial. c) Corrected AUC ($AUC_{Corrected}$) was calculated as the sum of the area corrected for the control trial from the E10 to R30 time points. Comparisons of AUC were made between groups (nOb vs. Ob) and between trials using Student's *t*-tests.

Correlation Analysis

Pearson product moment correlation was used to determine whether resting GH, GH_{Peak}, GH $AUC_{Baseline}$, and GH $AUC_{Corrected}$ were related to resting, baseline, and $AUC_{Baseline}$ values for insulin and glucose and baseline E_2, years since menopause, body weight, BMI, percentage of body fat, and waist circumference.

CHAPTER IV

RESULTS

Metabolic Measurement Systems

Pilot investigations using mixed gases of known concentrations, as determined by the Scholander method (119), tested inter- and intra-instrument reliability for the two metabolic measurement systems used in this study. The Quinton Q-Plex I Metabolic Measurement System (Quinton, Bothell, WA) was used for all maximal exertion tests, while all prolonged exercise and control trials were completed using the TrueMax 2400 Metabolic Measurement System (Consentius Technologies, Sandy, UT). Inter-instrument reliability scores showed no significant difference between the two systems in the mean values of F_EO_2 ($P>0.99$), F_ECO_2 ($P>0.99$), $\dot{V}O_2$ ($P>0.96$), and $\dot{V}CO_2$ ($P>0.97$) over 20 measurements. Additionally, inter- and intra-instrument reliability was further evaluated during serial sampling of known gases. No significant differences ($P>0.96$) in the fractional gas concentrations were found between six repeated measurements of three gas mixtures within and between the two systems.

Subject Characteristics

Table 2 shows the characteristics of the nOb, Ob, and combined nOb and Ob groups. Of the 19 total study participants, 17 were Caucasian, 1 was African American, and 1 was Hispanic. Overall, there was no significant difference between the nOb and Ob groups for age ($P=0.90$), height ($P=0.49$), absolute $\dot{V}O_{2max}$

Table 2. Subject characteristics (mean ± SE).

Characteristic	Total (N=19)	Non-Obese (N=10)		Obese (N=9)
Age (y)	56.3 ± 0.9	56.2 ± 1.4		56.4 ± 1.2
Height (cm)	164.3 ± 1.4	165.2 ± 2.0		163.2 ± 2.1
Weight (kg)	76.5 ± 4.2	64.0 ± 3.8	‡	90.4 ± 4.6
BMI ($kg \cdot m^{-2}$)	28.3 ± 1.5	23.3 ± 1.0	‡	33.9 ± 1.5
Waist:Hip Ratio	0.8 ± 0.0	0.8 ± 0.0	†	0.9 ± 0.0
Body Fat (%)	43.4 ± 2.3	37.4 ± 3.1	†	50.2 ± 1.2
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	1.6 ± 0.1	1.5 ± 0.1		1.8 ± 0.1
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	22.1 ± 1.1	23.6 ± 1.5		20.5 ± 1.4
$\dot{V}O_{2max}$ ($mL \cdot kg\ FFM^{-1} \cdot min^{-1}$)	39.1 ± 2.0	38.6 ± 2.0		39.6 ± 2.9
%$\dot{V}O_{2max}$ during exercise	69.9 ± 0.1	69.3 ± 1.5		70.5 ± 1.1
Maximal Heart Rate	165.9 ± 2.7	167.2 ± 3.5		164.4 ± 4.2
% HR_{max} during exercise	78.2 ± 1.4	77.2 ± 1.7		79.3 ± 2.4
Years since menopause	9.6 ± 1.1	10.4 ± 1.7		8.7 ± 1.5

Significantly different from the obese group, †P<0.01; ‡P<0.001.

(P=0.11), relative $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$, P=0.15; $mL \cdot kg\ FFM^{-1} \cdot min^{-1}$, P=0.76), %$\dot{V}O_{2max}$ maintained during exercise (P=0.55), and years since menopause (P=0.47). The Ob group had a significantly higher body weight (P<0.001), BMI (P<0.001), waist-to-hip ratio (P=0.005), and %BF (P=0.002) compared to the nOb group.

Resting Concentration

Resting values for measured blood parameters, determined as the mean value of all time points during the control trial, are shown in Table 3 for the nOb, Ob, and combined nOb and Ob groups. Serum GH concentrations at rest were not significantly different between the nOb and the Ob group (P=0.56). The Ob group had significantly higher serum insulin (P=0.009), blood glucose (P=0.01), blood

Table 3. Mean (± SE) resting blood concentrations.

Resting	Total (N=19)	Non-Obese (N=10)		Obese (N=9)
Growth Hormone ($ng \cdot mL^{-1}$)	2.9 ± 0.2	2.8 ± 0.2		3.0 ± 0.2
Insulin ($\mu IU \cdot mL^{-1}$)	17.3 ± 1.9	13.2 ± 1.6	†	22.4 ± 2.9
Glucose ($mmol \cdot L^{-1}$)	4.7 ± 0.1	4.4 ± 0.1	*	4.9 ± 0.1
Estradiol ($pg \cdot mL^{-1}$)	1.1 ± 0.6	1.9 ± 1.0		0.2 ± 0.2
L-Lactate ($mmol \cdot L^{-1}$)	0.92 ± 0.1	0.78 ± 0.1	*	1.07 ± 0.1
Glycerol ($mg \cdot dL^{-1}$)	6.0 ± 1.0	4.1 ± 1.0	*	8.1 ± 1.6
Hematocrit (%)	39.6 ± 0.5	40.2 ± 0.6		39.1 ± 0.7

Significantly different from the obese group, *P<0.05; †P<0.01. Resting values are the mean of all time points within the control trial.

lactate (P=0.02), and serum glycerol (P=0.04) concentrations compared to the nOb group. Serum E_2 levels, measured at the P10 time point were not significantly different between the nOb and the Ob group (P=0.13). Considering the postmenopausal status of these women, very low E_2 concentrations were expected. In fact, the expected normal range for postmenopausal women is 0-14 $pg \cdot mL^{-1}$ (41). In the current investigation, the baseline E_2 concentrations were 1.9 ± 1.0 $pg \cdot mL^{-1}$ for the nOb and 0.2 ± 0.2 $pg \cdot mL^{-1}$ for the Ob groups, verifying low E_2 concentrations and the postmenopausal status of these women. Resting hematocrit levels for both groups were within the normal range for healthy adults (35-48%) and were not significantly different between groups (P=0.26).

Dietary Analysis

The analysis of the self-reported dietary intake for the three days immediately preceding the prolonged exercise trial and control trial revealed no significant difference in total caloric intake, percent of calories form protein, percent of calories from carbohydrate, and percent of calories from fat between the two treatment trials and between the two groups ($P>0.05$). Table 4 shows the mean (± SE) self-reported caloric intake values for the Ob and nOb groups and the two groups combined.

Caloric Expenditure

Evaluation of the caloric expenditure data (Table 5) found significant between-trial differences for total kcal, exercise kcal, exercise kcal from CHO, exercise kcal from fat, and exercise RQ ($P<0.001$). Between-group differences were seen over the prolonged exercise trial. The Ob group expended a significantly greater total kcals ($P=0.05$) and kcals during exercise ($P=0.01$), compared to the nOb group. There were no significant group differences in kcal from CHO, kcal from fat, or RQ at any point during either trial.

Table 4. Self-reported total caloric intake and percent of kilocalories from protein, carbohydrate, and fat (mean ± SE).

Caloric Intake	Total (N=19)	Non-Obese (N=10)	Obese (N=9)
Total Caloric Intake (kcal)			
Prolonged Exercise Trial	1949.5 ± 103.1	1881.1 ± 112.1	2025.4 ± 182.8
Control Trial	1705.0 ± 79.5	1668.8 ± 92.4	1745.6 ± 137.6
Protein (%)			
Prolonged Exercise Trial	15.1 ± 0.9	15.5 ± 1.3	14.6 ± 1.3
Control Trial	15.5 ± 0.8	16.6 ± 1.3	14.2 ± 0.9
Carbohydrate (%)			
Prolonged Exercise Trial	50.0 ± 2.0	49.7 ± 3.3	50.2 ± 2.2
Control Trial	52.3 ± 1.8	53.1 ± 2.8	51.4 ± 2.3
Fat (%)			
Prolonged Exercise Trial	33.7 ± 1.6	33.2 ± 2.5	34.3 ± 1.9
Control Trial	30.9 ± 1.4	29.4 ± 1.9	32.6 ± 1.8

Table 5. Caloric expenditure, substrate utilization and respiratory quotient (mean ± SE).

	Prolonged Exercise Trial		Control Trial	
Energy Expenditure	Non-Obese (N=10)	Obese (N=9)	Non-Obese (N=10)	Obese (N=9)
Total Caloric Expenditure	268.3 ± 8.1	* 303.0 ± 14.4	132.6 ± 5.8	‡ 136.9 ± 8.7
Pre-Exercise Period				
Caloric Expenditure	25.9 ± 1.5	25.9 ± 3.3	24.2 ± 0.8	24.8 ± 1.7
Carbohydrate (kcal)	11.6 ± 1.1	13.1 ± 1.8	10.4 ± 0.8	11.0 ± 1.6
Fat (kcal)	14.3 ± 2.2	12.8 ± 2.5	13.8 ± 1.3	13.8 ± 1.2
RQ	0.84 ± 0.02	0.85 ± 0.02	0.83 ± 0.01	0.83 ± 0.01
Exercise Period				
Caloric Expenditure	150.8 ± 6.6	† 184.7 ± 10.0	28.9 ± 0.9	‡ 29.5 ± 2.0
Carbohydrate (kcal)	100.2 ± 10.4	118.8 ± 17.5	13.4 ± 0.9	‡ 14.5 ± 2.3
Fat (kcal)	50.6 ± 8.5	65.9 ± 14.3	15.6 ± 1.2	‡ 15.0 ± 1.6
RQ	0.90 ± 0.02	0.92 ± 0.02	0.84 ± 0.01	‡ 0.84 ± 0.02
Recovery Period				
Caloric Expenditure	86.2 ± 3.5	92.5 ± 6.5	79.4 ± 4.7	82.6 ± 5.2
Carbohydrate (kcal)	42.3 ± 4.0	44.0 ± 7.3	35.0 ± 2.1	38.3 ± 4.4
Fat (kcal)	43.9 ± 4.0	48.4 ± 5.4	44.4 ± 4.0	44.2 ± 3.3
RQ	0.84 ± 0.01	0.84 ± 0.02	0.83 ± 0.01	0.83 ± 0.01

Significantly different from the obese group, *P<0.05; †P<0.01; Significantly different from the prolonged exercise trial for both groups, ‡P<0.001.

Metabolic Response to Exercise Treatment

The mean oxygen consumption ($L \cdot min^{-1}$) was not significantly different during the pre-exercise period or recovery period between the prolonged exercise trial and control trial and the Ob and nOb groups were not significantly different at any time point within a given trial. $\dot{V}O_2$ during the exercise period of the prolonged exercise trial was significantly higher than the control trial for both groups (Table 6). Peak blood lactate values during the prolonged exercise trial were significantly higher than during the control trial for the Ob (mean ± SE, 2.6 ± 0.4 and 1.2 ± 0.1 $mmol \cdot L^{-1}$, respectively) (P=0.01) and the nOb groups (mean ± SE, 1.8 ± 0.2 and 1.0 ± 0.1 $mmol \cdot L^{-1}$, respectively) (P<0.001). Hematocrit values during the prolonged exercise trial did not change by more than 3.5% between consecutive time points for any individual with a mean shift between all time points of 0.04% and a mean difference of 0.12% between the initial and final measurement of the trial.

Table 6. Mean (± SE) $\dot{V}O_2$ during prolonged exercise trial and control trial.

$\dot{V}O_2$ ($L \cdot min^{-1}$)	Total (N=19)	Non-Obese (N=10)	Obese (N=9)
Pre-Exercise Period			
Prolonged Exercise Trial *	0.20 ± 0.01	0.21 ± 0.01	0.19 ± 0.01
Control Trial	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
Exercise Period			
Prolonged Exercise Trial †	1.13 ± 0.05	1.04 ± 0.05	1.24 ± 0.09
Control Trial	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
Recovery Period			
Prolonged Exercise Trial *	0.21 ± 0.01	0.20 ± 0.01	0.22 ± 0.02
Control Trial	0.19 ± 0.03	0.19 ± 0.01	0.19 ± 0.01

*Significantly difference from Exercise Period (P<0.001); †Significantly different from Control Trial (P<0.001).

Growth Hormone

Baseline values were defined as the mean between the P30 and P10 time points of each trial. There was no significant difference in baseline GH concentrations between the prolonged exercise trial and the control trial within each group (P=0.71 and P=0.36 for the Ob and nOb groups, respectively), and there were no significant between-group differences (P=0.16 for the prolonged exercise trial and P=0.44 for the control trial). The GH_{Peak} concentration of the exercise trial was defined as the highest GH value seen at any time point. The GH_{Peak} concentration of the control trial was the GH concentration at the time point corresponding to the GH_{Peak} concentration of the prolonged exercise trial. Peak GH concentrations were also established for GH concentrations corrected for the control trial ($Peak_{Corrected}$). There was no significant difference between groups for $Peak_{Corrected}$ GH concentrations (P=0.12) and GH_{Peak} concentrations during the prolonged exercise trial (P=0.30) and the control trial (P=0.46). However, GH_{Peak} concentrations were significantly higher during the prolonged exercise trial compared to the control trial for both Ob and nOb groups (P<0.001). During the prolonged exercise trial, the mean GH_{Peak} concentration for each group was seen at the R0 time point. Table 7 shows the baseline, peak, $Peak_{corrected}$, and R0 values for GH (mean ± SE). The repeated measures ANOVA revealed significant main effects for trial (P<0.001), and time (P<0.001) and a trial x time interactions (P<0.001). No significant effect was seen for group (P=0.769). To further examine the trial x time interaction, estimated marginal means and *t*-tests with Bonferroni adjustments were calculated.

Table 7. Growth hormone concentrations (mean ± SE)

Growth Hormone ($ng \cdot mL^{-1}$)	Non-Obese (N=10)	Obese (N=9)
Baseline		
Prolonged Exercise Trial	3.1 ± 0.2	3.9 ± 0.6
Control Trial	3.6 ± 0.6	4.2 ± 0.6
Peak		
Prolonged Exercise Trial ‡	12.3 ± 2.1	9.3 ± 1.9
Control Trial	4.0 ± 0.6	4.7 ± 0.8
$Peak_{Corrected}$	10.0 ± 2.0	5.7 ± 2.2
R0 Time Point		
Prolonged Exercise Trial ‡	11.5 ± 2.2	8.3 ± 1.9
Control Trial	2.2 ± 0.2	2.5 ± 0.1

‡Significantly different from the control trial, P<0.001.

Growth hormone concentrations during the pre-exercise period were similar between the prolonged exercise trial and the control trial for the Ob and nOb groups (P=0.168). The concentrations of GH at the E20, R0, R10, and R30 time points were significantly higher during the prolonged exercise trial compared to the control trial in both groups (P<0.001). Despite a lack of statistically significant group differences, the GH concentrations of the Ob group increased to a lesser extent during exercise compared to the nOb group (mean difference at the R0 time point, 3.2 $ng \cdot mL \cdot min^{-1}$; P>0.25). Figure 1 shows the mean (± SE) GH concentration for the nOb and Ob groups during the prolonged exercise trial and the control trial and individual GH concentration response curves are shown in Appendix I.

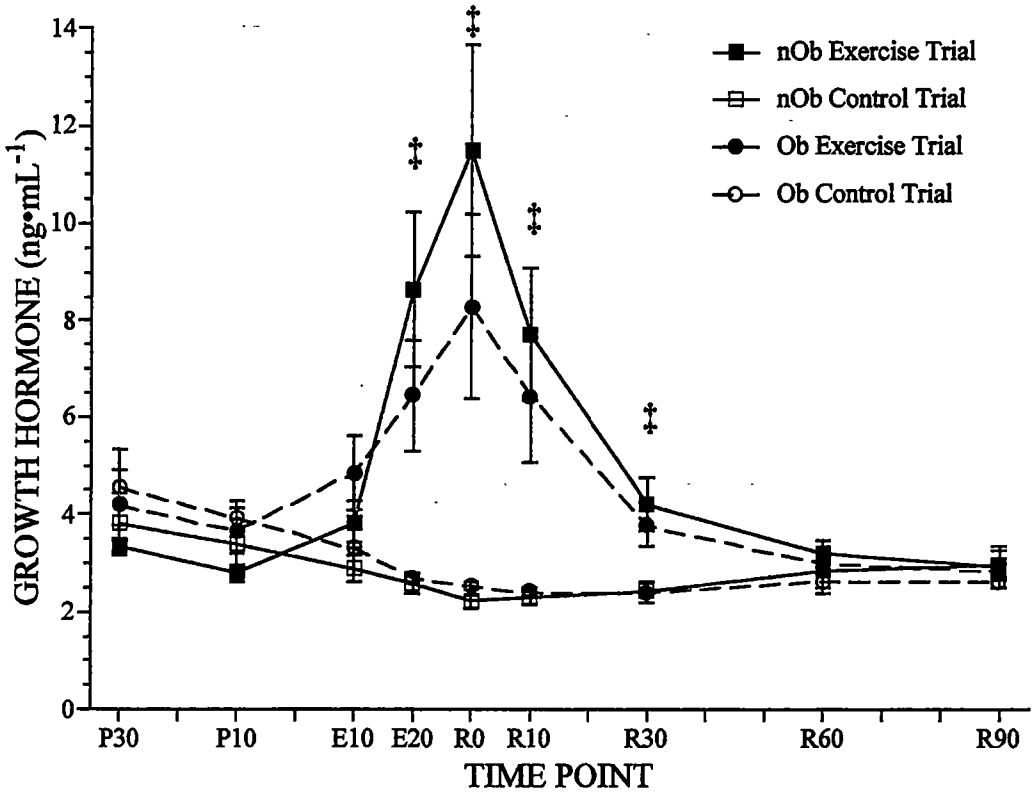

Figure 1. Growth hormone concentration ($ng \cdot mL^{-1}$) of the prolonged exercise and control trials for the obese (Ob) and non-obese (nOb) groups (mean ± SE). Significantly different from the control trial, ‡P<0.001.

Total AUC, baseline adjusted AUC for the control and prolonged exercise trials and $AUC_{corrected}$ were determined for GH concentrations (Table 8). The results of the t-test showed that the $AUC_{Baseline}$ for GH was significantly higher during the prolonged exercise trial compared to the control trial for both the Ob and nOb groups (P=0.01 and P=0.007, respectively) (Figure 2). It was found that the GH $AUC_{Baseline}$ was not significantly different between the Ob group and the nOb group during the prolonged exercise trial (P=0.16) or for the control trial (P=0.39). There was also no significant difference between the Ob and nOb groups for GH $AUC_{corrected}$ (P=0.31).

Table 8. Growth hormone area-under-the-curve (mean ± SE).

Growth Hormone AUC ($min \cdot ng \cdot mL^{-1}$)		Total (N=19)	Non-Obese (N=10)	Obese (N=9)
AUC_{Total}				
Prolonged Exercise Trial	†	684.5 ± 53.6	707.6 ± 82.2	658.8 ± 71.1
Control Trial		429.7 ± 21.2	421.6 ± 37.7	438.7 ± 18.4
$AUC_{Baseline}$				
Prolonged Exercise Trial	†	198.3 ± 59.9	278.8 ± 73.7	108.9 ± 91.3
Control Trial		-116.7 ± 40.1	-82.7 ± 48.8	-154.5 ± 65.8
$AUC_{Corrected}$		210.2 ± 42.8	256.8 ± 54.4	176.7 ± 52.5

†Significantly different from the control trial, P<0.01.

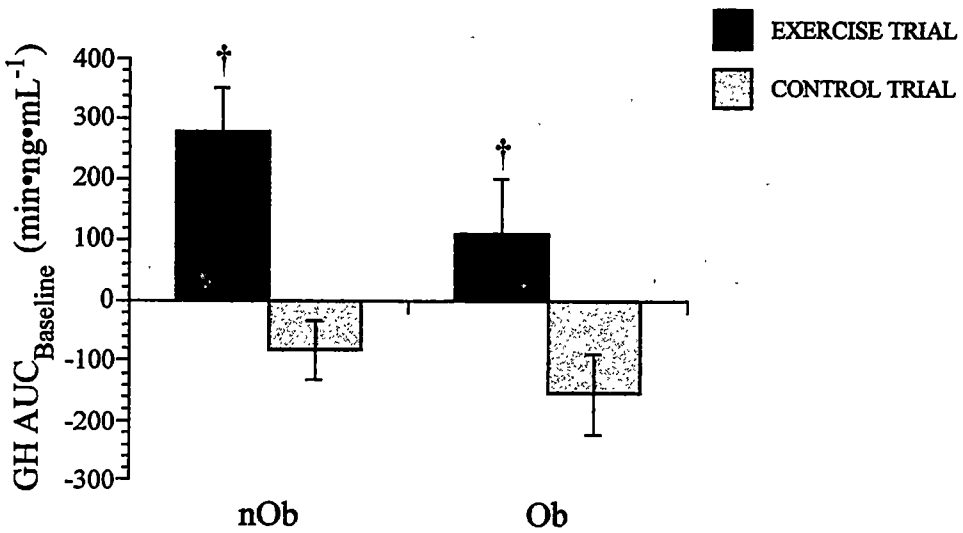

Figure 2. Growth hormone area-under-the-curve ($min \bullet ng \bullet mL^{-1}$) corrected for baseline concentration of the prolonged exercise and control trials for the obese (Ob) and non-obese (nOb) groups (mean ± SE). Significantly different from the control trial, †P<0.01.

Insulin

There was no significant difference in baseline insulin levels between the prolonged exercise trial and the control trial within each group (P=0.42 and P=0.96 for the Ob and nOb groups, respectively), although the Ob groups had significantly higher baseline insulin concentrations for the prolonged exercise trial (P=0.03) and for the control trial (P=0.005) compared to the nOb group. Table 9 shows the baseline, R0, and AUC values for insulin (mean ± SE). The repeated measures ANOVA showed that there was a significant trial x time interaction (P<0.001) and a significant group effect (P=0.02), but no significant trial x time x group interaction (P=0.06). Further analysis revealed that the insulin concentration of the Ob group was significantly higher than the nOb group during the prolonged exercise trial (P<0.001) and the control trial (P<0.001) but there was no significant difference between trials (i.e., no main effect for trial) for the Ob group (P=0.80) or the nOb group (P=0.17). Figure 3 shows the mean (± SE) insulin concentration for the nOb and Ob groups during the prolonged exercise trial and the control trial. The trial x time interaction is generated by the steady decline of insulin concentrations from the P30 to the R0 time points during the prolonged exercise trial followed by an abrupt rise in insulin concentration at the R10 time point (Figure 2). For insulin $AUC_{Baseline}$, there was no significant effect for group (P=0.91) or trial (P=0.11).

Table 9. Insulin concentrations (mean ± SE).

Insulin	Non-Obese (N=10)		Obese (N=9)
Baseline ($\mu IU \cdot mL^{-1}$)			
Prolonged Exercise Trial	13.7 ± 1.7	*	20.8 ± 2.6
Control Trial	13.6 ± 1.6	†	24.3 ± 3.2
R0 Time Point ($\mu IU \cdot mL^{-1}$)			
Prolonged Exercise Trial	11.5 ± 1.5		16.7 ± 2.9
Control Trial	11.8 ± 1.3		21.4 ± 3.1
$AUC_{Baseline}$ ($min \cdot \mu IU \cdot mL^{-1}$)			
Prolonged Exercise Trial	180.1 ± 103.5		374.9 ± 237.9
Control Trial	93.5 ± 90.0		-68.0 ± 147.4

Significantly different from the obese group, * P<0.05; † P<0.01.

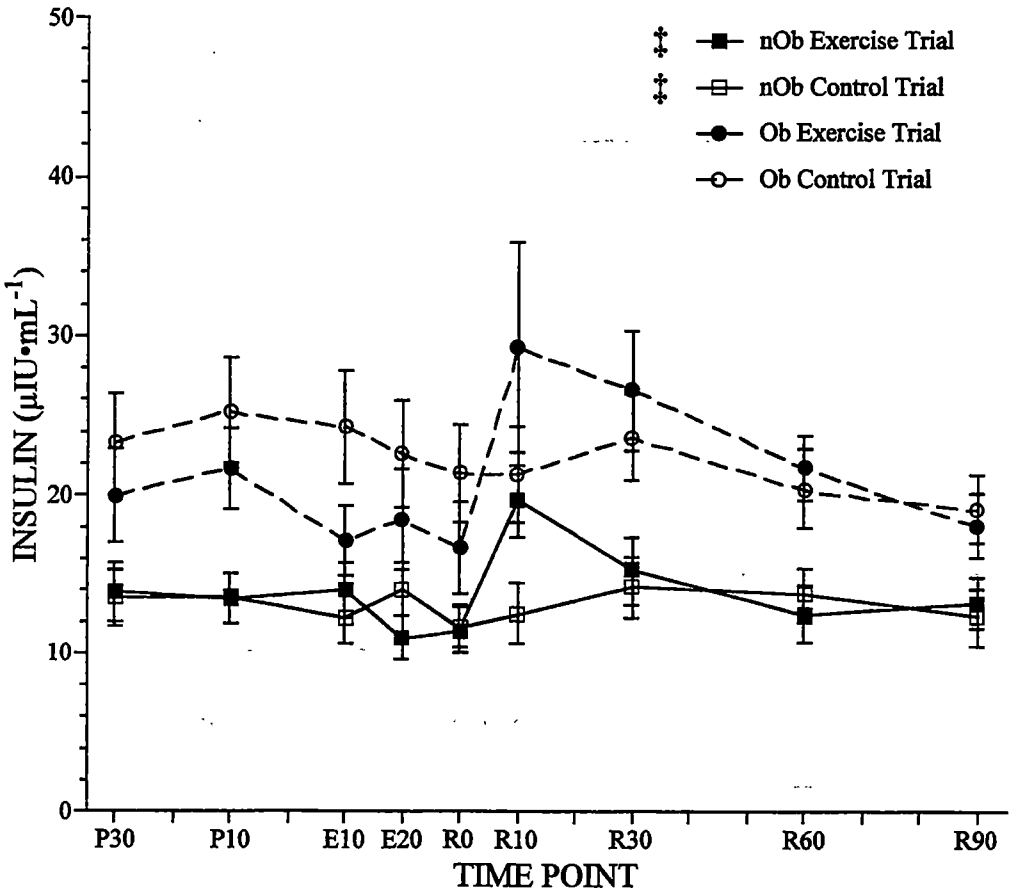

Figure 3. Insulin concentration ($\mu IU \cdot mL^{-1}$) of the prolonged exercise and control trials for the obese (Ob) and non-obese (nOb) groups (mean ± SE). Significantly different from the obese group, ‡P<0.001.

Glucose

There was no significant difference in baseline glucose levels between the prolonged exercise trial and the control trial within each group (P=0.44 and P=0.47 for the Ob and nOb groups, respectively). The Ob group had significantly higher baseline glucose concentrations compared to the nOb group for the prolonged exercise trial (P=0.03) and the control trial (P=0.03). Table 10 shows the values for glucose at baseline, R0, and $AUC_{Baseline}$ (mean ± SE). Figure 4 shows the mean (± SE) glucose concentration for the Ob and nOb groups during the prolonged exercise trial and the control trial. The repeated measures ANOVA showed no significant trial effect on glucose concentrations between the prolonged exercise trial and the control trial (P=0.613). There was a significant interaction for trial x time (P=0.001) and time (P=0.017). For both groups, the mean glucose concentration at the R10 time point was significant higher than the R0 time point (P<0.01). Similar to the baseline glucose values, the Ob group had significantly higher glucose concentrations compared to the nOb group (P=0.012) demonstrating a significant group effect. No significant difference was found for glucose $AUC_{Baseline}$ between the prolonged exercise trial and the control trial for the Ob and nOb groups (P=0.26 and P=0.14, respectively). There was also no significant difference in glucose $AUC_{Baseline}$ between the two groups for the prolonged exercise trial (P=0.78) or the control trial (P=0.80).

Table 10. Glucose concentrations (mean ± SE).

Glucose	Non-Obese (N=10)		Obese (N=9)
Baseline ($mmol \cdot L^{-1}$)			
Prolonged Exercise Trial	4.4 ± 0.1	*	4.8 ± 0.2
Control Trial	4.5 ± 0.1	*	5.0 ± 0.2
R0 Time Point ($mmol \cdot L^{-1}$)			
Prolonged Exercise Trial	4.3 ± 0.1		4.6 ± 0.2
Control Trial	4.5 ± 0.1		4.8 ± 0.2
$AUC_{Baseline}$ ($min \cdot mmol \cdot L^{-1}$)			
Prolonged Exercise Trial	51.4 ± 9.8		47.5 ± 9.4
Control Trial	30.0 ± 9.6		33.2 ± 7.8

*Significantly different from the obese group, P<0.05.

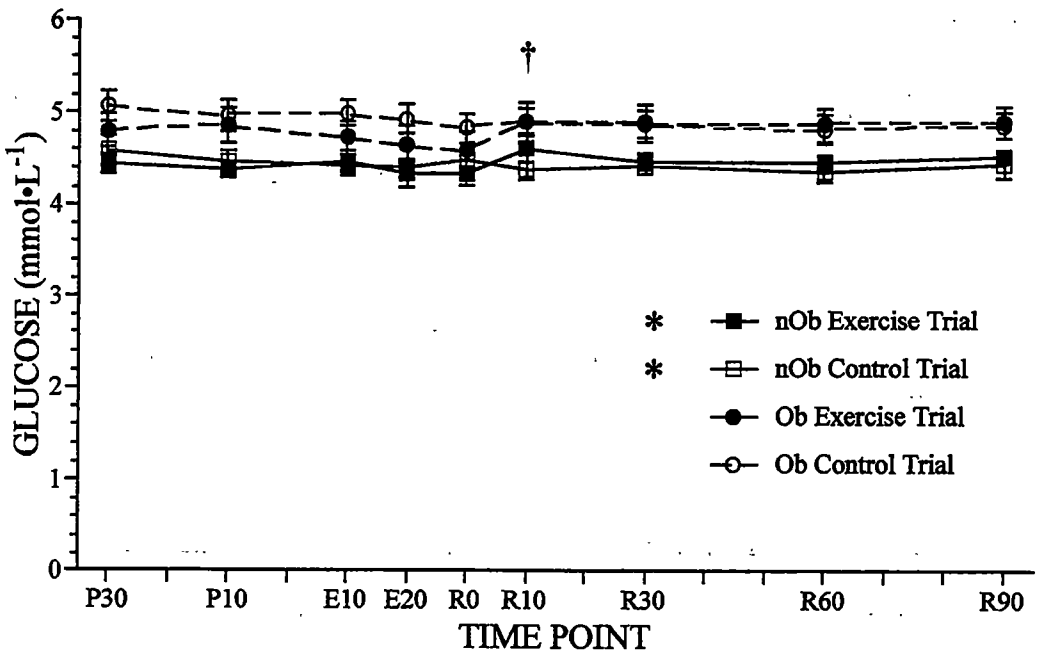

Figure 4. Glucose concentration ($mmol \cdot L^{-1}$) of the prolonged exercise and control trials for the obese (Ob) and non-obese (nOb) groups (mean ± SE). Significantly different from the obese group, *P<0.05. R10 time point of prolonged exercise trial significantly different from the R0 time point for both groups, †P<0.01.

Glycerol

Table 11 shows the glycerol values at baseline, R0, and AUC (mean ± SE). Glycerol concentrations at baseline were not significantly different between the prolonged exercise trial and the control trial for the Ob group (P=0.91) and the nOb group (P=0.60). The baseline glycerol concentrations of the Ob group were significantly higher than the nOb group for the prolonged exercise trial and control trial (P=0.05 and P=0.03, respectively). The repeated measures ANOVA for glycerol revealed a significant trial x time interaction (P<0.001) and significant effect for trial, time, and group (P<0.001, P<0.001, and P=0.04, respectively). Figure 5 shows the mean (± SE) glycerol concentration for the Ob and nOb groups during the prolonged exercise trial and the control trial. Further assessment found that glycerol concentrations were significantly higher during the prolonged exercise trial compared to the control trial (P<0.001) and that the Ob group had a significantly higher glycerol concentration than the nOb group during both trials (P<0.05). During the prolonged exercise trial, glycerol concentration increased significantly with each subsequent time point from P10 to R0 and then decreased significantly from the R0 to R60 time points (P<0.05). No significant change was found for glycerol concentrations between adjacent time points during the control trial (Figure 5). The glycerol concentrations of the Ob group were found to be significantly different between the prolonged exercise trial and the control trial at the E10, E20, R0, R10, and R30 time points (P<0.05) and at the E10, E20, R0, and R10 time points for the nOb group (P<0.05). There were significant differences in

Table 11. Glycerol concentrations (mean ± SE).

Glycerol	Non-Obese (N=10)		Obese (N=9)
Baseline ($mg \cdot dL^{-1}$)			
Prolonged Exercise Trial	2.9 ± 0.7	*	7.1 ± 1.9
Control Trial	3.4 ± 0.8	*	7.4 ± 1.5
R0 Time Point ($mg \cdot dL^{-1}$)			
Prolonged Exercise Trial †	17.7 ± 3.1		20.7 ± 2.3
Control Trial	3.3 ± 0.9	*	7.9 ± 1.5
$AUC_{Baseline}$ ($min \cdot mg \cdot mL^{-1}$)			
Prolonged Exercise Trial †	610.1 ± 86.7		665.1 ± 122.3
Control Trial	164.9 ± 50.5		199.4 ± 68.9

*Significantly different from the obese group (P<0.05); †Significantly different from the control trial (P<0.001).

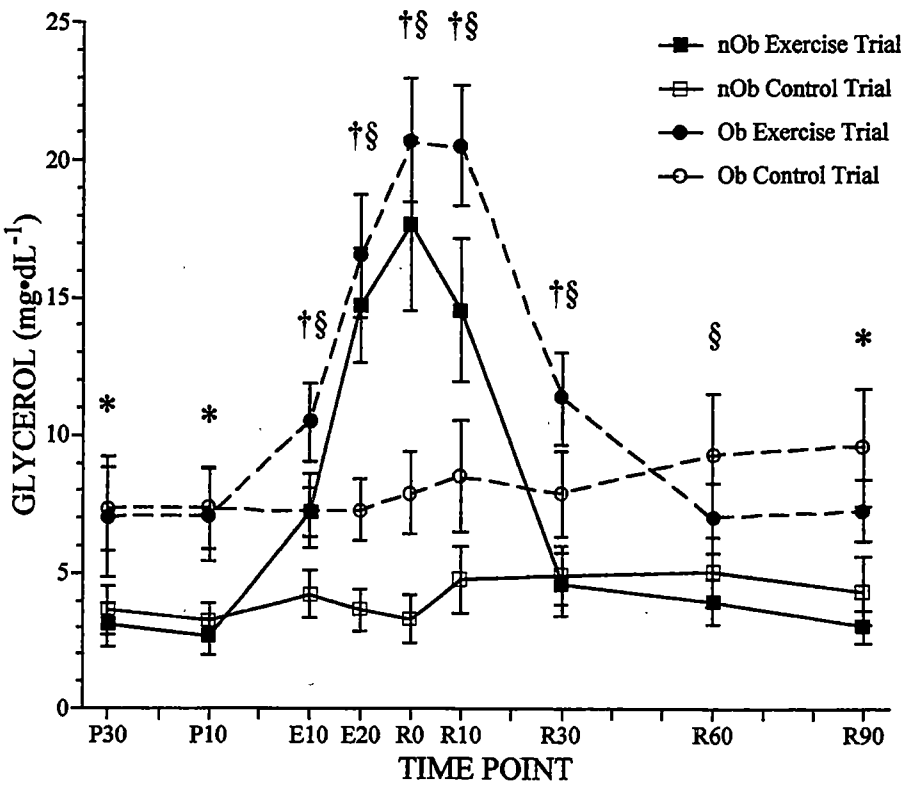

Figure 5. Glycerol concentration ($mg \cdot dL^{-1}$) of the prolonged exercise and control trials for the obese (Ob) and non-obese (nOb) groups (mean ± SE). Significantly different from the obese group, *P<0.05. Prolonged exercise trial was significantly different from the control trial for both groups, †P<0.05. Significantly different from preceding time point during the prolonged exercise trial for both groups, §P<0.05.

the glycerol $AUC_{Baseline}$ between the prolonged exercise trial and control trial for the Ob ($P=0.004$) and the nOb group ($P<0.001$). Glycerol $AUC_{Baseline}$ was not significantly different between the Ob and nOb groups during the prolonged exercise trial ($P=0.71$) or the control trial ($P=0.69$).

Relationship Between Variables

It was hypothesized that GH would be inversely associated with insulin, glucose, and body composition, and positively related to E_2. Pearson product moment correlation analysis of the data found that GH was not significantly related to resting, baseline or $AUC_{Baseline}$ values for insulin or glucose, and E_2 was not found to be related to any GH variable. The number of years since menopause was found to have a significantly negative relationship with resting GH, and weight was inversely related to GH_{Peak}, GH $AUC_{Baseline}$, and GH $AUC_{Corrected}$. Body mass index and waist circumference were also inversely related to GH_{Peak} and GH $AUC_{Corrected}$, and %BF had a significant inverse correlation with GH_{Peak} concentrations (Table 12).

Table 12. Correlation with growth hormone at rest, peak and area-under-the-curve.

Correlate	Resting GH			GH_{Peak}			GH $AUC_{Baseline}$			GH $AUC_{Corrected}$		
	r	P		r	P		r	P		r	P	
Estradiol	-0.02	0.94		-0.20	0.42		0.05	0.86		-0.12	0.64	
Years Since Menopause	-0.46	0.05	*	-0.20	0.42		-0.31	0.20		-0.23	0.34	
Weight	-0.16	0.52		-0.54	0.02	*	-0.47	0.04	*	-0.51	0.02	*
Body Mass Index	-0.10	0.68		-0.53	0.02	*	-0.40	0.09		-0.48	0.04	*
Body Fat	-0.20	0.43		-0.51	0.02	*	-0.33	0.17		-0.41	0.08	
Waist Circumference	-0.20	0.42		-0.54	0.02	*	-0.41	0.08		-0.47	0.04	*
Resting Insulin	-0.13	0.63		-0.19	0.45		-0.27	0.28		-0.14	0.59	
Baseline Insulin	-0.25	0.31		-0.42	0.08		-0.20	0.42		-0.31	0.21	
Insulin AUC	0.05	0.85		-0.23	0.37		-0.34	0.17		-0.28	0.26	
Resting Glucose	0.11	0.65		-0.28	0.24		-0.35	0.14		-0.26	0.29	
Baseline Glucose	0.26	0.30		-0.31	0.19		-0.19	0.44		-0.25	0.30	
Glucose AUC	0.43	0.07		0.19	0.45		-0.03	0.90		0.08	0.75	

Resting GH - resting growth hormone; mean growth hormone concentration of entire control trial. GH_{Peak} - peak growth hormone; highest growth hormone concentration at any time point during the prolonged exercise trial. GH $AUC_{Baseline}$ - growth hormone area under the curve adjusted for baseline growth hormone concentrations. GH $AUC_{Corrected}$ - the area under the curve of the E10 to R30 time points, adjusted for the control trial.

CHAPTER V

DISCUSSION

Major Findings

It is clear from the examination of these data, that there is much variability in the exercise-induced GH response of postmenopausal women. A higher degree of variance is common in endocrinological investigations and the advanced age and altered gonadotropic function associated with menopause may have accentuated the individual variability. The major findings of this study are that an exercise intensity of 70% $\dot{V}O_{2max}$ is, on average, an effective stimulus for the elevation of GH concentrations in postmenopausal women during 30 minutes of treadmill exercise and that this response is correlated with body composition variables.

Comparison of Results to Predicted Outcomes

Based on the available literature, it was originally hypothesized that GH would be related to E_2, insulin, and glucose concentrations, and body composition (Table 12). Variables that were related to GH were the number of years since menopause, body weight, %BF, BMI, and waist circumference. A longer duration (y) since menopause was inversely related to resting GH concentrations ($r=-0.46$, $P=0.049$). A heavier body weight was correlated with lower GH_{peak} ($r=-0.54$, $P=0.02$), lower GH $AUC_{baseline}$ ($r=-0.47$, $P=0.04$), and lower GH $AUC_{Corrected}$ ($r=-0.51$, $P=0.02$). In support of previous studies (3, 25, 32, 53, 77, 134), increased

levels of body fat were negatively related to GH_{Peak} (r=-0.51, P=0.02) and a higher BMI was inversely related to GH_{Peak} (r=-0.53, P=0.02) and GH $AUC_{Corrected}$ (r=-0.48, P=0.04). Waist circumference, suggested as an indicator of visceral adiposity (90), was inversely correlated with GH_{Peak} (r=-0.54, P=0.02) and GH $AUC_{Corrected}$ (r=-0.47, P=0.04). Kanaley et al. (77) reported significantly lower peak GH and 6-hour integrated GH concentration in obese young women compared to lean young women in response to 30 minutes of endurance exercise. The peak GH concentration of postmenopausal women in response to 1-hour of mild walking (1 mph) was reported to be inversely related to percentage of ideal body weight both before and after estrogen supplementation (37). Several studies have shown the GH response to GHRH to be negatively related to body composition (3, 8, 25, 37, 53). These data support the hypothesis that GH is negatively impacted by increased body weight, BMI and %BF.

Some studies have reported a significant relationship between E_2 and GH (20, 65-67, 84, 134). In this study, E_2 was not related to resting GH, GH_{peak}, GH $AUC_{Baseline}$, or GH $AUC_{Corrected}$. The mean resting E_2 concentration of the combined Ob and nOb groups of this study was extremely low (mean ± SE, 1.0 ± 0.6 $pg \cdot mL^{-1}$; range 0-11.4 $pg \cdot mL^{-1}$) with 10 of 19 subjects having E_2 concentrations that were undetectable. Therefore, with these compressed E_2 values, it is not surprising that a correlation between E_2 and GH was not found. Previous studies indicating a significant relationship between E_2 and GH used subject populations of men and women with a wide range of ages (66, 134), eumenorrheic women (67), or

postmenopausal women using supplemental estrogen (84). Several studies have focused on the effects of E_2 treatment and have reported enhanced GH secretion following estrogen therapy (37, 47, 84). Perhaps if these previous studies had isolated the older women or used other groups in which the E_2 range was low, the relationship between E_2 and GH would have been lost. It appears that a disperse range of E_2 values is necessary in order to show the relationship to the GH response.

There was also no apparent relationship between GH and resting, baseline, and $AUC_{Baseline}$ values for insulin or glucose. In spite of significantly elevated insulin and glucose concentrations in the Ob group compared to the nOb group (Table 2; Figures 3 and 4), the large variance in GH and insulin and the minimal change in glucose concentration over the trial period may have masked any possible GH relationships with insulin and glucose. Investigations involving dietary manipulation have found elevated insulin and glucose levels to lower GH concentrations (21, 60, 105, 127).

A second hypothesis stated that obese postmenopausal women would have lower baseline, resting, and peak GH concentrations compared to non-obese postmenopausal women and lower GH AUC. For these study groups, with very different body compositions (mean difference of 10.6 BMI units), the baseline, resting, and peak GH concentrations were similar between groups (P=0.16 P=0.56, and P=0.30, respectively). It remains a possibility that the baseline and resting GH concentrations of both study groups would have been determined low, had a premenopausal control group been added to the study design. Ho et al. (65)

reported that older women had lower resting 24-hour integrated GH concentrations compared to younger women. For the current study, GH_{Peak} concentrations and GH AUC were somewhat lower in the Ob group compared to the nOb group, but, despite being directionally as expected, statistical significance was not achieved.

The GH response curve for exercise shown in Figure 1 is typical of the expected pattern seen in the literature (11, 49, 77, 82, 84, 107, 143). Growth hormones levels were low at rest, increased with the onset of exercise, peaked at the cessation of exercise, and returned to baseline during recovery. As expected, serum GH concentrations during the exercise treatment increased significantly above the pre-exercise period and increased to a lesser degree in the Ob group compared to the nOb group, but the extreme level of variance within the subject population made it impossible to establish statistical significance between groups. A close evaluation of the data shows that the variance within a group for a given GH variable was sometimes as large as the mean value. This was particularly true with the AUC data (Table 8). Individual GH response curves are illustrated in Appendix I and graphically demonstrate the wide range of responses within the groups. A visual inspection of the individual plots suggests that 6 of the 19 participants in this study appear to have had little or no response to the exercise stimulus (4 Ob and 2 nOb). Conversely, 7 of 19 individuals demonstrated GH_{Peak} concentration approaching or exceeding the reported values for younger (20, 77) and older (84) women. To isolate the effect of the exercise treatment, the GH concentrations of the prolonged exercise trial were corrected for control trial concentration. While this increased the

mean difference between groups for peak GH concentration (mean difference ± SE difference; GH_{Peak}, 3.3 ± 1.4 and $Peak_{corrected}$, 4.7 ± 1.4 ng·mL^{-1}), there was no influence on the variance and statistical significance was still not attained (P=0.12). Correcting AUC for the control trial decreased the AUC value for the nOb group and increased the AUC value for the Ob group (Table 8). This resulted in an overall decrease in the mean difference between groups, had little effect on the variability (mean difference ± SE difference; $AUC_{Baseline}$, 169.9 ± 116.3 and $AUC_{Corrected}$, 80.2 ± 75.6 min·ng·mL^{-1}) and weakened the between-group difference (P=0.16 and P=0.31 for $AUC_{Baseline}$ and $AUC_{Corrected}$, respectively). The significant increase in GH concentrations seen as a result of the exercise treatment are consistent with previously reported studies of older women (14, 84, 100), but the lack of a significant group effect, as defined by a measure of obesity, is not supported by research in younger women (77).

Prior to the current study, only one published investigation was identified as addressing the GH response to exercise in obese women. It was reported that the GH response to exercise in young adult women was attenuated by obesity (77). Direct comparisons between values obtained in different laboratories are difficult due to variations between laboratory techniques. Growth hormone concentrations fluctuate throughout the day and in response to a number of stimuli, but typically expected resting plasma GH concentrations are 1-5 ng·mL^{-1} (50). In light of this, Kanaley et al. (77) found baseline serum GH concentrations of 1.8 and 0.6 ng·mL^{-1} for their nOb and Ob groups, respectively, and peak GH concentrations in response

to exercise of 13.7 and 6.8 $ng \cdot mL^{-1}$, respectively; an approximate 2-fold difference between groups at baseline and for peak concentration. These researchers also reported a nearly 2-fold difference in the 6-hour integrated GH concentration (77). Although the peak GH concentrations found in the current study were lower than those reported for non-obese women by Kanaley et al. (77); a 2-fold difference was seen between groups for mean GH $Peak_{Corrected}$ and GH $AUC_{Baseline}$ but did not show significance due to the large variance. Despite extensive similarities between study protocols, the current study showed more modest results for the other GH variables. Although directionally as expected, the magnitude of the difference between the nOb and Ob groups was 26.8% for GH_{Peak}, 6.9% for GH AUC_{Total}, and 31.2% for GH $AUC_{Corrected}$. The most obvious differences between subject populations of this and the above study are age and menstrual status of participants. The eumenorrheic women of Kanaley's study (77) were younger (~34 years) than those in the current study and the BMI of the obese and non-obese groups were 33.3 ± 1.0 and 21.6 ± 0.6 $kg \cdot m^{-2}$, respectively. In the present study, the mean age of all participants was 56.3 ± 0.9 years and the BMI of the Ob and nOb groups were 33.9 ± 1.5 and 23.3 ± 1.0 $kg \cdot m^{-2}$, respectively.

In a group of postmenopausal women similar to those of the current study, Kraemer and associates (84) investigated the effect of HRT on the GH response to a 30-minute bout of treadmill exercise at 80% of $\dot{V}O_{2max}$. While all participants in the current study were free of hormone and hormone mimetic medications, the control group (NHRT) of the HRT study had similar characteristics for age (53.0 ± 3.0 y),

height (164.8 ± 1.4 cm), weight (70.4 ± 5.2), BMI (25.9 ± 2.0 $kg \cdot m^{-2}$), and $\dot{V}O_{2max}$ (23.8 ± 2.8 $mL \cdot kg^{-1} \cdot min^{-1}$) as the nOb group of this study. A 2-fold increase in GH concentrations from baseline (2.51 ± 1.05 $ng \cdot mL^{-1}$) to peak (4.9 ± 1.2 $ng \cdot mL^{-1}$) was reported in the NHRT group. Again, while it may not be appropriate to make a direct comparison between values, there appears to be a greater exercise-induced GH response in both the nOb and Ob groups of this study compared to the NHRT group. A 4- and 2.5-fold increase in GH concentration was seen from baseline to peak for the nOb and Ob groups, respectively. Although the correlational data of the present study (e.g., significant inverse association between body composition and GH concentrations) support the proposed hypotheses, the lack of between group differences in GH response is in conflict.

The final hypothesis stated that the altered GH concentrations of the Ob group would be associated with differences in substrate utilization during exercise and recovery. A summary of caloric expenditure and substrate utilization is shown in Table 5. The data failed to reveal a significant difference in GH concentrations at rest, baseline, peak, and AUC; therefore it is not appropriate to conclude that the Ob group had altered GH levels. Additionally, the data in Table 6 show that there were no significant group differences in pre-exercise and recovery caloric expenditure, and no significant group differences in kcal utilized from CHO, kcal utilized from fat, and RQ values during the pre-exercise, exercise, and recovery periods. The significantly greater total kcal expenditure for the prolonged exercise trial by the Ob group is explained by the greater total work necessary to carry the additional body

weight during exercise. The lack of a difference in substrate utilization during exercise and recovery between the Ob and nOb groups is supported by other research. A study of substrate utilization during submaximal (50 and 75% $\dot{V}O_{2max}$) treadmill exercise and a graded maximal exercise test reported no difference in substrate use between obese and non-obese women at rest or at the same relative submaximal exercise intensity (121). Prolonged cycle ergometry also failed to generate a significant difference in substrate use in non-obese, obese, and post-obese women (42). Glycerol has been interpreted as a marker for lipolysis, and FFA levels are indicative of fat metabolism (64, 115). As expected, the serum concentration of glycerol increased during exercise. However, this increase was likely a result of sympathetic nervous system activation rather than GH facilitated lypolysis (18, 33). Luskey et al. (93) found a one to three hour delay before circulating FFA concentrations increase following GH stimulation at rest. The 120-minute trial duration following the onset of exercise used in this protocol was not sufficient to anticipate changes in FFA resulting from elevated GH levels. These data support previous research suggesting that body composition does not impact substrate utilization during rest, exercise, and recovery.

Possible Confounding Variables

As with any study, there are a number of factors capable of influencing the endocrine system that may cloud the results. These include: group selection; fitness level of individuals; previous training; choice of stimulus; and dietary intake.

Subject Group Selection

The Ob and nOb groups shared a number of similar characteristics (Table 2). The study design required the recruitment of apparently healthy postmenopausal women meeting specifically identified criteria. The groups were intended to be similar in age, height, years since menopause, and $\dot{V}O_{2max}$, and differ in characteristics related to obesity. Body mass index was used as the primary inclusionary standard, was used to define the subject groups, and the mean difference between the obese or non-obese groups was over 10 BMI units. As a result, the mean body weight of the Ob groups was more than 25 kg heavier than the nOb group and the mean difference in %BF was greater than 10%. Although weight and BMI are easily documented, the estimation of body fat in postmenopausal women is problematic. Whole body plethysmography, the estimation technique utilized in this study, is based on the two-component body composition model and assumes that the densities of fat and FFM are consistent between individuals. It has been well established that bone density decreases with age and that postmenopausal women are at a greater risk of osteoporotic complications (27, 149). With this in mind, it is reasonable to expect that this subject population had some degree of compromised bone density. Anecdotally, while conducting individual consultations, following testing, for the discussion of results, several participants mentioned having had below normal values during a bone density test. The prospect of an overestimation of %BF is evident in Table 2

and more obvious when analyzing individual data (not shown). The mean %BF for the nOb group (37.4 ± 3.1 %) is oddly elevated in relation to the mean BMI (23.3 ±1.0 $kg \cdot m^2$). While possible, it is less likely that the Ob group had experienced the same degree of loss in bone density as the nOb group based on the increased loading of the bones during normal locomotion as a byproduct of the Ob groups higher body weight. Despite the limitations in %BF estimation, there was a significant inverse relationship between %BF and GH_{Peak}.

There are some distinct physiological differences between the Ob and nOb groups of this study. Obesity is strongly associated with a number of pathologic conditions and risk factors for cardiovascular disease. The majority of those conditions such as diabetes, hypertension, thyroid disorders, and cardiovascular abnormalities were controlled for during the recruitment process and maximal exercise stress test. Despite the rigorous selection process, the Ob group had significantly higher insulin, glucose, and glycerol concentrations compared to the nOb group (Table 2). Although still within clinically accepted limits, the Ob group expressed classic characteristics of insulin resistance and dyslipidemia commonly associated with obesity. In addition to elevated insulin levels, Rasmussen and coworkers (109) found significantly lower 24-hour spontaneous GH release profiles, GH responses to hypoglycemia, and basal IGF-1 levels in young obese subjects while comparing them to age- and gender-matched controls. Unfortunately, these data do not contain 24-hour GH profiles or other GH stimuli for comparison. From the comparison of groups, it is clear that there were significant physiological

differences between the Ob and nOb groups. These differences (elevated insulin, glucose, and glycerol) are commonly associated with obesity but in these subjects were unrelated to the GH response.

Fitness Level

There is contradictory evidence supporting altered GH secretion related to fitness level and training history (20, 59, 69, 82, 141). The participants of this study were all physically inactive (moderate intensity activity <1-day/week) and had not participated in regular exercise during the previous six months. The results of the $\dot{V}O_{2max}$ test indicate that the fitness levels of these women were well below average for their age and were categorized in the bottom 20% of the population (1). Additionally, the data in Table 6 shows that there was no significant difference in $\dot{V}O_{2max}$ values between groups. These data clearly show that there was no difference in the fitness levels of the two groups and therefore, can be eliminated as a potential confounder.

Effectiveness of the Exercise Treatment

The duration and intensity of an exercise bout has been suggested to have a vital role in the stimulation of a GH response (107). The exercise treatment used in this study was of a sufficient intensity and duration to elicit a GH response in most women. Additionally, blood lactate values were significantly elevated above resting concentrations during exercise (P=0.01). The efficacy of the treatment is evident

from the significantly elevated GH_{Peak} concentrations during the prolonged exercise trial (mean ± SE, 10.9 ± 1.4 $ng \cdot mL^{-1}$) compared to the control trial (mean ± SE, 4.4 ± 0.5 $ng \cdot mL^{-1}$) for the combined Ob and nOb groups ($P<0.001$). The significant difference ($P<0.001$) in GH AUC between the prolonged exercise trial and control trial also support the treatment intensity as being sufficient to stimulate a GH response (combined group means ± SE, 193.8 ± 58.2 and -118.6 ± 40.4 $ng \cdot mL^{-2}$, respectively) and the exercise-induced GH response of these subject groups is similar to the literature (11, 49, 77, 82, 84, 107, 143). Despite the potent exercise stimulus utilized by this study, some of these postmenopausal women had minimal or no GH response to exercise (Appendix I). Since it is uncommon to have individual data reported in the literature, it is difficult to address response vs. non-response questions. Correlational analysis of these data was unable to reveal an apparent pattern or variable relationship that could be positively identified as an indicator for non-response.

Consistency of the Protocol

When comparing groups of individuals, it is imperative that each group is exposed to the same treatment. The testing protocols implemented for this investigation were followed with excruciating attention to detail and consistency. During subject testing, each individual was subjected to two trials; each initiated precisely at 0800 h to ensure that each sample collection and treatment was conducted at the exact same time of day. All blood samples were collected within

±3 minute of the designated time and all samples of the second trial were collected within ±2 minutes of the corresponding time point of the first trial. Individuals were asked to perform 30 minutes of treadmill walking at 70% of their predetermined $\dot{V}O_{2max}$. The prescribed exercise intensity was continuously monitored and tightly controlled for this study. As described in Table 2 the relative intensity of exercise during the prolonged exercise trial was nearly identical between groups for $\%\dot{V}O_{2max}$ and $\%HR_{max}$. Additionally, the $\dot{V}O_2$ data provided in Table 6, in conjunction with the blood lactate values (not shown), further support the similarity in exercise intensity performed by the Ob and nOb groups.

Dietary Influence on Growth Hormone Secretion

Diet is believed to play a vital role in endocrine secretion patterns. Nutritional status has been stated as one of the most important determinants of circulating IGF-1 concentrations (70). Starvation and hypoglycemia are known to cause increases in GH and elevated blood glucose levels suppress GH secretion (21, 61). The effect of a high fat diet has been associated with an increased GH response to exercise and a high carbohydrate diet showed no change (21) or a suppressive effect (61), while acute hyperglycemia has been reported to inhibit GH secretion through activation of somatostatin (105). To account for dietary influences on GH concentrations, participants in this study were asked to maintain dietary records (Table 4). It would appear that diet did not have an influential role in the outcome of this study since there was no difference between the Ob and nOb groups for any

of the dietary intake variables.

Variability Within the Subject Group

There exists an inherently large inter-individual variability for endocrine parameters. A review of the literature produced a seemingly appropriate estimation of the anticipated variance for postmenopausal women and an a-priori power analysis identified the number of subjects per group necessary to show significance at the 95% confidence interval to be five (N=5 per group). Conversely, an a-posteriori evaluation of the actual variance increased the number of subjects needed for each group to 62 in order to show significance with 95% confidence.

Typical of any large-scale research study with several intermediary steps, there are several areas in which small errors in measurement compound into large variances. To limit errors related to metabolic equipment, several comparisons were made to reference gases with concentrations measured by the most accurate method available. Ventilation volumes were compared to calibrated cylinder volumes. All equipment was thoroughly calibrated before each test and calibration stability assessed following completion of the trial. The accuracy of the treadmill to maintain speed and grade was frequently evaluated and expiratory tubes, facemasks, mouthpieces, and breathing valves continuously monitored for leaks. For subject testing, each investigator was required to undergo extensive training and retraining of specific tasks and the same four investigators conducted all trials. Blood sampling and sample preparations were scheduled, timed, and performed in an

identical manner each time to maintain uniformity. The benefits of such rigors are evident in the measured metabolic results and in the consistent timing of the trial protocols.

The blood assay procedures required a high degree of precision and consistency. The reliability of the pipettors to deliver a consistent volume was assessed and the %CV was less than one half of one percent for volumes of 100 μL or more. The %CV was higher for the 10 μL pipettor (~3%) but still well within acceptable limits. The size of each assay was limited to the smallest number of tests possible to minimize the end-of-run effect and control samples were tested at the beginning and end of each assay. In general, the %CV found for each assayed variable was similar to that reported by the manufacturer. There was little that could be done to enhance the sensitivity of the commercially available assay kits, but care was taken in the selection of products to ensure that the precision was maximized.

Some level of human error is inevitable in all research. To limit this contribution to the overall error, a very limited number of extensively trained investigators were involved in the collection of data. Considering the degree of attention given to equipment and procedures and the meticulous manner in which all protocols were conducted, it is firmly believed that the level of variability within the study was true variance for the sample group and may be indicative of the variability within postmenopausal women.

Future Research

Looking beyond the hypothesized outcomes, the purpose of this study was to establish a basis for future research. This project was intended to describe the acute exercise-induced GH response in postmenopausal women as it related to obesity. The direction of this line of research is to determine a means for elevating 24-hour GH secretion in older women through physical activity, pharmacology, or a combination of each. A future study should thoroughly examine the 24-hour GH secretory profile of postmenopausal women at rest and with a single or multiple intermittent bouts of endurance exercise. Past research has show that the large GH peak seen during a single extended (~4-hour) bout of moderate intensity endurance exercise is nullified by a compensatory reduction in nighttime GH secretion results in no change in 24-hour GH concentrations (81). Perhaps a single bout of shorter duration or intermittent bouts of low intensity exercise sufficient to stimulate a GH response would induce an increase in the overall 24-hour GH secretory profile. Since age and obesity are associated with decreased GH concentrations, and considering GH role in bone maintenance, protein synthesis, and substrate availability; it is of a public health interest to identify methods for elevating GH levels.

Conclusions

The results of this study indicate that a 30-minute bout of treadmill exercise at 70% of $\dot{V}O_{2max}$ is sufficient to elicit a significant elevation of serum GH concentrations in untrained postmenopausal women and that the GH response to exercise is negatively related to body weight and body fat. The response pattern of GH to exercise parallels the anticipated response as indicated by previous research in obese (77) and postmenopausal (84) women. Although no between group differences in GH response were found, the correlational data and the somewhat lower GH response in the obese women lends support for the hypothesis that obese postmenopausal women have lower Peak GH concentrations and less GH AUC in response to endurance exercise. Within each group of these postmenopausal women, a potent exercise stimulus elicited a range of GH response from none to what would be expected of young, lean women (Appendix I). An analysis of these data was unable to positively identify any characteristics or relationships that were indicators for non-response. These data may suggest that large variability is normal among postmenopausal women. This was the first study to focus specifically on obese postmenopausal women, and it is clear from the results that this remains a much-needed area of research.

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APPENDICES

APPENDIX A

INFORMED CONSENT FORM

TITLE OF THE STUDY: Effect of Body Composition on Growth Hormone Response to Exercise in Post-menopausal Women

PURPOSE

You are invited to participate in a study, the purpose of which is to examine how growth hormone levels change in response to exercise in post-menopausal women. Growth hormone is a substance made and released by your brain that helps control many body functions including how fat is used for energy. This form explains the procedures, risks and benefits of participation. If you have any questions about the information found in this form, please ask a member of the research team before signing.

PROCEDURES

You will attend 3 testing sessions at least one week apart. The first session will be held in Rehabilitation Services testing center at the University of Tennessee Medical Center, Knoxville. The other sessions will be held in the Human Performance Laboratory, room 310 of the Health, Physical Education, & Recreation (HPER) Building on the University of Tennessee campus. Each session will last approximately 4.0 hours.

Maximal Exercise Test

During the first session, you will complete a questionnaire on your health history and exercise patterns and your height, weight, and maximal exercise capacity will be assessed. You should avoid any strenuous physical activity for the 48-hour period prior to testing. You should also avoid eating for at least 3 hours prior to maximal testing.

The maximal exercise stress test will be performed on a motorized treadmill. Initially you will walk at a slow pace and the speed of the treadmill will be gradually increased each minute up to a brisk walk and then remain constant. The slope of the treadmill will then be increased in small intervals each minute until you have reached the limit of your ability. You will breathe through a mouthpiece and your expired air will be measured to determine how many calories you are using. Your heart rate, heart rhythm, and blood pressure activity will be monitored throughout the test and a physician will be present for your safety.

Prolonged Exercise Test and Control Test

The second and third sessions will be an exercise trial and a control trial. For the 3 days before these tests, you will write down all the food and drink you consume for the analysis of your diet.

On the day of the prolonged exercise trial, you will report to the laboratory at 7:00 AM after not eating or drinking anything for at least 8 hours (you are free to drink water) and having avoided all physical activity and alcohol consumption during the previous 48 hours. When you arrive in the lab you will sit in a reclined position while a needle is placed into a forearm vein for the collection of several small blood samples. During the test, 10 small blood samples will be collected and you will breathe through a sealed facemask or mouthpiece. The total amount of blood sampled over the entire test will be equal to about 1/4 cups (2 ounces). This small amount of blood is 1/8 the amount taken during blood donation and is not dangerous to your health. Once the test is started, you will remain in the reclined position for a 30-minute pre-exercise period while 2 resting blood samples are collected. Following the pre-exercise period, you will be asked to walk on a treadmill at a moderately hard pace for 30 minutes during which time 3 more blood samples will be collected. At the end of the exercise period, you will once again sit in a reclined position for a 90 minutes recovery period while 5 more blood samples are taken. At the end of recovery, the needle will be removed, ending the session.

The control trial will be exactly the same as the exercise trial except that you will remain resting during the entire 2.5-hour period. At the end of this period your body fat will be measured. Your body fat will be determined using the Bod Pod® procedure. The Bod Pod® procedure consists of measuring height, weight, and body volume. Body volume will be measured as you sit for approximately 3 minutes in a sealed chamber. While in the chamber, you will be able to breathe normally and see your

surroundings. For this measurement you will need to wear either a swimming suit or your underwear. We will also measure the distance around your waist and hips.

BENEFITS OF PARTICIPATION

As a participant in this study, you will learn your percent body fat, number of calories burned at rest, average number of calories you eat each day, physical fitness level, and gain knowledge about hormone levels within your body and how these levels change in response to exercise. This information can assist you in weight management, development of an exercise program, and decrease your potential risk of disease. This is important information from a health-related perspective. An additional benefit for you will be one semester free membership in The Center for Physical Activity & Health's exercise program, which is maintained by the Department of Exercise Science and Sport Management.

RISKS OF PARTICIPATION

The potential risks that may occur with participating in this research include those associated with exercise testing and blood sampling procedures. The potential risks associated with a maximal graded exercise test include: fatigue, muscle strains, irregular heart beats, 0.01% chance of death (in cardiac patients), 0.02% risk of irregular heart beats requiring hospitalization (in cardiac population), and abnormal increases in blood pressure. To minimize these risks, a physician will be present for your maximal exercise tests, your heart rate and rhythm, and your blood pressure will be monitored during your entire test. There is also a risk of local muscle and/or joint soreness following the prolonged exercise trial. A warm-up period will decrease the likelihood of this occurrence and a research team member will advise you on stretching exercises to minimize muscle soreness. There may be some minor skin irritation (redness) caused by the electrodes used for monitoring your heart rhythm. The blood sampling procedure may cause some localized bruising and/or tenderness of the arm and there is a small risk of infection. A trained technician will perform the insertion of the needle for blood sampling.

RIGHT TO ASK QUESTIONS AND/OR WITHDRAW FROM THIS STUDY

If you have questions or concerns at any time during the course of this investigation or after you complete this study, you may contact George A. King, M.S. at (865) 974-6040, Dixie L. Thompson, Ph.D. at (865) 974-8883, or the Institutional Review Board of the University of Tennessee Medical Center at (865) 544-9781. Mr. King's office is located in room 136B HPER building and Dr. Thompson's office is located in room 347 HPER Building.

As a volunteer in this study, it is your right to withdraw from this investigation at any time. If you decide to withdraw from this study, you will in no way be penalized.

PRIVACY OF RECORDS

Only you, Mr. King, Dr. Thompson, and individuals directly involved in this research will have access to your results. All data collected in this study will be coded by subject number rather than by name will be kept in a locked file in Dr. Thompson's office. The results of the research will be published; however, no publication will contain information that will allow you to be identified.

AUTHORIZATION

By signing this form, I am indicating that I have read this form, understand its contents, and have received a copy of this form for my files. I have also been given the opportunity to ask questions to clarify my role in the study. My signature indicates that I agree to serve as a subject in this research study.

_____ _____

Participant's signature Date

_____ _____

Investigator Date

_____ _____

Witness Date

APPENDIX B

Health Status Questionnaire

Please complete the following questions as accurately as possible.

Date of Birth: _____/_____/_____ Age:_____yr.

Average number of hours worked per week:
☐ Less than 20 ☐ 20-40 ☐ 41-60 ☐ over 60

More than 25% of time spent at work is: (mark all that apply)
☐ Sitting at a desk ☐ Lifting or carrying loads ☐ Standing
☐ Walking ☐ Driving

Medical History

Please mark any who have died of heart attack before age 50 years:
☐ Father ☐ Mother ☐ Grandparent ☐ Brother ☐ Sister

Date of last physical exam: _____/_____/_____

Date of last physical fitness test: _____/_____/_____

Date of last menstrual cycle: _____/_____/_____

Please mark and date all surgeries you have had:

☐ Back	_____/_____	☐ Heart	_____/_____
☐ Kidney	_____/_____	☐ Eyes	_____/_____
☐ Joint	_____/_____	☐ Neck	_____/_____
☐ Ears	_____/_____	☐ Hernia	_____/_____
☐ Lung	_____/_____	☐ Hysterectomy	_____/_____
☐ Other	_____		_____/_____

Please mark all of the following for which you have been diagnosed or treated by a physician or health professional:

☐ Alcoholism	☐ Emphysema	☐ Kidney problem
☐ Anemia, sickle cell	☐ Epilepsy	☐ Liver disease
☐ Anemia, other	☐ Eye problems	☐ Lung disease
☐ Asthma	☐ Gout	☐ Mental illness
☐ AIDS	☐ Hearing loss	☐ Neck strain

- ☐ Back Strain
- ☐ Bleeding trait
- ☐ Bronchitis, chronic
- ☐ Cancer
- ☐ Cirrhosis, liver
- ☐ Concussion
- ☐ Congenital defect
- ☐ Diabetes
- ☐ Heart problem
- ☐ Heart murmur
- ☐ Hepatitis
- ☐ High blood pressure
- ☐ Hypoglycemia
- ☐ High Cholesterol
- ☐ Infectious mononucleosis
- ☐ Joint problems
- ☐ Obesity
- ☐ Phlebitis
- ☐ Rheumatoid arthritis
- ☐ Stroke
- ☐ Thyroid problem
- ☐ Ulcer
- ☐ Other_____

Please mark all medications taken during the past 6 months:

- ☐ Blood thinner
- ☐ Diabetic
- ☐ Diuretic
- ☐ Insulin
- ☐ Epilepsy medication
- ☐ Heart medication
- ☐ High blood pressure medication
- ☐ Hormones
- ☐ Other_____
- ☐ Other_____
- ☐ Other_____
- ☐ Other_____

Please mark any of the following symptoms you have had recently:

- ☐ Abdominal pain
- ☐ Arm or shoulder pain
- ☐ Breathless with slight exertion
- ☐ Blurred vision
- ☐ Blood in urine
- ☐ Burning sensations
- ☐ Chest pain
- ☐ Cough up blood
- ☐ Difficulty walking
- ☐ Dizziness
- ☐ Feel faint
- ☐ Frequent urination
- ☐ Leg pain/numbness
- ☐ Low blood sugar
- ☐ Low-back pain
- ☐ Palpitation or fast heart beat
- ☐ Shortness of breath
- ☐ Significant emotional problem
- ☐ Swollen joints
- ☐ Unusual fatigue with normal activity
- ☐ Weakness in arms

Health-Related Behaviors

Do you smoke? ☐ Yes ☐ No

If yes, How much do you smoke per day?

Cigarettes: ☐ 40 or more ☐ 20-39 ☐ 10-19 ☐ 1-9

Cigar or pipe only: ☐ 5 or more or any inhale

☐ Less then 5, none inhaled

Do you currently exercise regularly? ☐ Yes ☐ No

During the past 6 months did you exercise regularly? ☐ Yes ☐ No

How many days per week do you accumulate 30 minutes or more of moderate activity?

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

How many days per week do you normally spend at least 20 minutes in vigorous exercise?

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

Can you walk 3 miles briskly without fatigue? ☐ Yes ☐ No

Can you jog 3 miles continuously at a moderate pace without discomfort?

☐ Yes ☐ No

Are you currently on a diet or program specifically designed for weight loss?

☐ Yes ☐ No

During the past 3 months have you been on a diet or program specifically designed for weight loss? ☐ Yes ☐ No

During the past 3 months has your body weight changed more than 4 pounds?

☐ Yes ☐ No

APPENDIX C

Date _____ Subject ID _____

VO2 Max Test Protocol

Supine ECG
Sitting ECG
Standing ECG

Resting BP

Stage	Speed	Grade	Predicted VO2 (ml/kg/min)	Blood Pressure SBP	Blood Pressure DBP
1	2.0	0%	7.36		
2	2.5	0%	9.20		
3	3.0	0%	11.04		
4	3.5	0%	12.88		
5	3.5	2%	16.26		
6	3.5	4%	19.64		
7	3.5	6%	23.02		
8	3.5	8%	26.40		
9	3.5	10%	29.78		
10	3.5	12%	33.16		
11	3.5	14%	36.54		
12	3.5	16%	39.92		
13	3.5	18%	43.30		
14	3.5	20%	46.68		
15	3.5	22%	50.06		

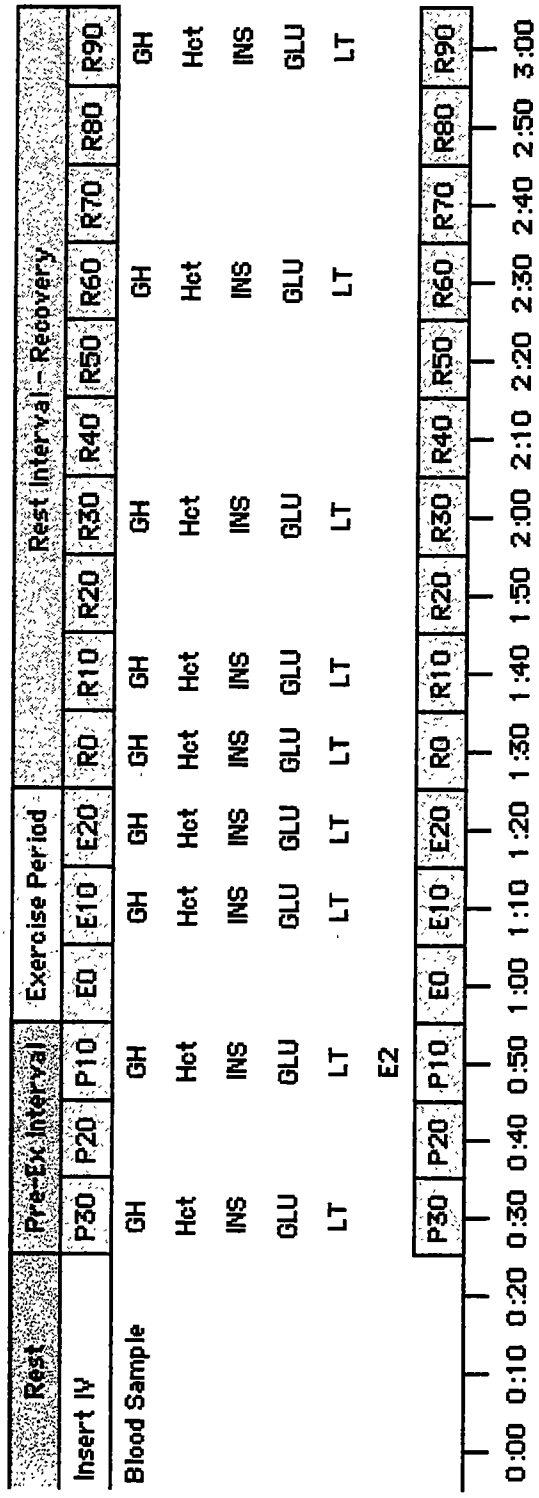
The initial speed of the treadmill will be 2 mph and grade will be 0%. Speed will be increased by 0.5 mph each minute up to 3.5 mph then grade will be increased 2% each minute until volitional exhaustion. Measure BP and ECG at end of each stage

NOTES _____

APPENDIX D

Prolonged Exercise Test Protocol

Protocol Time Line



PROTOCOL CHECK LIST

1. Turn on Metabolic System
    - Make sure pump is on
    - Turn on computer
    - Let system warm-up at least 30 min
2. Check YSI analyzer
    - Make sure there is sufficient buffer for analysis
    - Make new buffer solution is necessary
        - let new buffer stabilize for several hours before analyzing blood
3. Gather blood collection supplies
    - Gloves - 1 Box
    - Sharps Container
    - Alcohol Pads - 1 Box
    - Gauze Pads - 1 Box
    - Tourniquet
    - Cloth Tape 1/2" - 2 Roles
    - IV Catheters 20 g x 1 1/4" - 3 Catheters
    - Extension Set 20" - 3 Sets
    - Infusion Plug - 3 Plugs
    - IV Bag 250cc 0.9% Sodium Chloride - 1 Bag
    - Hypodermic Needles 20 g x 1 1/2" - 1 Box
    - Syringe 12cc - 35 syringes (need 28 for protocol)
    - Red Top Vacutainer Blood Tubes 5 ml - 15 Tubes (need 9 for protocol)
    - Test Tube Rack
    - YSI Blood Tubes - 15 Tube (need 9 for protocol)
    - Small Tube Rack
    - Capillary Tubes - 1 Jar (at least 20 tubes)
    - Crit-O-Seal - 1 Tray
    - Large beaker with 20% bleach solution for waste dilution
4. Set up resting blood sampling area
    - Position chair
    - Place spill paper on floor
    - Make breathing valve with face-mask
5. Set up treadmill
    - Place blood spill paper on floor
    - Make breathing valve with mouth-piece
    - Hang valve with inspired and expired tubes
6. Meet subject
    - Recheck file and paperwork
    - Make sure consent form is signed
    - Answer any questions
    - Ask if health history has changed

- Ask subject to use restroom/get drink of water
  - instruct subject that they will not be able to use restroom for about 3 hours
  - can drink water
  - have subject put on HR monitor
  - adjust height of mouthpiece on treadmill to match subject
- Set subject in resting position
  - subject should be reclined comfortably

7. Start IV and BEGIN REST interval
   - Flush extension tube
   - Rip tape strips
   - Draw syringe to flush catheter
   - Clean rubber draw site on IV fluid bag with alcohol (allow to dry)
   - Connect 20 g needle to 12 cc syringe
   - Draw 10 cc of saline from IV fluid bag
   - Recap needle and leave needle on syringe
   - Insert catheter, connect extension tube, and flush with saline
   - Secure catheter and tube in place
   - Record TIME of starting REST Interval
   - Draw saline for next catheter flush
8. Flush IV every 10 min. with 10 cc of saline
   - Clean infusion plug with alcohol and let dry
   - Insert needle of 12 cc syringe into infusion plug
   - Draw plunger of syringe back to make sure catheter is not clotted
   - Push plunger in slowly until all 10 cc have been injected
   - Record TIME and amount of saline used to FLUSH
   - Draw saline for next catheter flush
9. Calibrate Metabolic Cart
   - Run Flow Meter Cal
   - Run Gas Cal
   - Input subject data
   - Connect subject to face-mask
10. BEGIN PRE-EXERCISE Interval
    - Record time starting
11. **P30** - Pull blood sample
    - Clean infusion plug with alcohol and let dry
    - Insert needle of 12 cc syringe into infusion plug
    - Draw plunger of syringe back to make sure catheter is not clotted
    - Flush tube with 2 cc of saline
    - Draw 6 cc from subject and DISCARD
      - put waste from syringe into bleach solution
      - put syringe in sharps container
    - Insert second 12 cc syringe into infusion plug

- Draw 5 cc blood SAMPLE
- Fill 2 capillary tubes and place in Crit-O-Seal
- Place 1-2 drops in YSI tube and mix vigorously
  - (do not fill above the 0.2 mark)
- Place YSI tube in rack
- Place remaining blood in Red Top tube and mix gently
- Place Red Top tube in rack
- Flush IV with 10 cc saline
- Push plunger in slowly until all 10 cc have been injected
- Record TIME of sample collection
- Record amount of saline used to FLUSH tubing
- Record amount of blood drawn for WASTE sample
- Record amount of blood taken for SAMPLE
- Draw saline for next catheter flush

12. **P20** - Flush IV with 10 cc saline
    - Record TIME of flush
    - Record amount of saline used to FLUSH tubing
    - Draw saline for next catheter flush
13. **P10** - Pull blood sample
    - Record TIME, FLUSH, WASTE, SAMPLE
    - Draw saline for next catheter flush
14. **P5** - Have subject stand up and move to treadmill for warm-up period
    - Remove face-mask
    - Disconnect resting expired breathing tube from metabolic cart
    - Have subject straddle treadmill belt
    - Connect subject to mouth-piece
    - Put on nose-clip
    - Connect expired breathing tube from treadmill to metabolic cart
    - Move HR receiver from resting position to treadmill position
    - Start belt at 2.0 mph and have subject begin walking
    - Monitor HR and keep below 110 bpm
15. **E0** - BEGIN EXERCISE Interval
    - Increase exercise intensity to 70% of VO_2max
    - Increase speed (do not go above 3.5 mph)
    - Increase grade
    - Monitor HR and VO_2 to maintain prescribed intensity
16. **E10** - Pull Blood sample
    - Record TIME, FLUSH, WASTE, SAMPLE
    - Draw saline for next catheter flush
17. **E20** - Pull Blood Sample
    - Record TIME, FLUSH, WASTE, SAMPLE
    - Draw saline for next catheter flush

18. **R0** - Pull Blood Sample
    - Record TIME, FLUSH, WASTE, SAMPLE
    - Draw saline for next catheter flush
19. BEGIN RECOVERY Period
20. **R0** - have subject move back to rest position
    - Stop treadmill
    - Remove mouth-piece and nose-clip and give subject a towel
    - Have subject return to reclined position
    - Give subject a small drink of water if desired
    - Disconnect expired breathing tube from metabolic cart
    - Connect expired breathing tube from face-mask to metabolic cart
    - Place face-mask on subject
    - Move HR receiver from treadmill to rest position
21. **R10** - Pull Blood Sample
    - Record TIME, FLUSH, WASTE, SAMPLE
    - Draw saline for next catheter flush
22. **R20** - Flush IV
    - Record TIME, FLUSH
    - Draw saline for next catheter flush
23. **R30** - Pull Blood Sample
    - Record TIME, FLUSH, WASTE, SAMPLE
    - Draw saline for next catheter flush
24. **R40** - Flush IV
    - Record TIME, FLUSH
    - Draw saline for next catheter flush
25. **R50** - Flush IV
    - Record TIME, FLUSH
    - Draw saline for next catheter flush
26. **R60** - Pull Blood Sample
    - Record TIME, FLUSH, WASTE, SAMPLE
    - Draw saline for next catheter flush
27. **R70** - Flush IV
    - Record TIME, FLUSH
    - Draw saline for next catheter flush
28. **R80** - Flush IV
    - Record TIME, FLUSH
    - Draw saline for next catheter flush
29. **R90** - Pull Blood Sample
    - Record TIME, FLUSH, WASTE, SAMPLE
30. END TEST
    - End Test on metabolic cart
    - Print test

- Save test to disk

31. Remove face mask
    - Give subject a drink and a towel
32. Remove IV
33. Release Subject
34. Clean-up
    - Clean all breathing valves and tubes
    - Clean any spilled blood
    - Put away blood collecting supplies
    - Pick up all trash and straighten up lab area
    - Put biohazard trash into biohazard box
    - Pour bleach solution down drain with a large amount of water

Blood Sample Preparation

1. Let Red Top Tubes sit for at least 30 min
2. Centrifuge blood sample between 30 and 60 minutes following collection
    - Spin for 15 min
3. Pipette serum off cells and place in properly marked cryule vials
    - Check/recheck/match labels on blood sample tube and cryule vials
    - Put 500 μL in the cryule vial marked GH (growth hormone)
    - Put 500 μL in the cryule vial marked Ins (insulin)
    - Put 500 μL in the cryule vial marked Est (estrogen)
    - Put remaining serum in cryule vial marked FA (glycerol)
4. Place all cryule vials in storage box
    - Label/check label of storage box
5. Put samples in deep freezer for storage

Glucose and Lactate measurements

1. Place YSI analyzer in RUN Mode and let machine self-calibrate
2. Measure samples from standards
    - 5 mM lactate
    - 15 mM lactate
    - 200 mM glucose
    - 500 mM glucose
    - 900 mM glucose
3. Analyze each sample twice
    - Record lactate and glucose values on protocol data sheet in mM/L
4. Re-run standards when finished with test samples
5. Place YSI analyzer in STANDBY Mode
    - Empty waste container
    - Check buffer level to ensure it is not low

Hematocrit Measurement

1. Place micro-hematocrit tubes in centrifuge
    - note the location of tubes and label accordingly
2. Spin samples for 5 min
3. Read hematocrit values for samples and record on protocol data sheet
    - Make sure to match the sample with the appropriate protocol time point

APPENDIX E

GROWTH HORMONE ASSAY PROCEDURE

1. Remove samples from deep freezer at least 2 hours in advance and allow to completely thaw to ambient temperature.
2. Bring all Reagents to room temperature.
3. Set up a series of consecutively numbered (1-62) clean glass test tubes.
   - Label order of each assay will be
     - Tubes 1, 2 = Blank tubes
     - Tube 3, 4 = Zero tubes (0.0 ng/mL)
     - Tubes 5, 6 = Standard 1 (0.5 ng/mL)
     - Tubes 7, 8 = Standard 2 (1.0 ng/mL)
     - Tubes 9, 10 = Standard 3 (2.5 ng/mL)
     - Tubes 11, 12 = Standard 4 (5.0 ng/mL)
     - Tubes 13, 14 = Standard 5 (10.0 ng/mL)
     - Tubes 15, 16 = Standard 6 (25 ng/mL)
     - Tubes 17, 18 = Standard 7 (50 ng/mL)
     - Tubes 19, 20 = Control 1 PRE
     - Tubes 21, 22 = Control 2 PRE
     - Tubes 23-40 = Subject Samples EXERCISE
     - Tubes 41-58 = Subject Samples REST
     - Tubes 59, 60 = Control 1 POST
     - Tubes 61, 62 = Control 2 POST
4. Add 200 µL of Silver Vial (Polypeptide Diluent) to Tubes 1 and 2 (blank tubes).
5. Add 100 µL of Silver Vial (Polypeptide Diluent) to Tubes 3 and 4 (0 tubes).

6. Add 100 μL each of Green Vials (hGH Standard) into respective tubes (Tubes 5-18).
    - Tube 5, 6 = 0.5 ng/mL
    - Tube 7, 8 = 1.0 ng/mL
    - Tube 9, 10 = 2.5 ng/mL
    - Tube 11, 12 = 5.0 ng/mL
    - Tube 13, 14 = 10.0 ng/mL
    - Tube 15, 16 = 25.0 ng/mL
    - Tube 17, 18 = 50.0 ng/mL
7. Add 100 μL of Control Serum to respective tubes (Tubes 19-22).
    - Tube 19, 20 = Control 1
    - Tube 21, 22 = Control 2
8. Add 100 μL of Subject Serum to respective tubes (Tubes 23-58).
    - Tube 23, 24 = P30 Ex
    - Tube 25, 26 = P10 Ex...
    - Through tube 39, 40 = R90 Ex
    - Tube 41, 42 = P30 R
    - Tube 43, 44 = P10 R...
    - Through tube 57, 58 = R90 R
9. Add 100 μL of Control Serum to respective tubes (Tubes 59-62).
    - Tube 59, 60 = Control 1
    - Tube 61, 62 = Control 2
10. Add 100 μL of Gold Vial (Anti-hGH) to tubes 3-62.
    - DO NOT ADD GOLD VIAL TO TUBES 1, 2
11. Add 100 μL of Blue Vial (hGH ^{125}I) to each tubes (Tubes 1-62).
12. Vortex all tubes thoroughly to mix sample
13. Incubate sample at 37° C for 2 hours in oven
14. Find Red Vial (Precipitant Solution) and Mix Thoroughly
    - Shake if necessary
15. Add 500 μL of Red Vial (Precipitant Solution) to each tube (1-62)
16. Vortex all tubes thoroughly to mix sample

17. Centrifuge all tubes at 1000 x g (2300-2500 RPM) for 15 minutes.
    - Refrigerated centrifuge at 6-8° C
18. Immediately after centrifugation, Aspirate the tubes with aspirator with trap
19. Count the remaining precipitant in the tubes in a gamma counter for 1 minute
20. Record count on Data Collection Sheet
21. Calculate Growth Hormone Concentration

APPENDIX F

INSULIN ASSAY PROCEDURE

1. Remove samples from deep freezer at least 2 hours in advance and allow to completely thaw to ambient temperature.
2. Reconstitute the Blue Label Vial (lyophilized insulin-^{125}I) with 5.0 of distilled water.
    - Gently inject DI water
    - Allow to sit at room temperature for 60 minutes
    - Allow reagent to warm to ambient temperature
3. Bring all standards to room temperature
4. Bring all Coated Tubes to room temperature
5. Combine Blue Label Vial (insulin-^{125}I) and Tan Label Vial (insulin buffer) in clean glass Erlenmeyer flask.
    - Add Blue Label Vial to Erlenmeyer flask
    - Rinse Blue Label Vial with Tan Label Vial solution 3 times
    - Add rinsed Tan Label Vial Solution in Blue Label Vial to Erlenmeyer Flask
    - Add remaining Tan Label Vial Solution into Erlenmeyer Flask
    - Mix thoroughly
    - Allow to sit at ambient temperature for 1 hour
6. Set up a series of labeled Anti-Insulin Tubes for Blank, Standard, Serum, and Control
    - Label order of each assay will be
        - Tubes 1, 2 = Standard 1 (0.0 μIU/mL)
        - Tubes 3, 4 = Standard 2 (5.5 μIU/mL)
        - Tubes 5, 6 = Standard 3 (15.0 μIU/mL)
        - Tubes 7, 8 = Standard 4 (35.0 μIU/mL)
        - Tubes 9, 10 = Standard 5 (70.0 μIU/mL)
        - Tubes 11, 12 = Standard 6 (175.0 μIU/mL)
        - Tubes 13, 14 = Standard 7 (310 μIU/mL)
        - Tubes 15, 16 = Control 1 PRE
        - Tubes 17, 18 = Control 2 PRE

- Tubes 19-36 = Subject Samples EXERCISE
- Tubes 37-54 = Subject Samples REST
- Tubes 55, 56 = Control 1 POST
- Tubes 57, 58 = Control 2 POST

7. Add 100 μL of each standard into respective tubes
8. Add 100 μL of Controls to control tubes
9. Add 100 μL of Serum to respective tubes
10. Pipet 900 μL of Tracer/Buffer Solution from Erlenmeyer flask into each tube
    - Be careful not to touch pipettor to side of Erlenmeyer flask
11. Vortex all tubes thoroughly to mix sample
12. Incubate sample at room temperature for 18 hours
13. Aspirate the tubes using aspirator with trap
14. Rinse each tube with 4 mL of DI water
15. Aspirate the tubes using aspirator with trap
    - Empty aspirator trap every 2 assay's
    - Pour contents of trap into LIQUID RADIOACTIVE WASTE container
16. Count the empty tubes in a gamma counter for 1 minute
17. Record count on Data Collection Sheet
18. Calculate Insulin Concentration

APPENDIX G

ESTRADIOL ASSAY PROCEDURE

1. Remove samples from deep freezer at least 2 hours in advance and allow to completely thaw to ambient temperature.
2. Reconstitute the control with 5 mL of distilled water.
3. Bring all standards to room temperature.
4. Bring Coat-A-Count tubes to room temperature.
5. Set up a series of labeled tubes for blank, standards, serum, and controls.
   - Label order of each assay will be
     - Tubes 1,2 (total) 0 µL = Total Counts Tube
     - Tube 3, 4 (NSB) 100 µL = Standard A 0.0 pg/mL
     - Tube 5, 6 100 µL = Standard A 0.0 pg/mL
     - Tube 7, 8 100 µL = Standard B 20.0 pg/mL
     - Tube 9, 10 100 µL = Standard C 50.0 pg/mL
     - Tube 11, 12 100 µL = Standard D 150.0 pg/mL
     - Tube 13, 14 100 µL = Standard E 500.0 pg/mL
     - Tube 15, 16 100 µL = Standard F 1800.0 pg/mL
     - Tube 17, 18 100 µL = Standard G 3600.0 pg/mL
     - Tube 19, 20 100 µL = Control 1
     - Tube 21, 22 100 µL = Control 2
     - Tube 23, 24 100 µL = Control 3
     - Tube 25-100 100 µL = Subject Serum
     - Tube 101, 102 100 µL = Control 1
     - Tube 103, 104 100 µL = Control 2
     - Tube 105, 106 100 µL = Control 3
6. Pipet 100 µL of the zero calibrator A into tubes 1-6.
7. Pipet 100 µL of each calibrator B - G into respective tubes.
8. Pipet 100 µL of serum into respective tubes.
9. Pipet 100 µL of each control into respective tubes.

10. Add 1000 μL (1.0 mL) of ^{125}I Estradiol to all tubes 1-106.
    - No more than 10 minutes should elapse while adding ^{125}I E_2.
11. Thoroughly Vortex all tubes.
12. Incubate for 3 hours at room temperature
13. Aspirate tube 3-106 using aspirator with trap.
    - DO NOT ASPIRATE TUBES 1, 2
14. Count tubes in gamma counter for 1-minute.
15. Record counts on data sheet.
16. Calculate E_2 concentrations.

APPENDIX H

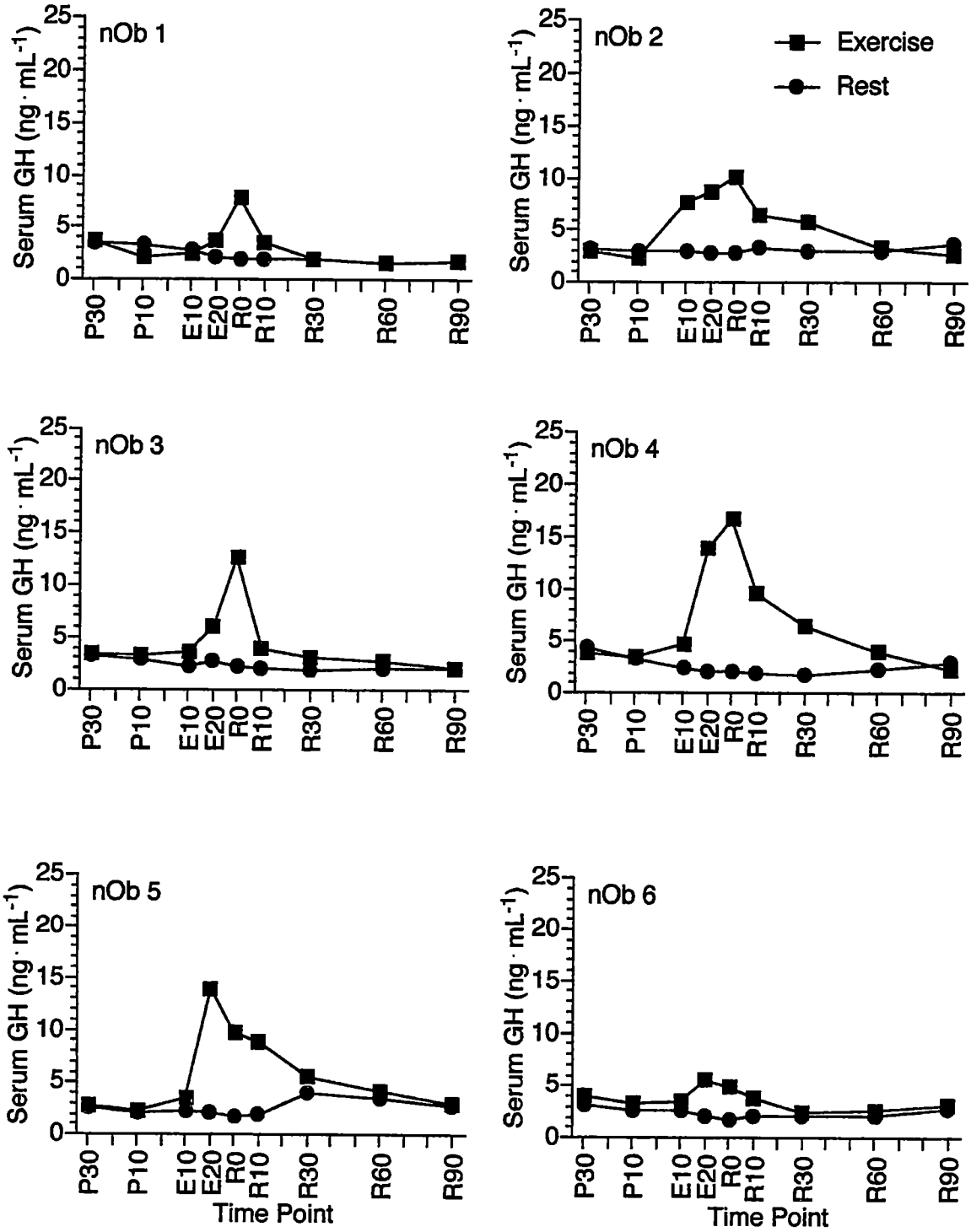
GLYCEROL ASSAY PROCEDURE

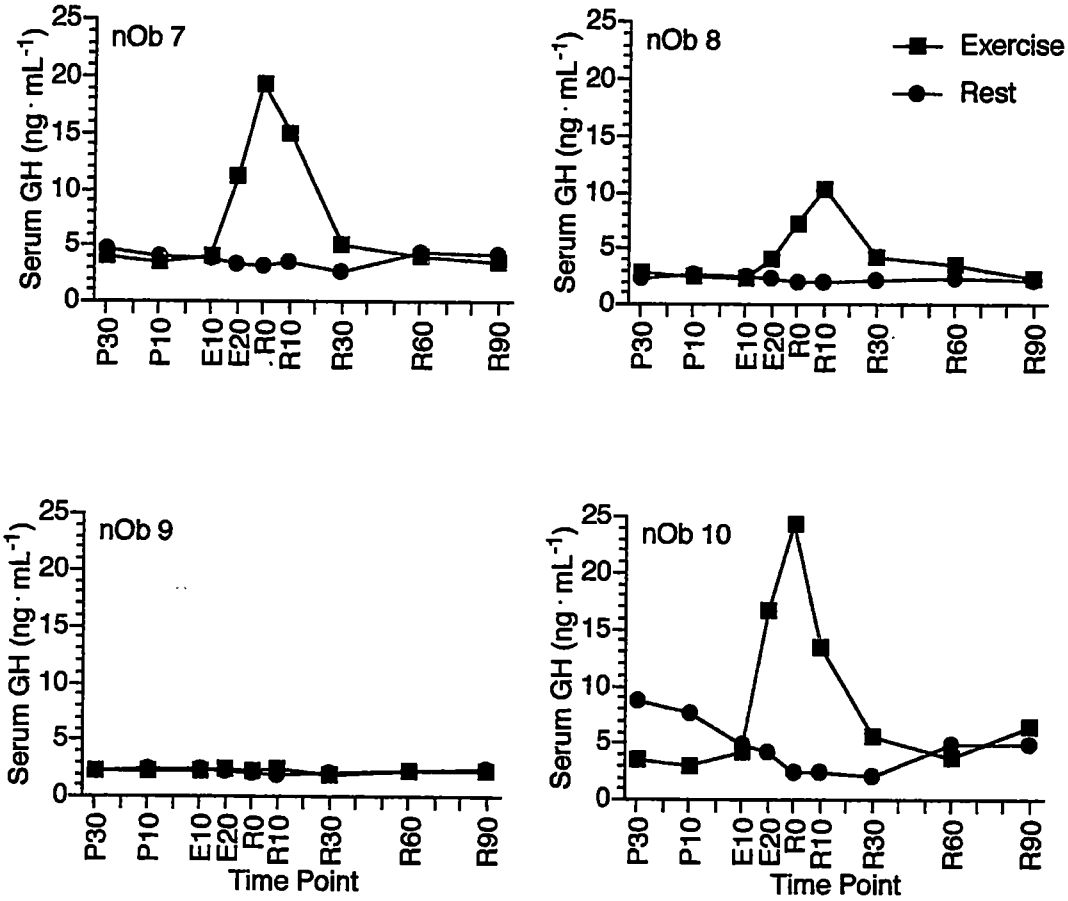
1. Remove samples from deep freezer at least 12 hours in advance and allow to completely thaw to ambient temperature.
2. Turn on spectrophotometer and set to a wavelength of 540 nm and let warm-up for at least 30 minutes.
3. Set absorbance reading on spectrophotometer to zero with distilled water as reference.
    - Push right or left arrow key until "Abs" is visible in upper left corner of LED.
    - Push enter, numbers on LED should flash.
    - Using key pad numbers, set absorbance to λ 540.
    - Push enter, numbers should stop flashing.
4. Reconstitute Blank Reagent A (Triglyceride GPO-Trinder) with 50 mL of distilled water.
    - Remove vial cap and gently inject DI water
    - Immediately stopper the vial
    - Mix several times by inversion - DO NOT SHAKE
    - Store reagent in amber bottle to protect from light
    - Allow reagent to warm to ambient temperature
5. **WASH POWDER OFF GLOVES**
    - Rinse and dry gloves before touching cuvets
    - Only touch front and back of cuvet (cloudy sides)
    - Do not touch the clear sides

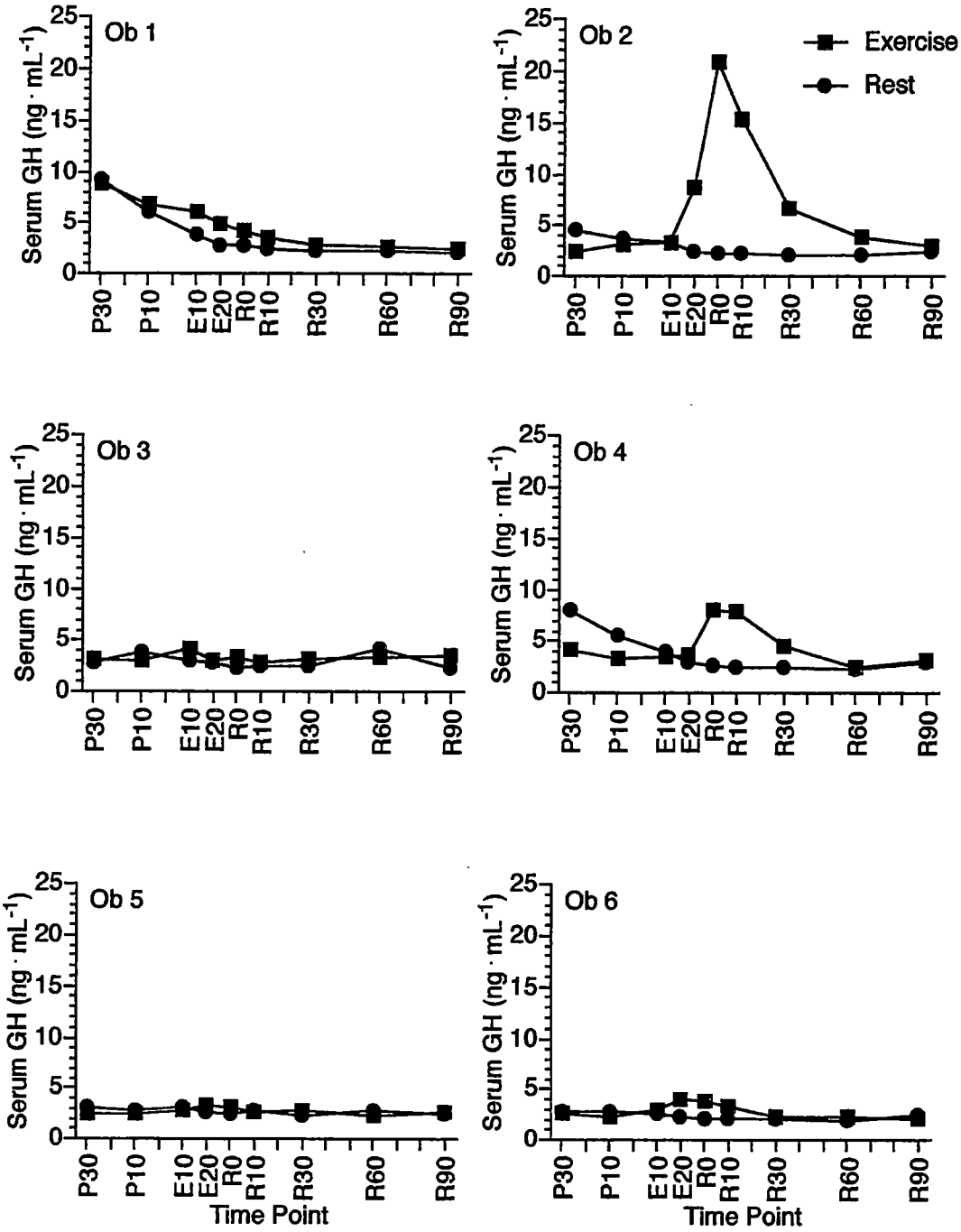
6. Set up a series of labeled cuvets for Blank, Standard, Serum, and Control
    - Label order of each assay will be
        - Tubes 1, 2 = Blank
        - Tubes 3, 4 = Standard 1 (250 mg/dL)
        - Tubes 5-6 = Standard 2 (500 mg/dL)
        - Tubes 7-24 = Subject Samples EX
        - Tubes 25-26 = Control 1
        - Tubes 27-44 = Subject Samples REST
        - Tubes 45, 46 = Blank
        - Tubes 47-48 = Standard 1 (250 mg/dL)
        - Tubes 49-50 = Standard 2 (500 mg/dL)
7. Pipette 1 mL of Blank Reagent A into each cuvet
8. Add 10 µL of DI water to blank cuvet
9. Add 10 µL of Standard to standard cuvet
10. Add 10 µL of Serum to subject cuvet
11. Add 10 µL of Control to control cuvet
12. Cover sample with parafilm
13. Mix sample by gentle inversion - DO NOT LET SAMPLE BECOME FROTHY
14. Incubate sample at ambient temperature for 15 minutes
15. Immediately read and record absorbance of sample
    - Open lid to spectrophotometer
    - Place cuvet in holder with clear sides in light path
    - Close lid and allow to stabilize
    - Record reading from LED
16. Calculate Glycerol Concentration

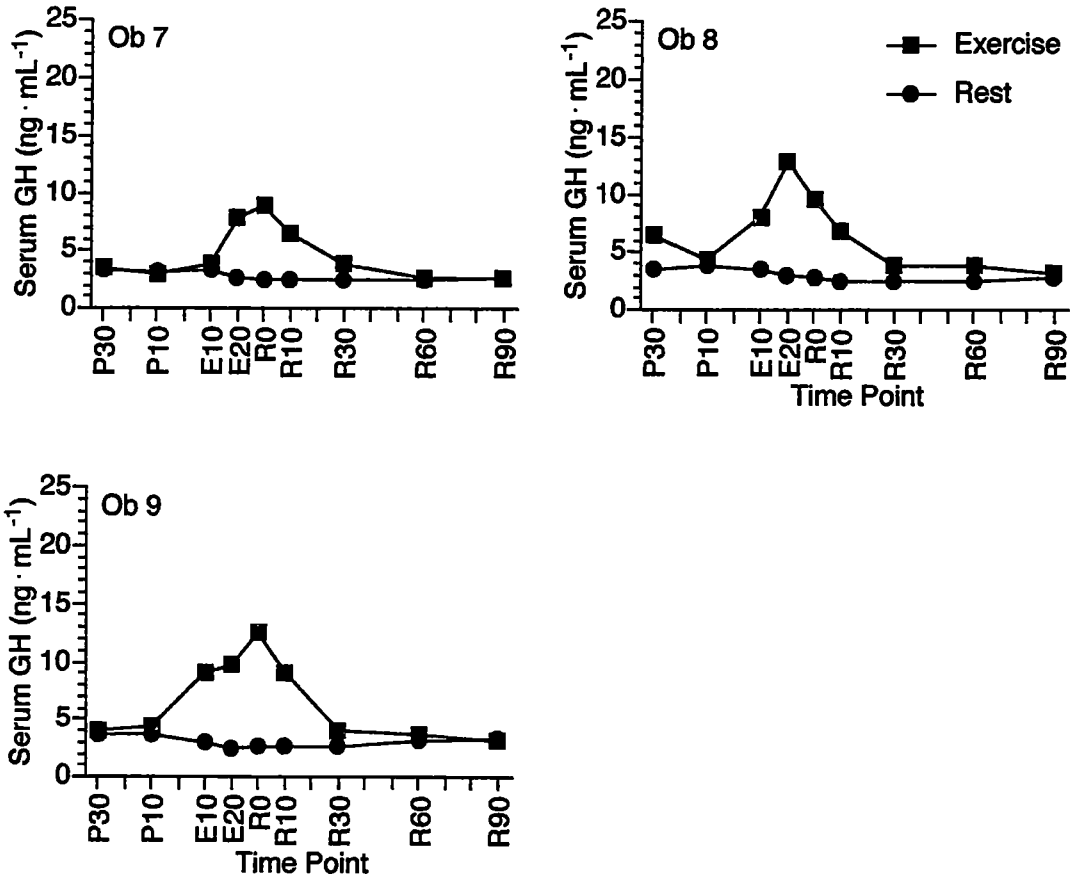
APPENDIX I

IDIVIDUAL GROWTH HORMONE PLOTS




nOb 7
nOb 8
nOb 9
nOb 10
Exercise
Rest
Serum GH (ng · mL-1)
0
5
10
15
20
25
P30
P10
E10
E20
R0
R10
R30
R60
R90
Time Point


Ob 1
Ob 2
Ob 3
Ob 4
Ob 5
Ob 6
Exercise
Rest
Serum GH (ng · mL-1)
0
5
10
15
20
25
P30
P10
E10
E20
R0
R10
R30
R60
R90
Time Point


Ob 7
Ob 8
Ob 9
Exercise
Rest
Serum GH (ng · mL-1)
25
20
15
10
5
0
P30
P10
E10
E20
R0
R10
R30
R60
R90
Time Point

VITA

George A. King was born in Los Angeles, California where he lived until nine years of age when his family relocated to Denver, Colorado. Following high school graduation in 1986, George served in the United States Army for three years as a medical specialist. Upon receiving an honorable discharge from the military, he entered college at Colorado State University. In 1994, George earned his Bachelor of Science degree in Exercise & Sport Science and remained at Colorado State University to work towards a graduate degree. He served as a graduate teaching assistant until graduating in 1996 with a Master of Science degree in Exercise & Sport Science. In the fall of 1997, George continued his graduate studies in exercise physiology at The University of Tennessee, Knoxville, where he worked as a graduate teaching associate and was responsible for the daily management of The Center for Physical Activity and Health. While in this position, George developed, implemented, and maintained a fitness program for the faculty and staff of the university; supervised graduate and undergraduate students working in the Center; engaged in community health promotion activities; and provided a wide variety of health assessment evaluation for the general public. George was also a recipient of the Arthur E. Yates Graduate Fellowship, which partially supported his dissertation research. Following the completion of his Doctor of Philosophy degree in Education in December 2000, he assumed a faculty position at The University of Texas as El Paso.