THE REGULATION OF GROWTH HORMONE SECRETION DURING DIFFERENT PHYSIOLOGIC STATES: INFLUENCE OF PHYSICAL ACTIVITY, ALPHA(α)-ADRENERGIC BLOCKADE AND CORE TEMPERATURE CLAMPING.

Michael Collins Cross

A thesis submitted in conformity with the requirements

for the degree of Doctor of Philosophy

Graduate Department of Community Health

University of Toronto

ABSTRACT

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy in the Graduate Department of Community Health, Faculty of Medicine at the University of Toronto, 1996.

The objectives of this thesis were to examine the regulation of GH secretion during different physiologic stimuli, such as physical activity, α-adrenergic blockade and alterations in core temperature. Specifically, we studied the GH response to several resistance exercise protocols utilizing different loads and frequencies. contraction types and muscle volumes. Similar resistance exercise protocols, with load and frequency of repetition variation, were completed during the infusion of the α-adrenergic blocker, phentolamine. We have also investigated the effect of core temperature during moderate intensity aerobic exercise on the subsequent responses of GH and selective immune system parameters. The findings of this thesis implicate a number of factors in the responses of GH to exercise, such as the α-adrenergic nervous system, products of accelerated glycolysis and the increase in core temperature that is observed during exercise. We also report a technique developed to separate the effects of metabolic heat production from that of energy expenditure during exercise, on the hormonal, immune and metabolic responses to a physical stressor.

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If I have seen further
It is by standing on the
shoulders of giants.

Sir Issac Newton.

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I would like to express my sincere gratitude to Dr. Walter VanHelder for his mentorship and assistance during the course of my graduate school experience. Dr. VanHelder's many accomplishments are a constant source of inspiration to me. He remains one of the world's truly fascinating and gifted persons, who has excelled in both academics and athletics. I thank him for his constant support and interest in my life and for the many long talks and discussions that we have had. May they never be replaced.

Dr. Michael Plyley has provided invaluable advice during my graduate school experience. He continues to be the last hope for successful undergraduate and graduate programs in applied physiology and health sciences in our department at the University of Toronto. I thank him greatly and wish him well in his struggle to protect what is remaining of what once was a successful and thriving academic program.

This thesis was completed under Department of National Defence research contract in order to find better ways to improve the preparedness of our military and special forces personnel. Unfortunately, the Defence Department remains under constant attack from both the political and public arenas. This thesis is partially dedicated to the military professionals who continue to give their lives in the defence of this nation, there is no greater honor. I remind the political influences that continue to erode the ability of our military to fulfill its role;

Freedom is not free.

JFK.

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People have suggested that I have accomplished great things in the last few years. The successful completion of two graduate programs at the same time, the PhD and MBA, combined with sailing for the Canadian National team, may seem like admirable achievements. However, I personally feel that my greatest achievement to date has been the relationship that I have developed with my wife, Dr. Tracy Elynn Hughes. She is my light, my friend and my love. Her dedication and devotion to me, at times when I needed it most, have been without fail. Her support of my studies and my sailing have been without comparison. Thanks, Tray.

And so now the race begins...

MCC.

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PRESENTATIONS AND PUBLICATIONS

The following two papers from this thesis have been publically presented:

1. Speed of muscle contraction affects growth hormone response to weightlifting exercise. American College of Sports Medicine, 38th Annual Meeting, May 29-June 1, 1991, Orlando, Florida USA.

2. Leucocyte and hormonal responses to sustained aerobic exercise with and without a core temperature clamp. International Society of Exercise and Immunology, 2nd International Symposium, November 17-18, 1995, Brussels, Belgium. *

* - Runner-up for best investigation of the conference.

The following papers from this thesis have been published, submitted or prepared for publication:

1. VanHelder, W.P., M.C. Cross, M.W. Radomski & T. VanHelder. (1991). Speed of muscle contraction affects growth hormone response to weightlifting exercise. *Med. Sci. Sports Ex.*, 4, S109.

2. Cross, M.C., M.W. Radomski, W.P. VanHelder, S.G. Rhind & R.J. Shephard. (1996). Leucocyte and hormonal responses to sustained aerobic exercise with and without a core temperature clamp. *Int. J. Sports Med.*, (In-Press).

3. Cross, M.C., M.W. Radomski, W.P. VanHelder, S.G. Rhind & R.J. Shephard. (1996). Endurance exercises with and without a thermal clamp: Effects on leucocyte subsets. *J. Appl. Physiol.*, (In-Press).

4. Cross, M.C., W.P. VanHelder, T. VanHelder & M.W. Radomski, (1995). Growth hormone and catecholamine responses to concentric leg exercise. *Med. Sci. Sport Exerc.*, (Submitted).

5. Cross, M.C., M.W. Radomski, T. McLennan & J. Zamecnik. (1996). Secretion of growth hormone: Influence of core temperature during aerobic exercise. *J. Appl. Physiol.*, (In-preparation).

6. Cross, M.C., W.P. VanHelder & M.W. Radomski. (1996). Role of α-adrenergic activation in the secretion of growth hormone during resistance exercise. *J. Appl. Physiol.*, (In-preparation).

ABBREVIATIONS

GH - Growth Hormone

GHRH - Growth Hormone Releasing Hormone

SS - Somatostatin

IGF - Insulin-like Growth Factor

CA - catecholamines

NE - Norepinephrine

EPI - Epinephrine

$\dot{V}O_2$ max - maximal oxygen uptake

O_2 D/A = $[\int_0^X \dot{V}O_2 \cdot dt] \cdot f$ - oxygen demand/availability ratio

1 RM - one repetition maximum (one complete lift during strength test)

5 RM - five repetition maximum (five complete lifts during strength test)

7 RM - seven repetition maximum (seven complete lifts during strength test)

10 RM - ten repetition maximum (ten complete lifts during strength test)

H-S - heavier loaded, lower number of repetitions and slower contractions

L-F - lighter loaded, higher number of repetitions and faster contractions

H-S (block) - heavier loaded, lower number of repetitions and slower contractions and completed under adrenergic blockade with Phentolamine (Rogitine, Ciba CA.)

L-F (block) - lighter loaded, higher number of repetitions and faster contractions and completed under α-adrenergic blockade with Phentolamine (Rogitine, Ciba CA.)

H-C - hot control trial - 80 min rest in 39° C water

H-E - hot exercise trial - 40 min exercise and 40 min rest in 39° C water

C-C - cold control trial - 80 min rest in 23° C water

C-E - cold exercise trial - 40 min exercise and 40 min rest in 23° C water

Δ - data normalized from time 0

CHAPTER 1

Introduction to the Study of Pituitary Growth Hormone

1-1. Growth Hormone Synthesis and Release:

Growth hormone (GH), also referred to as somatotrophic hormone or somatotrophin, is the major growth stimulating hormone of the body and is secreted from the anterior pituitary. The 191 amino acid (a.a.) monomeric variant of the GH molecule is the dominant representative of this family of growth promoting peptides. The daily production of GH in the anterior pituitary is approximately 0.4 to 1.0 mg and is stored in quantities of 5-15 mg throughout the anterior aspect of the adult human pituitary (Vance et al., 1986).

The secretion of GH from the anterior pituitary is directly regulated by the interaction of two hypothalamic hormones:

1. Growth Hormone Releasing Hormone (GHRH) - stimulatory
2. Somatostatin (SS) - inhibitory

The releasing hormones are secreted from the median eminence of the hypothalamus in response to distinct neurotransmitter stimuli. After these hormones are released from the hypothalamus, they are circulated through the hypothalamic-pituitary portal system to their target gland, the anterior pituitary. Ultimately, these controlling neurosecretory hormones interact with specific receptors on the surface of the somatotrophes, the GH producing cells of the anterior pituitary, where they carry out their respective stimulatory and inhibitory influences on the release of GH into circulation (Figure 1-1).

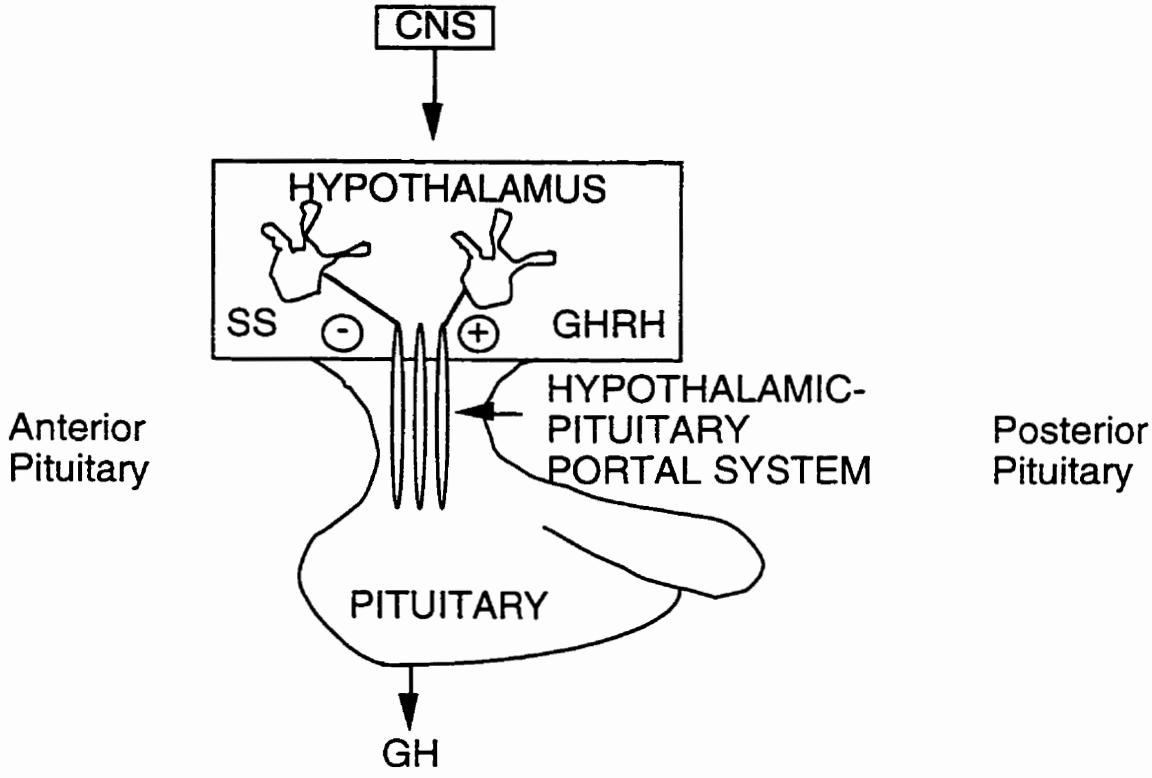

Figure 1-1: *Primary location and control of GH secretion.*

The GH stimulating hormone, GHRH, is produced by cells of the arcuate nucleus of the hypothalamus. After binding with specific receptors on the surface of the somatotrope cells, the 40 and 44 a.a. GHRH acts via the activation of adenyl cyclase to produce cAMP which eventually promotes the exocytotic release of the GH from the somatotrophes (Draznin et al., 1988). The pituitary stores of GH are divided into an immediately releasable form and a more stable compartmentalized hormone reserve (Stachura et al., 1989). The newly synthesized GH is the first to be released, and this is followed by the discharge of hormone previously stored in the vesicles. The mean half-life of GH in the circulation is approximately 16 min. This can be delayed or accelerated depending on whether the GH molecules are in free-form or bound to the GH-binding proteins (BP). The BP are known to prolong the half-life of the GH-complex by delaying metabolism (Baumann, 1991).

While somatostatin is primarily localized in the arcuate nucleus, it is also found in the ventro-medial, suprachiasmatic and paraventricular regions of the hypothalamus (Gabriel et al., 1987). Two forms of SS have been identified in the plasma, a 14 a.a. configuration from the hypothalamus, and a 28 a.a. form, which is secreted from the gastrointestinal (GI) tract tissues. After secretion from the median eminence, the hypothalamic SS is bound to its plasma BP, where its half-life is 1-3 min (Reichlin, 1983). While both SS forms have similar inhibitory qualities, the 28 a.a. GI variant suppresses GH secretion for a longer duration and has three times as many receptors in the anterior pituitary. However, it does appear that the 14 a.a. form is 2-3 times more abundant than the 28 a.a. configuration. Both SS variants block GH secretion after binding to two different anterior pituitary receptor sites (Strobl & Thomas, 1994).

The regulation of the two GH controlling hormones, GHRH and SS, is managed through a number of neurons interacting with the neurosecretory cells of the hypothalamus. Although a number of other neurosecretory substances have been implicated in the regulation of the regulatory hormones, such as serotonin, galanin, γ-aminobutyric acid and opioids, the major neurotransmitter systems involved in the secretion of GHRH and SS from the hypothalamus are:

1. Adrenergic - (norepinephrine and/or epinephrine)
2. Cholinergic - (acetylcholine)
3. Dopaminergic - (dopamine)

The current understanding of the neurotransmitter regulation of GH secretion is demonstrated by Figure 1-2. It is obvious that the neural regulation of the GH releasing factors is accomplished through an interaction of the prevalent neurotransmitters within the hypothalamus, with each system being involved in this regulation depending upon the interaction of specific stimuli.

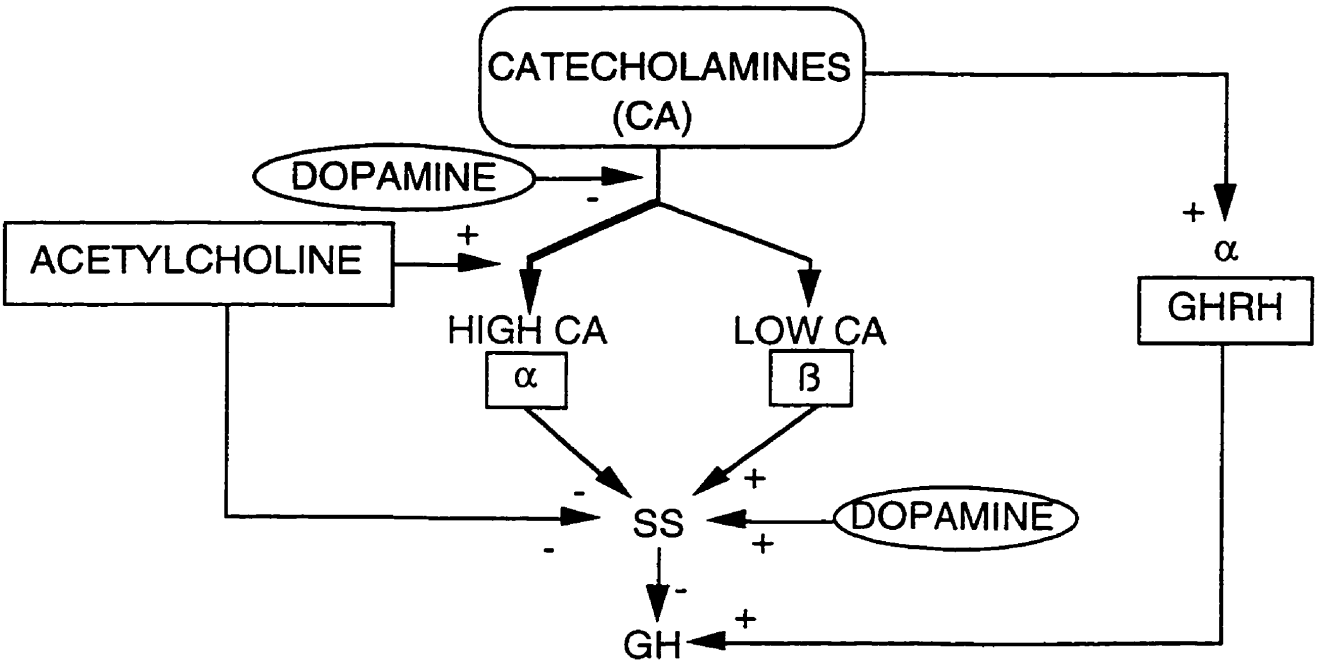

Figure 1-2: *Neurotransmitter control of SS and GHRH.*

It has been known for a number of years that pharmacologic enhancement or blockade of the synthesis/release of central nervous system catecholamines leads to changes in the circulating levels of plasma GH (Massara & Camanni, 1972). Increased concentrations of norepinephrine (NE), and potentially epinephrine (EPI), have been shown to lead to changes in the circulating levels of GH. The adrenergic regulation of pituitary GH has been determined to be both stimulatory and inhibitory in nature. The catecholamine system involves both GHRH and SS release or inhibition through activation of alpha (α) and beta (β) adrenergic receptors located in the hypothalamus (Figure 1-2).

The pharmacologic blockade of α-adrenergic activity, specifically α_2 receptors, by agents, such as phentolamine or yohimbine, inhibits the secretion of pituitary GH. Conversely, the use of adrenergic agonists, such as clonidine, an α_2 stimulator, leads to an increase in the circulating levels of GH (Martin & Millard, 1986). Interestingly, the use of methoxamine, an α_1-adrenergic

agonist, sharply reduces the amplitude of the GH pulses (Bluet-Pajot et al., 1993). While the release of GHRH is promoted by α_2-adrenergic stimulation, Devesa et al. (1990) have concluded that the α_2-adrenergic pathways control the release of GH mainly through the inhibition of SS release, rather than via an extensive stimulation of GHRH secretion.

Previous investigations have demonstrated that the role of β-adrenergic activity, specifically β_2 receptors, in the inhibition of GH secretion is due to the enhancement of SS release from the hypothalamus. The experimental infusion of β-adrenergic agonists, such as isoproterenol, has led to the increase in SS secretion, with a subsequent decrease in circulating GH levels (Kreig et al., 1988). Furthermore, β-adrenergic blockers, such as propranolol, enhance the release of GH induced by different stimuli, including insulin-induced hypoglycemia and amino acid ingestion (Kelijman et al., 1989).

Thus, adrenergic activation plays a dual stimulatory and inhibitory role in the overall regulatory control of GH secretion. This physiologic influence is dependent upon the concentration of catecholamines that are released into the neural system (Figure 1-2). With low catecholamine concentrations, the SS-producing neurons are stimulated to increase SS levels, which subsequently inhibit GH secretion (Devesa et al., 1992). In higher concentrations, catecholamine levels influence the hypothalamic cells by the inhibition of SS secretion and stimulation of the release of GHRH (Devesa et al., 1990).

Thus, it appears that adrenergic pathways function in two primary ways to control the release of GH:

1. Facilitatory - α_2 stimulation to decrease SS release
2. Inhibitory - β_2 stimulation to increase SS release

The adrenergic influence will either activate SS release via the β-adrenoceptors, which are sensitive to low catecholamine concentrations, or through the SS-inhibiting α_2-adrenoceptors, which respond only to excessively elevated levels of catecholamines.

A number of investigators have attributed a major role to the cholinergic pathways in the regulation of GH secretion (Casanueva et al., 1984). By blocking the action of acetylcholine at the central muscarinic receptors with agents, such as atropine, methscopolamine or pirenzepine, the release of GH is greatly reduced due to a number of stimuli, including arginine, clonidine, GHRH, exercise and slow-wave sleep. Furthermore, the use of pyridostigmine, an acetylcholinesterase inhibitor that prevents the breakdown of acetylcholine, or other cholinergic agonists, leads to a marked increase in circulating GH levels (Massara et al., 1984). It appears that the influence of the cholinergic system on the regulation of GH secretion is indirect in that the degree of cholinergic activity acts to condition the amount of adrenergic activity in the hypothalamus (Devesa et al., 1992). In this system, the secretion of the adrenergic neurotransmitters would be magnified or diminished by the influence of the cholinergic fibers interacting with the adrenergic fibers (Figure 1-2).

The dopaminergic system also modulates the release of GH, but does so in a bi-phasic manner, first inhibitory, and later, stimulatory. The use of metoclopramide, a central dopamine receptor blocker, has the ability to increase the release of GH from the anterior pituitary (Devesa et al., 1990). However, Vance et al. (1987) have reported that dopamine also acts to decrease the release of SS from higher portions of the hypothalamus and directly at the level of the median eminence. The use of centrally acting dopamine agonists, such as bromocriptine, leads to an inhibition of GH secretion, which is followed by a rebound stimulation (Miell et al., 1991). Thus, it is believed that the role of dopamine in the control of GH secretion depends on its effects on the adrenergic transmission to SS neurons in the hypothalamus (Devesa et al., 1992).

Growth hormone is normally secreted in episodic bursts or pulses occurring every 3-4 h throughout the 24 h cycle (Figure 1-3). The largest amplitude of these bursts results in a 4-fold increase in circulating GH levels approximately 60-90 min after the onset of sleep and in phase with the occurrence of slow wave sleep (Hartman et al., 1991). In contrast, the lowest levels of GH during sleep are recorded during the morning hours. This pulsatile pattern of GH secretion is important for the numerous metabolic actions of GH that result in normal growth. It is interesting that the target tissues are more sensitive to the frequency of GH secretory bursts than to the total amount of GH secreted throughout a given period (Robinson & Clark, 1987).

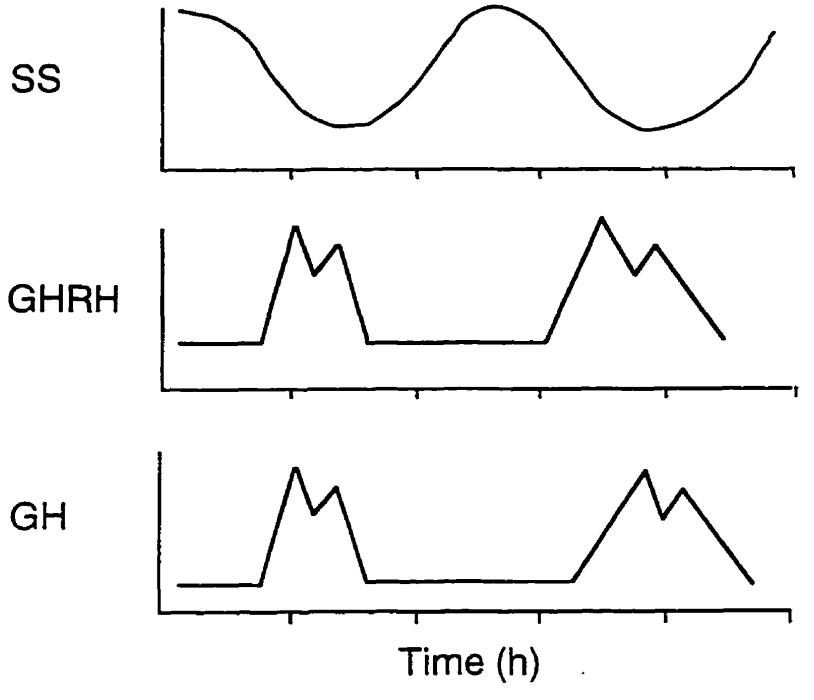

Figure 1-3: Ultradian rhythms of SS and GHRH and subsequent GH responses.

The peaks and troughs theory, developed by Tannenbaum & Ling (1984), was established to explain the typical GH regulation scheme where high levels of SS and low GHRH concentrations result in the inhibition of GH secretion (Figure 1-3). Conversely, low SS concentrations and high GHRH levels lead to increases in circulating GH concentrations. When

studied, the GHRH and SS concentrations appear to be 180 degrees out of phase with each other. As a consequence, the final GH level is a reflection of the interaction of both SS and GHRH. Although GHRH is needed for the GH bursts or pulses, the extent of SS withdrawal actually determines the amplitude of the GH surge (Tannenbaum et al., 1990). Constant GHRH infusion still produces peaks and troughs, as it appears that SS may be the ultimate regulator of GH during these events (Devesa et al., 1989).

1-2. Metabolic Actions of Growth Hormone:

Although GH is vital for the normal development during the pubertal growth spurt, the importance of GH in post-pubertal homeostasis has only recently been investigated (Herman-Bonert et al., 1995; Moller et al., 1995). Whereas the significance of GH in metabolic processes may be indisputable during the pubertal growth spurt, the actions of GH also remain important throughout adult life. Indeed, Moller et al. (1995) have suggested that a major part of the growth-promoting potential of GH may be secondary to the less conspicuous metabolic impact of the hormone. The normal physiologic actions of GH can be classified into the metabolism of lipids, carbohydrates and proteins.

The most striking effect after a single GH pulse is a steep increase in circulating levels of FFA and ketone bodies (Moller et al., 1995). Growth hormone affects the metabolism of fat by increasing its oxidation and release from adipose tissue into the circulation (Davidson, 1987). Typically, a doubling of baseline FFA values is achieved within 2-3 h after exposure to the GH pulse (Moller et al., 1990). The action of GH also tends to cause an increase in fat storage in the liver and a decrease in fat deposition throughout the adipose tissue (Strobl & Thomas, 1994).

The effects of GH on carbohydrate metabolism can be considered both acute and longer term. Initially, GH increases carbohydrate storage by increasing the availability of alternative

fuels, such as lipids (Davidson, 1987; Kostyo & Regan, 1976). Thus, as an increase in lipid oxidation is seen, a concurrent and proportional decrease in glucose oxidation occurs, which would increase the glucose storage levels (Moller et al., 1990). Longer term effects include the increased production of hepatic glucose via gluconeogenesis in order to increase blood glucose concentrations (Karlander et al., 1986) and the suppression of muscle glucose uptake (Moller et al., 1995). The action of GH also produces an impairment in hepatic and peripheral insulin sensitivity, thereby reducing the action of insulin on carbohydrate metabolism (Rizza et al., 1982).

The acute influence of GH on protein metabolism has not been well described, but can be considered to be both protein sparing and protein producing (Moller et al., 1995). The protein sparing effect of GH is similar to its effects on glucose metabolism in that the enhanced transport of lipids into the cell will provide alternative fuel supplies. This shift of energy systems promotes a decrease in the catabolism of structural proteins (Strobl & Thomas, 1994). Protein synthesis is enhanced by GH through an increased transcription and formation of mRNA, the stimulation of mRNA protein synthesis, and an enhanced transport of amino acids (a.a.) into the cell (Fryburg et al., 1991).

The mechanisms behind the actions of pituitary GH are best described by Green's Dual Effector Theory (Green et al., 1985). In this case, Green's group has stated that GH, and its secondary mediator, Insulin-like Growth Factor (IGF), work co-operatively to bring about the changes in growth and metabolism. For example, in bone, GH would stimulate the chondrocytes to differentiate, while the GH-induced IGF stimulation would lead to clonal expansion (Isaksson et al., 1982). The primary IGF protein, IGF-1, is a 70 a.a. long peptide (Bell et al., 1985). While the liver appears to be the most important source of circulating IGF proteins, recent investigations have demonstrated that the local production of IGF in various tissues, such as bone, heart, lung and pancreas, does occur (Isaksson et al., 1988). Thus, the GH-induced secretion of IGF appears to work as a paracrine locally while also maintaining endocrine effects via its hepatic production

and transport into the circulation.

The investigation of GH is now over a hundred years old (Crowe et al., 1910). Previously, it was felt that GH was of insignificant importance in normal adult homeostasis; it is now known that GH plays an important role in metabolic function throughout the life cycle (Salomon & Sonksen, 1987). Thus, the importance of GH in longitudinal bone growth has been supplemented by an understanding of the regulatory properties of GH on lipid, carbohydrate and protein metabolism. Furthermore, the role of GH in other physiologic functions, such as its influence on the immune system, is of growing importance. With this in mind, the control of GH during physical stress and its role in the metabolic adaptation during exercise are important areas of science and medicine. Although physical exercise is one of the most potent stimuli for the secretion of GH, the exact contribution of physical exercise in the secretion of GH has not been completely elucidated and continues to demand attention and investigation. At a general level, it appears that GH secretion is intensity dependent, with the higher intensity exercises producing the greatest responses in GH secretion. It has also been demonstrated that anaerobic activities tend to result in a more amplified secretion of GH than aerobic activities. The mechanisms of the exercise-induced release of GH remain undefined. The main objectives of this thesis were to examine the various factors involved in the regulation of GH during exercise, and attempt to clarify the mechanisms regulating GH release during exercise. Thus, both the characteristics and mechanisms behind the exercise-induced increases in GH have been investigated. The thesis is organized into four separate research projects that were conducted over the last six years at the Defence and Civil Institute of Environmental Medicine and in co-operation with the Mt Sinai Hospital and the University of Toronto.

CHAPTER 2

Overall Literature Review

2-1. Growth Hormone Responses To Physical Stress:

The use of physical exercise as a stimulus for the secretion of GH has been known and practiced for some time (Greenwood & Landon, 1966; Hunter & Greenwood, 1964; Hunter et al., 1965; Roth et al., 1963). However, previous investigations have demonstrated inconsistent hormonal responses both during different types of physical stressors, aerobic and anaerobic, and during continuous versus intermittent exercises. Certainly, it seems that not all types of physical exercise have the ability to stimulate an increase in the secretion of GH. The GH responses to a given physical stressor have been influenced by a number of factors including the intensity, duration, total work output and type of exercise test utilized. Additionally, age, gender, nutritional status, body composition profile, fitness level and environmental conditions, all play significant roles in the GH responses to any exercise challenge (Galbo, 1983).

The secretion of pituitary GH during physical exercise may have lipolytic, glucoregulatory, anabolic or other metabolic consequences, such as influences on the immune system (Galbo et al., 1981; Rhind et al., 1994; Terjung, 1979). Shepherd and Sidney (1975) suggested that the secretion of GH during prolonged exercise was required for the appropriate regulation of fuel availability. However, the hypothesis of the role of GH in the mobilization of free fatty acids as an important energy source has not been generally accepted because increases in serum free fatty acids are not evident until a substantial period of exercise time has elapsed (Galbo, 1983). In the past, a great deal of literature has suggested that GH was not important to continued fuel availability, since GH levels tended to return to baseline levels prior to the completion of long duration exercise (Koivisto et al., 1982). However, it is interesting to note that following an ultra-endurance triathlon race, consisting of a 3.9 km swim, 180 km bicycle ride and a 42 km run, GH levels were

still significantly elevated by the end of the race (Malarkey et al., 1993). The exact role of GH during exercise and its role in training adaptation needs to be addressed. To a varying degree, GH responds to both aerobic and anaerobic exercises.

2-2. Aerobic Exercise:

The use of aerobic exercise as a stimulus for GH secretion has been utilized in both scientific and clinical settings for some time (Roth et al., 1963). Buckler (1972) was one of the first investigators to suggest the use of physical exercise as a diagnostic test for GH deficiency in short-stature pediatric patients. Farrell et al. (1983) found that lower intensity treadmill exercise, 65 % and 80 % of $\dot{V}O_2$ max, for 20 min resulted in significantly greater increases in plasma GH concentration than a much shorter and more intense treadmill run of 100 % of $\dot{V}O_2$ max. Kindermann et al. (1982) proposed that moderate aerobic running of 50 min at the subjects' anaerobic threshold led to the enhancement of GH concentration over that found during short-term (1.5 min duration) anaerobic sprinting at 156 % of the subjects' $\dot{V}O_2$ max. Obviously, work output in these exercise bouts was quite different, making any conclusion about the stimulation of GH secretion impossible. However, in the literature, the exact elements of aerobic exercise that lead to increases in GH secretion have not been defined clearly. It has been suggested that the elevation of GH levels in response to aerobic exercise is intensity dependent (Galbo, 1983).

Lassare et al. (1974) investigated the exercise-induced GH secretion in response to a 1 h cycle ergometer ride with an intensity workload of 57 $W \cdot m^{-2}$ (i.e., $\dot{V}O_2$ = 3.8 $L \cdot min^{-1}$). In these untrained subjects, increases in the plasma GH level only occurred after an initial lag period of 15 to 20 min. Although a significant correlation was found between GH and the initial oxygen deficit, no relationship was established between the GH and glucose or free fatty acid levels during the exercise period. While the investigators acknowledged the possible contribution of lactate to the responses of plasma GH concentrations during the exercise period, they failed to report any

significant correlation between these two variables. Lassare et al. (1974) concluded that there was a relationship between the oxygen deficit and the subsequent GH responses during the exercise test, and that in order for GH to respond to exercise, a threshold of oxygen deficit must first be reached.

Later, Bloom et al. (1976) investigated the hormonal and metabolic responses to cycling in trained and untrained subjects. In this case, each subject completed four successive bouts of exercise at 30, 45, 60 and 70 % of $\dot{V}O_2$ max. Each exercise bout lasted for 8 min. While each of the subjects performed the identical exercise type and relative intensity, the untrained group responded with higher GH, catecholamine and lactate responses than the trained group. The GH concentrations increased continuously as the exercise intensity and duration continued to increase. The GH responses remained elevated for a time following the end of the exercise period, although the catecholamine and lactate levels had already started to decrease towards resting levels. The authors suggested that the differences in GH responses between the trained and untrained subjects were the result of differences in the responses of lactate (Bloom et al., 1976).

Koivisto et al. (1982) investigated the effect of training on the GH responses to a 3 h, low intensity cycle ride at 40 % of the subjects' $\dot{V}O_2$ max. The exercise intensity used in this testing was insufficient to produce any significant change in lactate levels. Although the six week training program resulted in an increase in the $\dot{V}O_2$ max by 20 %, a reduction in the plasma GH response to the prolonged 3 h exercise test was observed. In both the pre-training and post-training exercise tests, the GH level peaked at the 1 h mark, and subsequently decreased until the end of the testing session. Koivisto et al. (1982) declined to suggest a possible mechanism behind these findings, but eluded to the idea that changes in fuel metabolism might have been involved.

In 1988, Barreca et al. (1988) compared the GH responses to two types of exercise in both trained and untrained subjects. The first exercise consisted of cycling at 50 to 60 % of the

subjects' $\dot{V}O_2$ max for 2 h. The second exercise protocol tested was a progressive, incremental cycling activity using 50 W workload increases every 3 min until exhaustion was reached. It is interesting that, while the work output and duration between the two protocols were not identical, the peak GH increases were the largest in the exhaustive cycle ergometer ride in both groups of subjects. While Barreca et al. (1988) have suggested that the production of lactate was responsible for the response of GH, they failed to provide for lactate analysis in their methods and, therefore, cannot offer any data to substantiate their claims.

While investigating the GH responses to cycle exercise, Maresh et al. (1988) employed the use of a moderate level of workload intensity, 50 % of $\dot{V}O_2$ max, for 45 min. This testing protocol produced a 300 % increase in plasma GH levels over the pre-exercise base line values. Farrell et al. (1986) also had subjects exercise on a cycle ergometer, but at a greater aerobic intensity than Maresh et al. (1988). In this case, the investigators used an intensity equal to 70 % of the subjects' $\dot{V}O_2$ max, and then applied the exercise stimulus for 40 min. Although the work intensity was considerably higher than used by Maresh et al. (1988), Farrell et al. (1986) only reported a 166 % increase in serum GH level above the resting, pre-exercise control value.

In that same year, Hagberg et al. (1988) exercised young and old male subjects on a cycle ergometer at 70 % of their predetermined $\dot{V}O_2$ max. The exercise protocol lasted for 1 h in both groups and consisted of repeated blood sampling. The results indicated that GH secretion, while significantly elevated in both groups, was markedly lower in the older age group than in the younger group. The authors attributed the lack of GH response in the older group to the opinion that the older sedentary subjects experienced less physiologic stress than the younger group despite exercising at the identical intensity.

Viru et al. (1992) studied the dynamics of alterations in GH levels in response to prolonged

exercise. In this case, trained and untrained subjects completed a 2 h cycle ergometer test at an intensity equal to 60 % of $\dot{V}O_2$ max. Peak GH level was recorded at the 1 h mark and failed to increase thereafter. Viru et al. (1992) were unable to differentiate statistically the GH responses in the trained group from the responses of the untrained group.

The study of different types of exercise at an identical oxygen uptake has been attempted by a number of investigators. Karagioros et al. (1979) determined that, with an intensity maintained at 45 % of the subject's $\dot{V}O_2$ max, both continuous and intermittent exercise types led to similar GH responses. The continuous exercise group resulted in a GH increase that was 5-fold above the baseline value. In contrast, the intermittent exercise produced a GH response that was 6-fold above the resting GH level. The intermittent exercise also produced the greatest lactate response and an increase in core temperature. The investigation did not report any correlation between the observed GH level and the "remnants" of accelerated glycolysis, such as lactate, pyruvate or alanine. The low intensity nature of this activity failed to provide any real enhancement of the circulating lactate levels, and thus, the results of this investigation suggested that GH release was not related to the oxygen deficit of the exercise. The oxygen deficit, in this case, was calculated as the difference between the oxygen consumption during steady state exercise and the actual oxygen consumption during the transient phase of the first 8 min of exercise.

In order to investigate the differences in the hormonal responses between continuous and intermittent types of exercise, while limiting the influences of other stimuli, VanHelder et al. (1986) utilized a strictly controlled exercise protocol. In this case, the hormonal response to different types of low intensity, aerobic cycling exercise of equal oxygen uptake and duration were described. During cycling at 40 % of $\dot{V}O_2$ max, pedaling rates of 50 and 90 $rev \cdot min^{-1}$ were combined with varying resistance levels in order to equalize the total work performed between the two protocols. The results demonstrated that, when the oxygen consumption was maintained at identical levels, the continuous and intermittent cycling resulted in similar serum GH responses.

This investigation suggested that the control of GH secretion was not dependent upon the number of repetitions, or revolutions in this case, as no difference in the magnitude of GH secretion was observed. It is also interesting to note that there were no statistical differences in the plasma lactate responses between the continuous and intermittent exercise tests. VanHelder et al. (1986) suggested that their results provided indirect support for the hypothesis that the oxygen availability was one of the regulators of GH secretion during exercise. The authors suggested that the similar increases in GH during the continuous and intermittent exercise tests may have been the result of an initial oxygen deficit, as suggested by Lassare et al. (1974), or some other unidentified regulating factor.

While the immediate secretory response of GH to aerobic exercise of varying intensities has been demonstrated, the significance of these findings remain the focus of much debate. There is some speculation that aerobic training may induce an increase in the total 24 h secretion rate of GH (Rogol & Yesalis, 1992; Weltman et al., 1992). Conversely, Hackney et al. (1989) and Hurley et al. (1990) have disputed these claims; in fact, they suggest that the normal daily circulating GH levels are suppressed following endurance exercise training. This belief is supported by a number of other investigators (Galbo, 1983; Koivisto et al., 1982; Sutton & Lazarus, 1976).

The aerobic fitness level of the subject influences GH secretion. A number of investigators have found higher GH levels during exercise in untrained as compared to trained individuals, and that with training the GH response is decreased (Galbo, 1983). In studies completed by Hartley et al. (1972a), Kindermann et al. (1982), Koivisto et al. (1982) and Sutton (1978) the magnitude of the GH release has been shown to be inversely related to the individual's level of fitness. Physical training appears to result in a decreased stimulus or sensitivity for GH secretion during exercise.

However, results from Bunt et al. (1986) indicate that the GH response to exercise in trained subjects was of a greater magnitude than the responses seen in untrained controls, and that

these differences may be due to the type of training that the subjects participate in. This was in agreement with Snegovskaya and Viru (1993a) and Snegovskaya and Viru (1993b), who found greater GH concentrations in well-trained elite rowers than in age-matched, less trained counterparts. These investigators suggested that the functional capacity of the endocrine system was also improved with training. Vasankari et al. (1993) provided evidence suggesting that the resting GH level was more highly elevated in the trained than the untrained subjects, but they reported the exact opposite results 2 h into recovery from a 4 h cycle exercise test. These authors suggested that the higher GH level in the untrained subjects after exercise resulted from the exercise-induced stress on the pituitary which persisted longer in the untrained than the trained subjects. Unfortunately, no explanation has been given for these divergent findings.

The responses of GH during aerobic exercise seem to suggest that the intensity and duration of effort are the most important factors involved in the exercise-induced GH responses. Generally, the higher the intensity and longer the duration of the exercise stimulus, the greater the GH response. A short-term exercise, of either low or very high intensity, results in little GH response.

2-3. Anaerobic Exercise:

The use of higher intensity, anaerobic exercise as a stimulus for GH secretion provides more insight into the possible regulatory mechanisms behind the control of this hormone in response to physical stress. Anaerobic exercise activities are conducted at oxygen uptake levels exceeding the anaerobic threshold, and are characterized by higher levels of lactate, catecholamines and oxygen debt. Unfortunately, most of the research in this area has been directed at documenting the release of GH during various physical exercises, and as such has failed to advance the actual mechanism behind GH regulation during exercise (Kuoppasalmi et al., 1976; Vasankari et al., 1993).

An early study by Hartley et al. (1972b) examined the acute and training responses to intermittent cycle ergometry exercise at 75 % of the subjects' $\dot{V}O_2$ max. Each 20 min exercise period was separated by a 10 min rest period. This exercise:rest schedule was repeated until exhaustion. In the pre-training experiments, Hartley et al. (1972b) demonstrated that the peak increase in serum GH, which occurred at the end of the second interval (40 min), was 300 % above the resting values. The GH levels began to decrease during the next exercise session. After a seven week training program, the peak GH responses to the identical exercise test were more elevated than the responses of the pre-training testing. Hartley et al. (1972b) suggested that the improvement found in endurance times with training was the result of modifications of the endocrine system that resulted in a sparing effect on muscle glycogen while the supply of glucose and free fatty acids was enhanced.

Later, in a study of the hormonal responses during anaerobic exercises, Kuoppasalmi et al. (1976) examined the effects of strenuous maximal running bouts on the subsequent response of GH. All subjects completed a 15 min warm-up period, followed by three 300 m sprint intervals that were separated by a 5 min and a 3 min rest period. A 233 % increase in the circulating GH levels was found immediately after completion of the three sprints. Unfortunately, methodological short-comings, such as the infrequency of blood sampling (pre and post-exercise only), and the inclusion of a significant warm-up period, contributed little to the elucidation of the mechanism of GH regulation.

In an attempt to explain the opioid contribution to the hormonal responses observed during exercise, Grossman et al. (1984) investigated the use of opioid antagonist and placebo-control medications during a high intensity cycle ergometer test. The test, which was conducted at 80 % of the subjects' $\dot{V}O_2$ max for 20 min, was preceded by a significant warm-up period when the subjects cycled at 40 % of their $\dot{V}O_2$ max for 20 min. While the results of the control/placebo investigation demonstrated the ability of the moderately heavy exercise stimulus to increase serum

GH secretion by over 325 % above the pre-exercise base line values, the inclusion of the 20 min warm-up period could have affected the observations made by this group. As a result of using an opioid antagonist, Grossman et al. (1984) concluded that the endogenous opioids played no significant role in the GH responses to the exercise stimulus.

In order to control the number of variables interacting on the human system during the exercise-induced secretion of GH, VanHelder et al. (1984a) compared the hormonal responses to two types of cycling exercise, intermittent and continuous, of identical total external work output and duration, but varied in load and frequency characteristics. The anaerobic. intermittent cycling involved 1 min of exercise at 285 W followed by a 2 min rest period. This work-rest cycle was repeated a total of seven times during the length of the test. The comparative aerobic condition consisted of continuous cycling at 100 W for the entire 20 min testing period. The two protocols were equal in total external work output and duration. While the continuous cycling failed to produce an increase in GH levels, the intermittent exercise protocol resulted in significantly elevated GH and lactate levels during both exercise and recovery periods. The investigators speculated that an oxygen deficit was important in the increase of lactate and GH concentrations in the blood. Later, VanHelder et al. (1985) compared the GH responses to three different exercise protocols: leg squats, intermittent cycling, and continuous cycling. As before, the anaerobic, intermittent exercises, both weightlifting and cycling, produced significantly elevated GH responses, while the continuous, aerobic cycling exercise resulted in GH levels that remained relatively unchanged.

In general, the GH response to anaerobic exercise appears to be related to intensity. As the intensity of the exercise stimulus increases, GH concentrations increase. However, if the exercise is too intense, and therefore, extremely brief in duration, it will fail to produce any elevation of GH levels (Galbo, 1983). Hartley et al. (1972a) reported that plasma GH responses, during short term (5 min) heavy exercise at 100 % of $\dot{V}O_2$ max, were significantly lower than GH levels seen in

moderate exercise, 70 % of $\dot{V}O_2$ max, of a longer duration (8 min). Farrell et al. (1983) reported significantly augmented plasma GH responses to 20 min of treadmill exercise at an intensity of 65 % and 80 % of $\dot{V}O_2$ max compared to a shorter, maximal (100 % of $\dot{V}O_2$ max) treadmill run, although significantly higher lactate responses occurred during the maximal exercise test. Thus, there appeared to be an inverse relationship between the GH and lactate responses. Furthermore, the use of intermittent anaerobic exercise produced a significantly greater GH response than that seen with an exercise bout in which the total work output and duration were identical, but completed in a continuous fashion.

2-4. Resistance Exercise:

Although not studied as extensively as other forms of exercise, the classic form of anaerobic activity, high intensity resistance training has been shown to lead to the enhancement of circulating GH levels both during and following the resistance exercise (VanHelder et al., 1984b). In resistance training, the intensity of the exercise stressor, which is based on a percentage of the subjects' one repetition maximum (RM) load, appears to be one of the most important variables in determining the GH response to a particular exercise session.

One of the earliest investigations into the GH responses to resistance training was conducted by VanHelder et al. (1984b). In this investigation, the GH responses to two leg press resistance protocols were compared. The two protocols were of equal total external work output, duration and work-rest intervals, but, differed in load and frequency characteristics. In this case, an attempt was made to concentrate on only the load and frequency of contraction characteristics as the possible stimulatory influences, and therefore, control the number of interacting variables that might have influenced the GH responses during the exercise test. Through this investigation, it was demonstrated that the heavier loaded, lower repetition exercise led to an increased level of GH, while no statistically significant change in GH was found in the lighter loaded, higher repetition

exercise. In the former case, the GH concentration was correlated with the lactate response. VanHelder et al. (1984b) suggested that the lactate response observed during the heavier loaded, lower frequency exercise might have been responsible for the increase in GH secretion, and that the oxygen deficit that occurred during this specific exercise might be involved as an independent regulator of GH and lactic acid. It should be noted, however, that the heavier loaded, lower frequency exercise was completed at a slower speed of movement, whereas limb movements in the lighter loaded, higher frequency exercise protocol were completed at a higher velocity. In this case, a slow sustained concentric and eccentric contraction during the high load, lower repetition leg press exercise should have resulted in significantly different blood flow responses than the faster, concentric-only contractions of the lower load, higher repetition exercise. A reduction in blood flow to the active tissues would have resulted in increased fatigue and the accumulation of metabolic waste products, such as lactate.

In order to study the adaptive responses in the neuromuscular and hormonal systems during resistance training, Haakinen et al. (1988a) investigated the GH response in young males undergoing a strict weightlifting exercise program. The subjects were trained and tested at intensities that were between 70 and 100 % of their maximal lifting capacity, which was designated as the 1 RM. The GH concentration increased after the first 15 min of exercise and remained elevated for a time after the cessation of the weight-lifting test. Unfortunately, the authors only sought to describe the GH response during a single weight-lifting training session, and in so doing, failed to provide any further insight into the controlling mechanism for GH secretion. Subsequently, Haakinen et al. (1988b), in an attempt to improve the design of training programs for strength athletes, investigated the effect of two successive resistance training sessions in one day on the subsequent hormonal and metabolic responses. In order to simulate more closely the practice of high-performance athletes that train twice per day, each of the 2 h training sessions consisted of Olympic style lifts and were completed at 0900 h and 1500 h during that same day. While the serum GH levels were elevated in each trial, GH levels were significantly lower in the

second test than in the first test. Unfortunately, blood sampling consisted of only pre and post-training tests, and therefore, make it impossible to form an accurate conclusion regarding the control of the hormone response. Although the specific exercise type, total work output and work-rest intervals were not controlled between the two exercise sessions, Haakinen et al. (1988b) did demonstrate that a relative suppression of hormonal responses during the second exercise session, suggesting that training programs could be designed with these influences in mind.

A study of the hormonal response to resistance exercise in young and old untrained subjects was conducted by Craig et al. (1989). Their exercise protocol consisted of 3 sets of 10 repetitions at eight different resistance exercise stations. The secretion of GH increased in both exercising groups, although a greater elevation was observed in the younger group. The level of subject motivation played an important part in this investigation as the only instruction that the subjects received was to increase the work load when 10 complete repetitions could be executed in the final exercise set. Unfortunately, no strength test was recorded, thereby making it difficult to reach any conclusions on GH regulation or GH stimulation. Craig et al. (1989) speculated that the difference in GH response between the age groups might have been caused by either the natural process of aging or by an actual difference in training motivation in the older subjects which would have influenced the actual work intensity completed.

Kraemer et al. (1990) completed a well controlled and planned investigation into the effects of load, rest period length, and total work on a number of different hormonal systems, including serum GH, IGF and testosterone. Two resistance exercise protocols were studied; one based on a strength lifting routine, and the other based on a hypertrophy training program. The total work performed was identical within each of the two protocol types, but the load characteristics varied, consisting of either 5 RM or 10 RM, with rest intervals of either 1 or 3 min. Thus, each subject was tested on six different resistance exercise protocols. The highest GH and lactate responses were found with the 10 RM exercise and the shortest rest interval between each set, 1 min.

Kraemer et al. (1990) concluded that the hormonal response to the resistance training protocols were dependent on the specific characteristics within the resistance exercise protocols, and that a combination of factors related to anaerobic metabolism was involved in the regulation of GH secretion.

The hormonal responses to heavy resistance exercise have not been completely explored. It has been previously shown that this type of physical stress can be a significantly potent stimulus for the secretion of GH. However, not all resistance exercise protocols lead to increases in GH, nor is it known exactly what elements of resistance training are responsible for producing the GH responses. A number of possible exercise stimuli for GH secretion have been suggested. Hypoxia, hypoglycemia, decreased insulin levels and increased lactate levels, to name a few, have all been implicated as stimuli leading to the increase in GH secretion (Grossman et al., 1984; Miller et al., 1984; VanHelder et al., 1986).

2-5. Possible Stimuli for the Regulation of Exercise-Induced GH Response:

The factors controlling the secretion of GH during exercise have not been fully elucidated. A number of investigators have forwarded several theories (Deschenes et al., 1991; Galbo et al., 1983; Kjaer et al., 1987; VanHelder et al., 1987), including the oxygen demand/availability ratio, initial oxygen deficit, lactate or lactic acid, sympathetic output, and a motor cortex-induced central command hypothesis. To date, the only consensus is on the role that exercise intensity plays in regulating the GH response. Generally, as the intensity of the exercise increases, expressed either as a percentage of $\dot{V}O_2$ max or as the number of repetitions maximum used, increases in GH can be expected to occur. However, if the exercise is too intense, as in a short maximal sprint or one repetition exertion, GH does not respond.

The availability of blood glucose both before and during exercise has been implicated in the modulation of the GH response to exercise. When carbohydrate fluids were given before exercise, GH responses decreased (Cappon et al., 1993). Although hypoglycemia is a potent stimulus for GH secretion, blood glucose levels do not change greatly during exercise (Shephard & Sidney, 1975). Furthermore, GH levels have been shown to increase dramatically during exercise in the presence of stable blood glucose levels (Hunter et al., 1965). increasing glucose levels (VanHelder et al., 1984a), or already excessively elevated glucose levels (Berger et al., 1977). It is well known that GH levels during both rest and exercise are elevated, despite a prevailing hyperglycemia in poorly controlled diabetics (Berger et al., 1977; Hansen et al., 1971). Thus, it does not appear that fluctuations in blood glucose levels during exercise are the prime determinant of GH levels.

Until recently, the role of lactic acid during exercise was not believed to be important to the enhanced secretion of GH. Klimes (1977) infused lactic acid into subjects to disrupt the blood acid-base equilibrium but were unable to demonstrate a change in the subsequent GH concentrations. As a result of this and other work (Sutton et al., 1976), the role of lactic acid as a possible metabolic regulator of GH secretion has been challenged and discounted (Galbo et al., 1983). However, it must be remembered that the intracellular and plasma environments are quite different, and that plasma lactate changes during infusion cannot be assumed to reflect the status of the intracellular milieu, nor can infused lactate be expected to move into muscular tissues to trigger any chemical-metabolic receptors.

However, a recent investigation by Luger et al. (1992) has disputed the claims of Klimes et al. (1977) and Sutton et al. (1976). In concentrations equivalent to lactate production during exercise in the range of 70-90 % of $\dot{V}O_2$ max, Luger et al. (1992) infused sodium lactate into resting subjects and demonstrated a significant increase in circulating GH levels. The increases in GH closely paralleled the infusion and increases in lactate levels. Thus, Luger et al. (1992) have

challenged the view of Klimes et al. (1977) that has pervaded the scientific literature since its publication, and the role of lactate production in the regulation of GH during exercise must be reconsidered.

More recently, a study from Kraemer's group (Gordon et al., 1994) investigated the role of acid-base alterations in the responses of GH during exercise. In this study, Gordon et al. (1994) compared the GH responses to a maximal effort 90 s cycle ergometer test under two conditions, alkalosis and control. The control experiment resulted in significant GH elevations above resting values during the entire post-exercise recovery period (30 min). In contrast, the alkalotic trial demonstrated GH concentrations that were statistically above baseline levels only at the end of the recovery period. Significant differences in blood pH were also observed in the control and alkalotic conditions, with the more acidic control condition resulting in the greatest GH responses. Gordon et al. (1994) concluded that an increase in blood hydrogen ion concentration was partly responsible for the GH response to the acute high-intensity anaerobic exercise test.

By concentrating on the oxygen uptake and lactate production during different exercises, VanHelder et al. (1987) described a new factor related to GH secretion during physical stress, the oxygen demand/availability ratio. This ratio was described as the body's need for oxygen compared to the ability of the system to deliver it. Oxygen demand was calculated as the cumulative oxygen consumption during the exercise period (including the debt), while oxygen availability was considered the change in blood lactate levels during the same period (VanHelder et al., 1987). In their investigation, subjects performed seven sets of leg squats at 80 % of their 7 RM. The observed GH responses were highly correlated with the oxygen demand/availability ratio.

When the relationship of the oxygen demand/availability ratio to the exercise-induced GH response was applied by VanHelder's group (VanHelder et al., 1987) to a number of earlier

investigations, (Karagiorgos et al., 1979; Raynaud et al., 1981; Sutton, 1978; VanHelder et al., 1984a; VanHelder et al., 1984b; VanHelder et al., 1986), the correlation between the oxygen demand:availability ratio and the GH response was maintained during intermittent and continuous exercise, weightlifting and cycling, anaerobic and aerobic exercise, in fit and unfit subjects, and during hypoxic and normoxic conditions. VanHelder et al. (1987) concluded that a balance between the oxygen demand and availability at the muscle site was an important regulator of GH secretion during exercise.

Another theory for the control of GH secretion during exercise that has been suggested involves a feed-forward mechanism (Galbo, 1983), and is similar to that proposed for the control of breathing during exercise (Mateika & Duffin, 1995). In this case, a central command output would stimulate the release of GH from the anterior pituitary in conjunction with, or at least at the same time as, motor signals from the central nervous system activated via the given physical motor task (Kjaer et al., 1987). Previous investigations have demonstrated, as is the case in the control of breathing during exercise, the existence of a two-phase hormonal response, an initial fast-response which is followed by a slower and more sustained activation response. Kjaer et al. (1987) proposed that at the onset of exercise, impulses from motor centres in the brain (central command-fast response), as well as from the working muscles, elicit a work load-dependent increase in sympathoadrenal activation and in the pituitary hormone secretion. Although a number of investigators have suggested the existence of a central command mechanism, the intricacy of the central nervous system has thus far made it impossible to substantiate or reject (Galbo, 1983; Kjaer et al., 1987).

It has also been suggested that the core temperature responses accompanying exercise may be responsible for the secretion of GH. It is known that the hypothalamus is involved in the regulation of body temperature and that passive body heating, through the exposure to hot saunas, warm water or heating pad environments, results in an increase in the secretion of GH (Okada et

al, 1970; Okada et al., 1972b). It has been reported that the GH responses during exercise are augmented in the heat and blunted in the cold (Galbo et al., 1979). The true effect of increasing core temperature on the responses of GH during exercise remains to be fully determined as no investigation has been conducted under tightly controlled conditions.

While numerous investigations have been conducted to compare GH responses to various exercise types in an attempt to narrow the possible regulatory mechanisms behind GH regulation, the many differences in exercise-induced GH changes reported in the literature can be attributed to differences in experimental designs that have failed to equalize total work output, duration, work-rest intervals, or did not include an adequate frequency of blood sampling. Furthermore, the type of exercise is an important factor associated with changes in the plasma hormone concentrations, especially those hormones that regulate fuel metabolism. The physiological changes induced by, or accompanying exercise, such as pH, oxygen availability and deficit, as well as thermal status, may play an important role in the hormonal responses to exercise.

2-6. Conclusions:

In order to elucidate fully the control of GH secretion during physical stress, it is necessary to understand the possible hormonal interactions that take place. The GH response to any stimulating activity will depend on its influence during two physiologic situations:

1. A primary potentiation of GHRH while SS is low
2. A primary inhibition of SS and the secondary secretion of GHRH

Since it is unlikely that the GH response to exercise is the result of any direct pituitary stimuli other than via GHRH and SS, it can be speculated that the exercise stimulus operates through the neurosecretory control of these regulating hormones. Furthermore, the importance of properly

conducted, resting control blood samples cannot be stressed enough. Confounding factors, such as pre-exercise psychological stress levels, dietary intake, environmental temperature, sleep deprivation, posture and circadian rhythms all need to be addressed when investigating the influence of a specific exercise type on GH responses (Tremblay et al., 1995).

A great deal of research into the regulation of GH exists that has failed to address the importance of using properly controlled exercise trials. When specific elements of different exercise types are to be compared, the total work performed, duration, work-rest intervals and blood sampling must be adequately matched. Only under strictly controlled conditions can conclusions be made with regards to the regulatory mechanism in the secretion of GH during exercise. VanHelder et al. (1984a, 1984b, 1985, 1986, 1987) and Kraemer et al. (1987, 1988, 1989, 1990, 1993) have published the most tightly controlled research in this field, but the exact nature of the contributory mechanism(s) remain to be determined.

It appears that the majority of the research in exercise endocrinology has concentrated on documenting the hormonal responses to physical activity, while ignoring important questions on the control mechanisms involved in the system. The anabolic, lipolytic and glucogenic consequences of GH action are important bi-products to high performance and amateur athletes, and military personnel who want to optimize their physical training programs in restricted amounts of time. It is therefore meaningful to address the question of hormonal regulatory mechanisms during physical activity. By determining the factors that are most important in the secretion of GH during exercise, physical training protocols can be established that take advantage of high GH levels and the accompanying actions.

2-7. Thesis Objectives and Organization:

The objectives and organization of this thesis are as follows:

Chapter 3 - To define the role of muscle contraction velocity and work:rest intervals in resistance exercise on GH secretion. The hypothesis is that a higher load, lower frequency resistance exercise protocol will induce higher GH responses.

Chapter 4 - To determine the influence of muscle volume on GH responses to resistance exercise. The hypothesis is that larger muscle volumes will induce higher GH responses to resistance exercise than smaller muscle volumes under identical conditions.

Chapter 5 - To determine the role of the alpha-adrenergic nervous system in the secretion of GH during resistance exercise. The hypothesis is that blockage of alpha-adrenergic receptors during exercise will not inhibit the subsequent GH responses.

Chapter 6 - To examine the role of core temperature in the secretion of GH during moderate aerobic exercise. The hypothesis is that the core temperature increases during exercise are not responsible for the accompanying GH responses to exercise.

Note: Due to the distinct differences between males and females and the consideration of significant influences due to the hormonal fluctuations of the menstraul cycle, only males were studied in the four projects encompassing this investigation.

CHAPTER 3

The Role of Muscle Contraction Velocity and Work-Rest Interval Characteristics in the Growth Hormone and Catecholamine Responses to Concentric Leg Muscle Exercise

3-1. Abstract:

Objectives: The objectives of this study were to examine the hormonal and metabolic responses to two resistance exercise protocols of equal total external work output, duration and work-rest intervals but with different load and frequency characteristics. We hypothesized that the velocity of muscle contraction and the length of work-rest interval are important factors in the regulation of GH release during exercise. To minimize further confounding variables, an exercise design was used whereby only a concentric type of exercise using only one muscle group was investigated.

Design of the Study: Fourteen healthy men, aged 24.6 $\pm$ 2.5 years, randomly completed two intermittent resistance training exercise protocols of concentric-only leg extensions, each lasting 18.5 min. The heavier load-slower repetition protocol (H-S) consisted of 7 sets of 7 repetitions at 75 % of each subjects' 7 repetition maximum (7 RM). A lighter load-faster repetition (L-F) protocol consisted of 7 sets of 21 repetitions at one-third of the load of the H-S protocol. To determine the speed of movement characteristics, and deliver the appropriate workloads, all exercise sessions were conducted on a calibrated Ariel computerized ergometer.

Analyses: After resting samples were taken, peripheral venous blood was obtained every 5 min during the exercise period (5 samples) and every 10 min during the 40 min of recovery (4 samples). All plasma samples were analyzed for growth hormone (GH), cortisol, norepinephrine (NE), epinephrine (EPI), glucose, and lactate before, during and after each of the H-S and L-F leg

extensions protocols. Oxygen uptake was continuously monitored by breath by breath metabolic cart analysis. All values were adjusted for plasma volume changes (Dill and Costill, 1974).

Results: Total external work between the two exercise protocols was not significantly different, 11.4 $\pm$ 2.2 kJ for H-S and 11.3 $\pm$ 2.2 kJ for L-F. Speed of movement during the concentric phase of the leg extensions was 141.4 $\pm$ 15.9 deg$\cdot s^{-1}$ in the H-S protocol and 178.0 $\pm$ 17.3 deg$\cdot s^{-1}$ in the L-F protocol ($p<0.05$). Plasma GH was unchanged in the H-S trial, but increased in L-F after 15 min of exercise and remained elevated for 40 min of recovery ($p<0.001$). Cortisol followed a similar pattern in that there was a small response in H-S, while the L-F protocol produced significant increases during the exercise and recovery periods ($p<0.001$). After 5 min of exercise, NE was higher ($p<0.001$) in the L-F protocol than in the H-S trial and remained elevated to the end of the exercise period. The EPI responses were identical to those of NE. Glucose and lactate responses were higher in the L-F protocol than in H-S protocol ($p<0.05$). Significant correlations were found during the L-F protocol between GH responses and the oxygen demand/availability ratio ($r=0.89$, $p<0.05$), GH and NE ($r=0.67$, $p<0.05$), and GH and lactate ($r=0.64$; $p<0.05$).

Conclusions: The experimental design compared two resistance exercise protocols of equal total external work output, duration and work-rest intervals, but different load, rest periods between exercise repetitions, and speed of movement characteristics. The results demonstrated that GH responses during the resistance exercise are related to the speed of movement and the work-rest interval characteristics within each exercise set. The results also suggested that the adrenergic nervous system is involved in the regulation of GH as a significant correlation between the responses of GH and NE was demonstrated in the L-F protocol. In contrast, the H-S protocol failed to produce any significant responses in GH, NE or lactate during either the exercise or the recovery periods. The major finding of this study is that the velocity of muscle contraction as well as the interval between each repetition are important determinants of GH stimulation whereas previous studies have suggested that load and intensity were the key factors.

3-2. Introduction:

For over 50 years, it has been known that GH is essential for normal muscular growth and development. Although various mechanisms for the regulation of GH secretion during exercise have been proposed, many investigators have failed to control for exercise type, work-rest intervals, duration or total external work output (Karagiorgos et al., 1979; Kindermann et al., 1982; Kuoppasalmi et al., 1976; Shephard & Sidney, 1975). A vast majority of the research literature centers around the GH responses to aerobic, with occasional references to anaerobic running and cycling exercise. Given that these exercise types are not popularly known for producing enhancements in muscular growth, and given that GH acts as an anabolic hormone, it is unusual that research studies have concentrated principally on these exercise types. While it is true that GH also plays important physiologic roles in glucoregulation and lipolytic energy metabolism, and that hypoglycemia potentiates the exercise-induced GH response, it has been previously shown that these factors are not responsible for the observed response to exercise in all conditions. Thus, the role of GH in the adaptation to resistance exercise, and any possible regulatory mechanisms for its release, necessitates that this issue be addressed.

Investigations of the GH responses to heavy resistance exercise training, known to lead to muscular hypertrophy, have only recently appeared in the research literature. To date, the most in-depth and controlled studies on the hormonal responses to resistance exercise have been completed by a small group of investigators (Haakinen et al., 1989; Kraemer et al., 1988; VanHelder et al., 1987). The potential for GH to act as an important anabolic and glucoregulatory hormone in the adaptation to resistance exercise dictates that the exact regulatory mechanism(s) of its regulation be accurately described. By characterizing the components of resistance exercise that are important for an enhanced release of GH, military and civilian individuals seeking to utilize its anabolic and lipolytic properties, can better design their exercise training programs for the potentiation of GH response.

3-3. Growth Hormone Responses To Resistance Exercise:

A review of the GH response to resistance exercise by Kraemer (1988) attests to the absence of research in this area, as only two references were described. Early work on the GH response to resistance exercise was published by Skierska et al. (1976) and Lukaszewska et al. (1976). However, since these investigators failed to provide adequate blood sampling techniques, any conclusions based on their data must be examined critically. These authors studied subjects that were completing Olympic weightlifting exercises, but only sampled blood after the training session was completed and after a further 30 min of recovery had elapsed. The type, intensity and total work of the exercise sessions were not controlled nor recorded. To compound the limitations of these studies, the Olympic style of lifting used in the studies, is not known to produce maximal hypertrophic gains, but focuses principally on the power and strength components of adaptation during the weightlifting training. The research results reported by these investigators were merely descriptive of a particular exercise type and did not attempt to describe the possible regulator behind this physiologic response.

In the first well designed and controlled investigation into the hormonal responses of resistance training, VanHelder et al. (1984b) studied two protocols of leg press activity with identical work-rest intervals and total work expenditure. These authors designed the components of the two weightlifting protocols with the intention of comparing a high-load, low repetition (H-S) protocol to a low-load, high repetition protocol (L-F). VanHelder et al. (1984b) demonstrated that the heavier loaded, lower frequency exercise produced greater GH increases than the identical exercise type with a smaller load, higher frequency of repetitions. It was suggested that these increases may have been the result of the observed lactate or oxygen deficit responses. In this investigation, it was also demonstrated that a significant time lag of 16 min existed before increases in GH were observed in each of the subjects. While Galbo (1983) described this time-lag as a result of the long half-life (16 min) of GH, VanHelder et al. (1984) suggested that the observed

GH responses were diluted by the large central blood volume, and therefore, were not detected until reaching significantly higher amounts. The results of VanHelder et al. (1984b) suggested that the load and/or frequency of lifts were determinant factors in the regulation of GH levels during the leg press activity. Furthermore, observations made during the investigation eluded that the speed of movement within each of the protocols may also be responsible for the observed GH responses. While no data was collected to substantiate VanHelder's claims, the perception was that the heavy-slow (H-S) protocol was completed at a slower speed of movement than occurred during the light-fast (L-F) exercise.

Later, VanHelder et al. (1987) examined the responses of GH to leg squat activities. In this case, the hormonal and metabolic responses of seven sets of squats (total power output 600-900 W) were described. Utilizing 80 % of each individual's 7 RM, the subjects completed 7 repetitions within 30 s and then proceeded to rest for an additional 150 s before beginning the next exercise set. A total of 7 sets followed this work-rest schedule (30 s on and 150 s off). Significantly elevated GH levels were reported after 10 min of the exercise protocol. The peak GH levels of 4.95 $\pm$ 0.9 $\mu g \cdot L^{-1}$ were reached at 10 min into the recovery period. The investigators concluded that 7 sets of leg squats each utilizing 7 repetitions of the subjects 7 RM was a potent stimulus of the secretion of pituitary GH. Furthermore, VanHelder et al. (1987) found that the GH responses to the leg squat activity were directly related to the aerobic requirements of the exercise and indirectly to the ability of the body to meet those requirements. VanHelder's' group characterized this physiologic phenomena as the oxygen demand/availability ratio. The oxygen demand of the leg squat activity was calculated as the cumulative oxygen consumption, which was averaged every 5 min during the period of exercise. The oxygen availability of the activity was described as being inversely proportional to the normalized blood lactate concentration, which was also a cumulative index, for that particular sampling time (VanHelder et al., 1987). Thus, analysis of the oxygen demand/availability ratio demonstrated a highly significant correlation with the corresponding time-matched GH concentrations during the blood sampling of the leg squat

activity.

It is necessary to mention that, after further inspection of a number of previously published investigations, VanHelder et al., (1987) were able to demonstrate that the oxygen demand/availability ratio was applicable to a large variety of aerobic and anaerobic, continuous and intermittent, weightlifting and cycling exercises of durations ranging from between 20 to 60 min, in both fit and unfit subjects, and under both normoxic and hypoxic conditions (Karagiorgos et al., 1979; Lassare et al., 1974; Raynaud et al., 1981; Sutton & Casey, 1975; Sutton, 1977). These investigators suggested that differences between the oxygen demand and supply, indicative of conditions at the muscle site, represented an important regulator of GH secretion during a particular exercise (VanHelder et al., 1987).

While VanHelder et al. (1984) investigated the influence of load and frequency of lifts on the subsequent GH responses, Kraemer et al (1990) examined the impact of load, length of rest period and total work on the serum GH response patterns during the exercise period and throughout recovery from two different heavy resistance exercise protocols representing both hypertrophy and strength conventions (3 of each). In this case, variations in load (5 RM vs 10 RM) and rest period length (1 min vs 3 min) were combined to produce six exercise protocols that were closely matched for both total work effects and exercise order. The exercises utilized (eight in total) and the order used were identical between all of the testing protocols. In the hypertrophy series, each of the three exercise protocols consisted of 24 exercise sets, with the primary exercise protocol utilizing a 10 RM per set and a 1 min rest interval. The two control protocols, designated "load" (5 RM and 1 min rest) and "rest" (10 RM and 3 min rest), were also matched with the primary protocol for total work. In the strength series of experiments, each exercise protocol, primary, rest and load, consisted of 93 exercise sets. In this case, the primary exercise protocol utilized a 5 RM per set and 3 min rest period. The two corresponding control series, load (10 RM and 3 min rest) and control (5 RM and 1 min rest), were also matched with the primary workout

for total work effects. While the results of the strength series failed to separate the GH responses of the three specific protocols, the primary workout of the hypertrophy series, utilizing a 10 RM exercise set and 1 min rest intervals, resulted in the greatest GH responses within this protocol series peaking at 25.1 $\mu g \cdot L^{-1}$. Thus, in this case, a longer rest period (3 min) or a higher intensity (5 RM) exercise reduced the GH response observed in the hypertrophy series. Kraemer et al. (1990) supported the findings of VanHelder et al. (1984b) and concluded that the exercise that produced the greatest demands on anaerobic glycolysis also potentiated the serum GH responses. With this in mind, it was demonstrated that the blood lactate response was significantly influenced by the rest period length and duration of exercise. When the rest interval was increased from 1 to 3 min, lactate levels failed to increase. Conversely, when the exercise intensity and within-set exercise duration was changed from 10 RM to 5 RM, as it would take longer to complete 10 repetitions than 5 repetitions, the lactate responses were also reduced. The resistance exercise activities with the lower lactate response also resulted in a lower GH response. The combination of short rest periods (1 min) between sets and the higher intensity (10 RM) and longer duration within the exercise sets, resulted in a greater lactate response and subsequently, a greater GH response (Kraemer et al., 1990).

Later, Kraemer et al. (1991) compared the results of men and women under similar conditions of Kraemer et al. (1990), i.e. utilizing the identical eight exercises. They found that the hormonal response to a hypertrophy protocol, consisting of 10 RM and 1 min rest intervals, were comparable to that of a typical strength protocol of 5 RM and 3 min rest interval. While the female subjects exhibited significantly higher basal levels of GH in the pre-exercise analysis of both testing series, they failed to respond to any of the exercise stimuli within the strength series. The more anaerobic hypertrophy series, and incidentally higher total work, produced a clear and sustained elevation of GH in both the female and male subjects. Unfortunately, these two protocols were not matched for total work effects or duration, and therefore, the results must be viewed with caution. In this case, as was demonstrated by Kraemer et al. (1990), the protocol

which placed the greatest demands on anaerobic glycolysis, i.e., the hypertrophy protocol, also resulted in the greatest GH responses.

In an attempt to substantiate elements of this proposal, Gordon et al. (1994), part of Kraemer's group, manipulated the acid-base balance in subjects performing high intensity anaerobic cycling and examined the subsequent GH responses. Specifically, Gordon et al. (1994) investigated the acute effect of the increase in blood [H^+] on serum GH concentration after a short high-intensity anaerobic exercise bout of controlled intensity and duration. Previous investigations by Sutton et al. (1976), Sutton et al., (1969) and Vigas et al., (1974) had failed to demonstrate consistent increases in the levels of GH in response to lactate infusion of various concentrations. With this in mind, the authors believed that, since lactic acid dissociates completely at physiologic pH, any stimulus for GH secretion arising from lactic acidosis would probably act through either the lactate anion or hydrogen cation (H^+) and not the intact lactic acid (Gordon et al., 1994; Hultman & Sahlin, 1980). The experimental design allowed for the comparison of the GH responses to a maximal-effort cycle ergometer test of 90 s under both alkalotic and normal (control) conditions. Alkalosis was achieved through the ingestion of $NaHCO_3$ prior to beginning the cycle test. Under the control condition, the ingestion of a suitable volume of NaCl, acting as placebo, replaced the $NaHCO_3$. Through this investigation, it was demonstrated that the $NaHCO_3$-induced alkalosis suppressed the acute responses of serum GH to the high-intensity, anaerobic cycle test. The serum GH response rose less quickly and to a lesser extent than that observed when the identical exercise was carried out under placebo ingestion (Gordon et al., 1994).

While the response of blood glucose concentration was unaffected by either the substrate ingestion or the exercise tests in Gordon et al. (1994), whole blood lactate and pH levels were increased in the $NaHCO_3$ trial over those found with the placebo ingestion. The investigators believed that the increased lactate response was the result of an enhanced lactate efflux from the active tissue, both during and throughout recovery from the exercise stimulus. The efflux of

lactate through the active tissue sarcolemma has been described as a carrier-mediated pH gradient-sensitive transport process (Roth & Brooks, 1990). It is believed that this transport system is more active as the intracellular-extracellular [H^+] gradient is increased (Heigenhauser et al., 1991; Roth & Brooks, 1990). Thus, although the exercise intensity was identical between the two exercise conditions, different whole blood lactate responses were observed (Gordon et al., 1994). In this investigation, Gordon et al. (1994) were able to separate the influences of lactate and [H^+] on the exercise-induced GH increases. If lactate, specifically, was the regulator of GH secretion during exercise, the alkalotic condition, with the higher blood lactate level, should have produced the greatest GH response. Instead, the alkalotic condition, with the lowest [H^+] level produced the lowest GH response. These results consequentially implicate [H^+] as the regulator of GH during this exercise condition and not lactate responses, *per se*. However, a given lactate level in the blood does not adequately reflect the level of lactic acid within the active muscle cell. Unfortunately, as the authors failed to sample blood beyond the 30 min of recovery, important post-exercise hormonal responses may have been missed. Indeed, the slope of the serum GH response of the $NaHCO_3$ trial appeared to continue to increase at the last (30 min) post-exercise sampling, while the slope of the GH curve for the placebo trial was becoming less steep at the same time. Thus, quite widely varying responses of GH could have occurred after 30 min of recovery, but any information in this regard was lost due to the blood sampling schedule utilized by the authors. While a number of methodological concerns needed to be addressed in this investigation, these results do extend our knowledge of the regulation of GH during short-term, high intensity anaerobic cycling exercise.

3-4. Literature Conclusions:

Despite an infrequent blood sampling protocol during the actual exercise periods, which do not adequately describe the hormone secretion events, Kraemer et al. (1990; 1991) have suggested that protocols with more frequent repetitions lead to an enhanced GH response. Previous

investigations by VanHelder and co-workers into the regulation of GH secretion during exercise have controlled for the type of exercise, work-rest intervals, duration and total work output (1984b; 1985; 1986; 1987). These findings suggested that the oxygen demand/availability ratio, an indicator of anaerobic glycolysis, described physiologic events that would regulate the responses of GH during a number of different exercise types. In light of these previous investigations, and the work of Kraemer et al. (1990, 1991), it can be suggested that specific components of the exercise set/period, such as load, repetition number, speed of exercise movement, or length of the work-rest interval, may be important factors leading to the release of GH during exercise. It is these attributes of resistance exercise that need to be investigated.

While the investigation of GH secretion in response to resistance training has recently grown in popularity, considerably more research needs to be completed. It is known that the magnitude of the GH response to any exercise stimulus is related to the intensity, duration, total work output and fitness level of the subject (Bloom et al., 1978; Sutton et al., 1974; VanHelder et al., 1987). It is also becoming increasing more suspect that the regulation of GH secretion during heavy resistance exercise is, at least in part, the result of the accumulation of the bi-products of anaerobic glycolysis. VanHelder et al. (1987) have suggested that the GH responses during exercise were reflective of the relationship between the aerobic requirements of the exercise weighted by the inability of the human system to meet those requirements. Gordon et al. (1994) have provided convincing data that the regulation of GH during anaerobic exercise is based on the levels of $[H^+]$ observed during the exercise period. This would not dispute, but would in fact, substantiate the assumptions behind the regulation of GH by the oxygen demand/availability ratio.

3-5. Objectives:

The objectives of this investigation were to examine the:

i. Role of muscle contraction velocity in the hormonal and metabolic responses to two resistance exercise protocols of equal total external work output, duration and work-rest intervals but with varying the load and frequency characteristics.

ii. Role of catecholamines, specifically NE and EPI, in the regulation of GH secretion during two resistance exercise protocols.

iii. Role of the oxygen demand/availability ratio in the regulation of the GH secretion during different resistance exercise protocols.

3-6. Hypotheses:

The hypotheses tested in this investigation were:

i. The heavier load, slower repetition (H-S) protocol, with a lower velocity of muscle contraction would produce greater GH responses than a lighter load, faster repetition (L-F) protocol, with a higher velocity of muscle contraction.

ii. NE responses are not related to the GH responses.

iii. The oxygen demand/availability ratio is not correlated with the response of GH during either resistance exercise protocols.

3-7. Materials and Methods:

This study was completed at the Immunology and Physiology Laboratories of the Defence and Civil Institute of Environmental Medicine at the Canadian Forces Base Toronto. Some additional testing and analysis were also conducted at various departments within the University of Toronto.

i. Subjects: Fourteen healthy males, from the university and military populations that were not currently involved in any resistance training program, volunteered as subjects for this experiment. Each participant was informed of all of the risks associated with the experiment and signed an informed consent which was previously approved by the Ethics' Committees of the University of Toronto and the Defence and Civil Institute of Environmental Medicine. Medical screening excluded any subject with acute or chronic medical conditions. Subjects were requested to abstain from eating, smoking, caffeine and sexual activity for 12 h before any of the testing procedures. Each subject visited the laboratory on five separate occasions, three visits for physical tests and familiarization and two visits for protocol testing purposes.

ii. Physical Tests, Familiarization and Protocol Design: On the first laboratory visit, two weeks prior to beginning the experiment, the physical characteristics for each subject were established. The percentage of body fat of each subject was determined through the use of skin-fold thickness measurements according to the method of Durin & Womersley (1974). In addition, the predicted $\dot{V}O_2$ max was determined using a cycle ergometer exercise test (Astrand & Rodahl, 1977) (Table 3-1).

One week before the experiment, a seven repetition maximum (7 RM) strength test, similar to the strength test used by VanHelder et al (1984b), was performed by each subject using the Global Gym Quadriceps Extension apparatus (Global Gym & Fitness Equipment, Weston ON,

Canada). In this case, after a brief warm-up period, the subject completed 7 full leg extension lifts with a load he could easily accomplish. After a suitable rest period, the load was increased and the subject completed another 7 repetitions. This process was continued until the subject could no longer complete 7 full lifts. The penultimate load was then considered the 7 RM. The quadriceps strength testing also included a 1 RM test after the 7 RM had been determined. Each subject returned to the laboratory at the end of participation in the experiment to perform a re-test of both the 7 RM and 1 RM determinations. No significant differences were found between the pre and post testing results. The results are presented in Table 3-2.

After the strength testing, each subject was familiarized with the leg ergometer (Ariel Computerized Exerciser, Ariel Dynamics Inc. Model # M-4000, Trabuco Canyon, California, U.S.A.). The Ariel controls work loads throughout the range of extensions (preset at 70°) and calculates the velocity and acceleration characteristics of each concentric lift. As the Ariel suspends workloads during the relaxation phase of the movement, no data are collected during the eccentric phase of the knee extensions. To standardize the total external work, a laboratory technician applied an external force after each contraction to reset the apparatus to the starting position. Total external work output was calculated as the product of the load, number of lifts, and the distance of the extension movement (Table 3-2).

The 7 RM was employed as a bench mark for establishing the protocol characteristics, i.e., load and frequency. Seventy-five percent of the 7 RM was used to establish the work load for the two protocols utilized during the actual testing. The heavier load-slow repetition exercise protocol (H-S), consisted of 7 sets of 7 leg extensions using 75 % of the 7 RM. The lighter load-faster repetition (L-F) exercise protocol consisted of 7 sets of 21 extensions, but utilized a load equal to one-third of the load in the H-S.

Testing of the two protocols was conducted randomly, but separated by at least 7 days

(Figure 3-1). Acting as their own controls, each subject performed both protocols, with each protocol testing commencing at 0900 h following an overnight fast. The exercise sets began at 0, 3, 6, 9, 12, 15 and 18 min. The subjects performed the leg extensions, either 7 or 21, in 30 s then rested for another 150 s before beginning the next exercise set. This work-rest interval was used for all 7 sets. The two protocols were of equal total external work output and exercise duration with identical work-rest intervals (30-150 s), but different repetition and load characteristics.

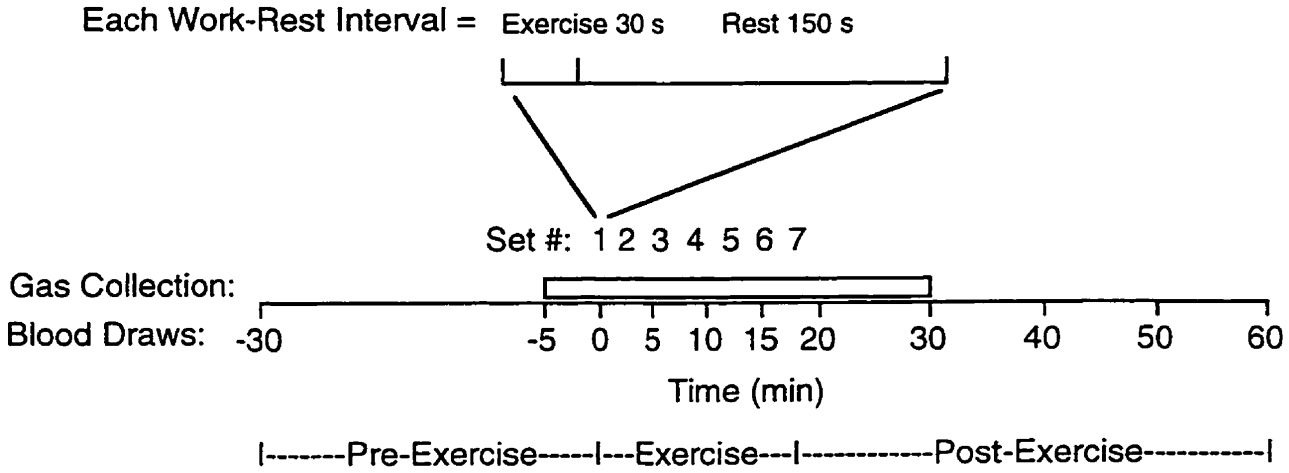

Figure 3-1: *Time sequences for exercise sets and blood sampling in H-S and L-F protocols.*

iii. Measures: A sterile I.V. catheter (Insyte 20G, 2.5 cm), fixed with an injection adapter (Medex 1 7/16"), was inserted into the antecubital vein 35 min before the protocol testing was to begin. The catheter was maintained patent with the use of a 0.6 cc heparin-saline locking solution (100 units•mL^{-1}) which was administered after each sample and removed before the next blood sample was obtained. Resting blood samples were taken from seated subjects at 30 and 5 min prior to each start of the protocol testing. After the 5 min, pre-exercise blood sample, each subject was seated on the Ariel. Venous blood sampling continued during the exercise period at 0, 5, 10, 15 and 20 min and throughout 10, 20, 30, and 40 min of recovery. Immediately after filling each

vacutainer tube, the contents were mixed by gentle repeated inversion. Blood samples for GH and cortisol were collected in 10 mL tubes containing 0.4 mL EDTA acid (15%) solution. Blood samples for catecholamine analysis were collected in 10 mL tubes containing 143 USP units heparin and 60 mg glutathione while blood samples for glucose were collected in 3 mL tubes containing 30 mg sodium fluoride (NaF) powder. A 25 µL sample from this tube was mixed with 200 µL of ice-cold perchloric acid ($HClO_4$) for lactate analysis. A 25 µL sample was also used to determine the hematocrit and hemoglobin levels for the calculation of blood and plasma volume changes. Each vacutainer was immediately centrifuged at 4° C and 2500 G for 15 min and the separated plasma frozen at -70° C until analysis. As each blood sampling interval consisted of 23 mL volume, the total blood volume obtained during each protocol test was 253 mL.

Oxygen consumption was measured by continuous sampling and gas analysis on a calibrated metabolic cart (Sensor Medics MMC Horizon System 4400, Anaheim CA, U.S.A.) 10 min before exercise, throughout the exercise testing and continuing until 10 min into the recovery period.

iv. Analysis: All subsequent biochemical analyses were performed in duplicate, except for GH which was performed in triplicate. Plasma GH and cortisol levels were analyzed using double-antibody radioimmunoassay kits, (Pharmacia, Uppsala, Sweden and Farmosgiagn Diagnostica, Espoo, Finland, respectively). The standard curve for GH was modified with the addition of three extra standards for low GH levels (0.25, 0.0125 and 0.00625 $\mu g \cdot L^{-1}$) in order to extend the curve to allow the detection of extremely low resting GH levels. Radioactivity was determined using a calibrated Cobra Auto-gamma Counter (Packard Model 5002, Meriden CT, U.S.A.). Catecholamine levels were analyzed on 5 mL plasma samples using high performance liquid chromatography and electrochemical detection (ESA HPLC, Coulochem II, Bedford MA, U.S.A.) according to the methods of Munoz et al (1989). Glucose levels were determined with a glucose enzyme kit (Boehringer Mannheim, Mannheim Germany) as described by Trinder (1969). Lactate

levels were determined with the use of a lactate enzyme kit (Boehringer Mannheim, Mannheim, Germany) as described by Maughan (1982). Colorimetric analysis for glucose and lactate was on the Gilford Stasar III Spectrophotometer (Gilford Laboratories INC., Oberlin OH, U.S.A.) and the Perkin-Elmer 650-10M Fluorescence Spectrophotometer (Hitachi Ltd., Tokyo, Japan), respectively. Hematocrit and hemoglobin levels were determined with the use of microhematocrit capillary tubes (Autocrit Ultra-3, Franklin NJ, U.S.A.) and the Hemocue β-Hemoglobinometer (Hemocue Photometer, Helsingborg, Sweden), respectively. The responses observed in each protocol were corrected for percent changes in plasma volume by the method of Dill & Costill (1974) in order to eliminate the influence of plasma volume shifts during the exercise and recovery periods.

The oxygen demand/availability ratio was calculated according to the method described by VanHelder et al. (1987). Oxygen demand was calculated by measuring the cumulative oxygen consumption over the time (t) of the exercise period and was expressed by the following equation:

$$\text{Oxygen Demand} = \int_0^x \dot{V}O_2 \cdot dt \text{ (where x = sampling time during exercise)}$$

Blood lactate concentration was measured at each corresponding time period and oxygen availability was expressed as being inversely proportion to the blood lactate concentration at that time:

$$\text{Oxygen Availability} = 1/f \text{ (where f = [lactate at time x]/[lactate at time 0]}$$

The oxygen demand/availability ratio (O_2 D/A) was then expressed as:

$$O_2\,D/A = [\int_0^x \dot{V}O_2 \cdot dt] \cdot f$$

and compared to the normalized changes in plasma GH levels during the exercise period.

v. Statistical Analysis: As no significant differences were found in any of the measured resting blood factors between -30, -5 and 0 min, all exercise and recovery levels were normalized with respect to the average of the three resting levels and compared using a repeated measures ANOVA (Superanova, Abacus Concepts Inc., Berkeley CA, U.S.A.). Post-hoc analysis was also completed using a means comparisons. The integrated area under the response curve was calculated using the trapezoidal rule for unequally space X values. In addition, relevant correlations for each hormone were also calculated and compared between the H-S and L-F protocols (Statview, Abacus Concepts Inc., Berkeley CA, U.S.A.). The level of statistical significance accepted was $p<0.05$. Mean data are presented using SE in all figures, and SD in all table, presentations. Where error bars do not seem to be presented, the particular error for that graph was too small to be included in that specific figure.

3-8. Results:

i. Subject Characteristics:

All of the fourteen subjects were able to complete all phases of the experimental design. The physical characteristics for each of the subjects are presented in Table 3-1.

TABLE 3-1:

Physical characteristics of subjects (n=14).

Characteristic (unit):	Mean ± SD	Range
Age (y)	24.6 ± 2.5	21-29
Body Mass (kg)	77.0 ± 7.5	68.0-93.6
Height (m)	1.79 ± 0.07	1.68-1.9
Body Mass Index (kg/m^2)	24.2 ± 2.5	18.8-27.9
Body Fat (%)	15.9 ± 2.8	10.3-23.5
$\dot{V}O_2$ max ($L \cdot min^{-1}$)	3.4 ± 0.4	2.8-4.3
$\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	44.1 ± 6.1	37.5-51.5

Data collection for this experiment began in mid-March and was completed by the beginning of May of that same year. Throughout the experiment, there were no significant changes in the subjects' fitness levels or medical conditions (Table 3-2).

The calibration of various laboratory devices (Ariel, metabolic cart, ect) was completed before and after each testing session and demonstrated no significant variation throughout the

length of the experiment. When applicable, biochemical analysis was completed immediately following the collection of the sample or following the amassing of a suitable pool of samples, in each case using appropriate controls.

TABLE 3-2:

Strength testing and workload determinations.

Characteristic (unit):	Mean ± SD	Range
Seven Repetition Maximum - 7RM (kg)	59.5 ± 11.6	45.0-75.0
One Repetition Maximum - 1RM (kg)	75.7 ± 18.5	54.5-115.9
Workload for H-S (kg)	44.7 ± 8.7	34.1-56.3
Workload for L-F (kg)	15.0 ± 2.7 *	11.3-18.6
Total Work for H-S (kJ)	11.4 ± 2.2	8.7-14.4
Total Work for L-F (kJ)	11.3 ± 2.2	8.7-14.3
Speed of Movement for H-S ($deg \cdot s^{-1}$)	141.4 ± 15.9	115.0-164.0
Speed of Movement for L-F ($deg \cdot s^{-1}$)	178.0 ± 17.3 **	155.5-225.0

* $p<0.05$ significance from H-S trial
** $p<0.005$ significance from H-S trial

ii. Speed of Concentric Movement:

The total work completed during each of the two protocols was not significantly different from each other (Table 3-2). The speed of concentric movement during the leg extensions was significantly higher ($p<0.005$) in the Light-Fast (L-F) protocol than the Heavy-Slow (H-S) protocol [178.0 ± 15.9 vs. 141.4 ± 17.3 $deg \cdot s^{-1}$ (mean, ± SD), respectively] (Table 3-2). The Ariel did not provide any data during the eccentric or relaxation phase of the movements, as the ergometer suspended the workload, and the apparatus was reset to the starting position.

iii. Growth Hormone (GH):

Resting GH concentrations were not statistically different between the two exercise protocols (0.89 $\pm$ 0.4 and 0.81 $\pm$ 0.4 $\mu g \cdot L^{-1}$ in H-S and L-F, respectively). However, statistically significant different patterns in the response of Δ GH were observed during both the exercise and recovery periods (Figure 3-2).

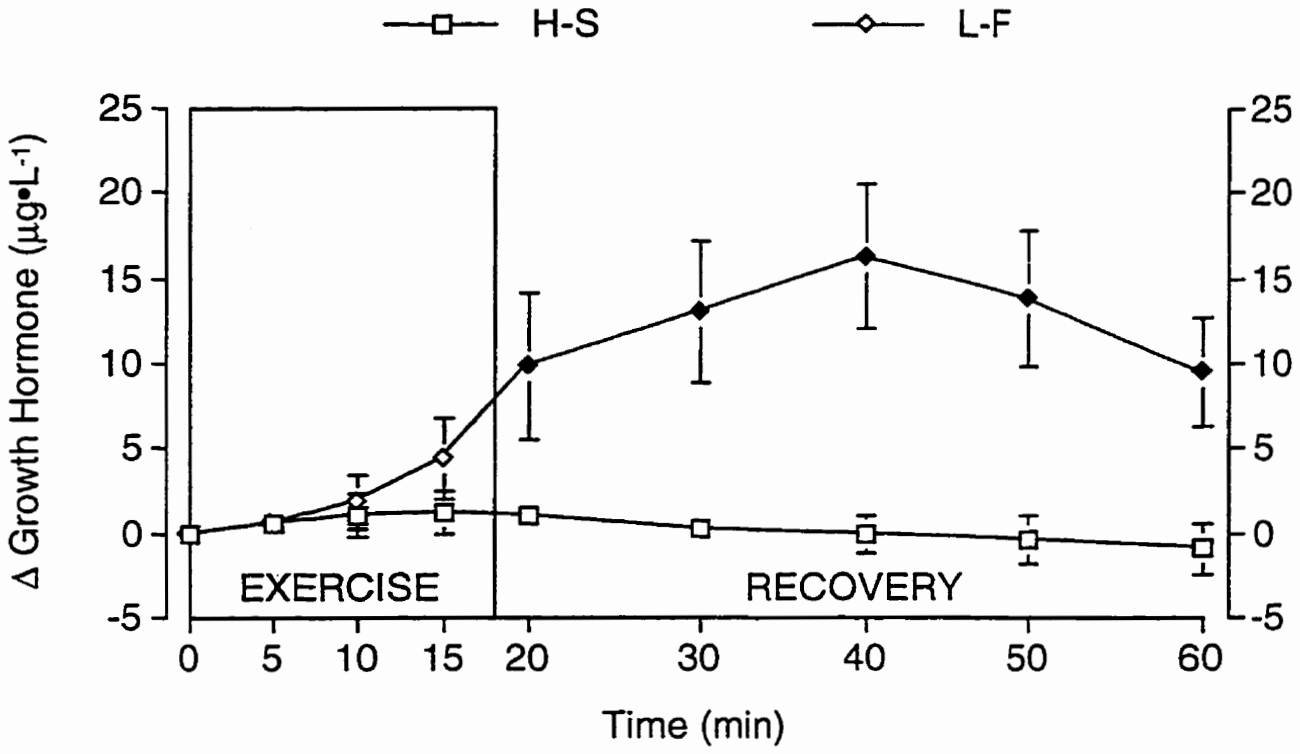

Figure 3-2: *Mean Δ GH responses in H-S and L-F protocols. Solid symbols indicate statistically significant responses between the H-S and L-F time pairs ($p<0.05$).*

Growth hormone failed to increase significantly beyond basal resting levels during the H-S protocol. However, after an initial lag period of 10 min from the beginning of exercise in the L-F protocol, GH increased to significant levels over the GH levels in the H-S trial by 15 min of

exercise. The responses of GH remained statistically elevated over the response observed in the H-S protocol, and continued to increase throughout the exercise period, reaching a peak at 20 min into the recovery period. After peaking at 16.3 ± 4.2 $\mu g \cdot L^{-1}$ at 40 min, GH values decreased to 9.6 ± 3.2 $\mu g \cdot L^{-1}$ after the 20 min of recovery.

The total area under the Δ GH response curve (60 min) for the exercise and recovery periods was 68-fold higher in the L-F protocol (617.4 ± 164.6 $\mu g \cdot L^{-1}$) than in the H-S protocol (37.8 ± 16.5 $\mu g \cdot L^{-1}$) ($p<0.001$) (Figure 3-3).

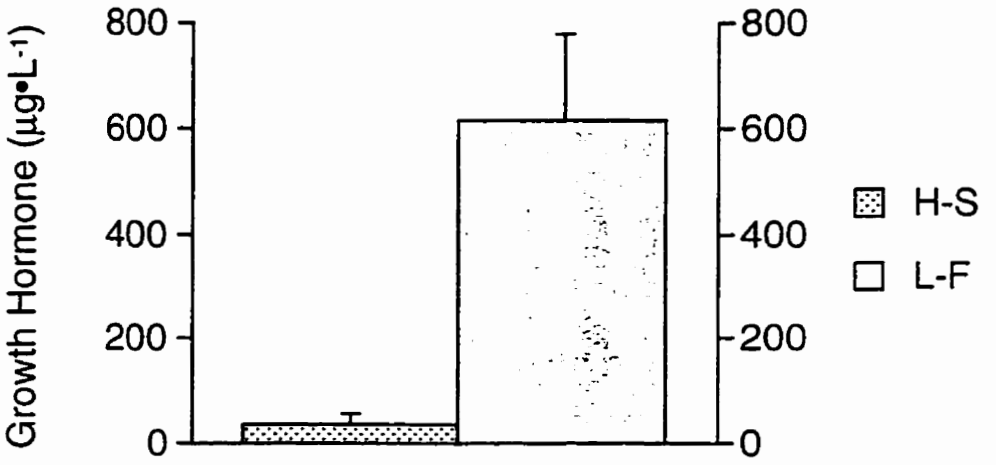

***Figure 3-3**: Incremental area of GH in H-S and L-F.*

iv. Cortisol:

The resting basal levels for cortisol were 239 ± 19.5 $nmol \cdot L^{-1}$ in H-S and 208 ± 22.4 $nmol \cdot L^{-1}$ in the L-F protocol, which were not significantly different from each other. In both of the resistance exercise protocols, Δ cortisol followed similar patterns to those responses observed for Δ GH (Figure 3-4). Following the pre-exercise sampling, the H-S and L-F exercise protocols produced significantly different Δ cortisol values.

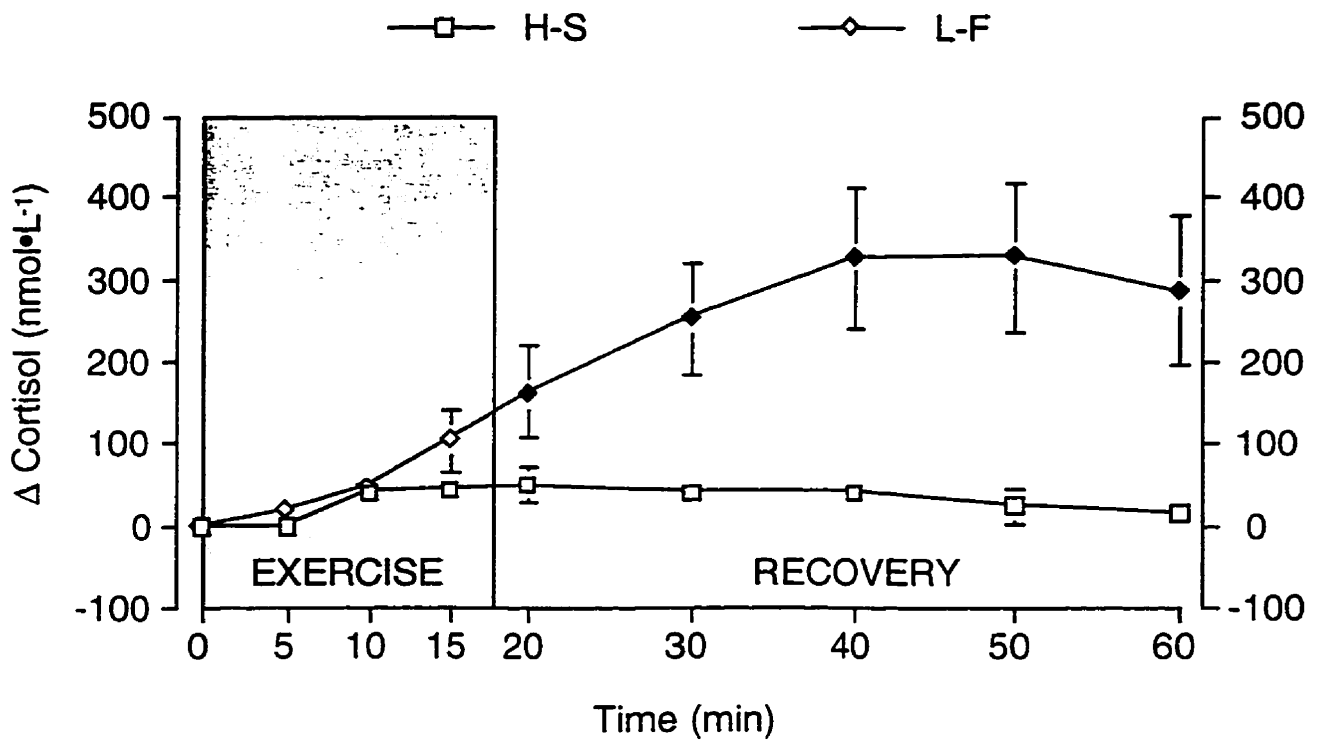

Figure 3-4: *Mean Δ cortisol responses in H-S and L-F protocols. Solid symbols indicate statistically significant responses between the H-S and L-F time pairs ($p<0.05$).*

Peak cortisol levels of 50.6 ± 21.7 nmol•L^{-1} occurred immediately at the end of the exercise period (20 min) in the H-S protocol and then proceeded to decrease throughout the 40 min of recovery to 15.5 ± 18.2 nmol•L^{-1}. The cortisol responses in the L-F protocol differed from those of the H-S protocol, becoming significantly more elevated in the L-F trial immediately following the exercise period (20 min). Peak levels in L-F occurred at the 40 min mark at 329.9 ± 91.2 nmol•L^{-1}, followed by a decrease to 287.6 ± 91.7 nmol•L^{-1} by the end of the recovery period (60 min).

The total area under the cortisol response curve (60 min) in the L-F protocol was 7-fold higher than in the H-S protocol (2205.5 ± 668.1 nmol•L^{-1} vs 13658.9 ± 3511.3 nmol•L^{-1}) ($p<0.001$) (Figure 3-5).

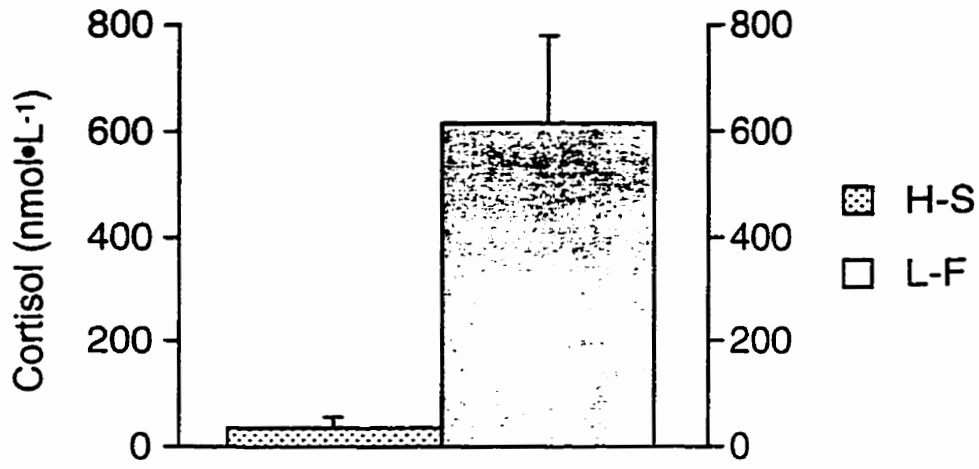

***Figure 3-5**: Incremental area of cortisol in H-S and L-F.*

v. Norepinephrine (NE):

Figure 3-6 describes the NE responses in the H-F and L-F protocols.

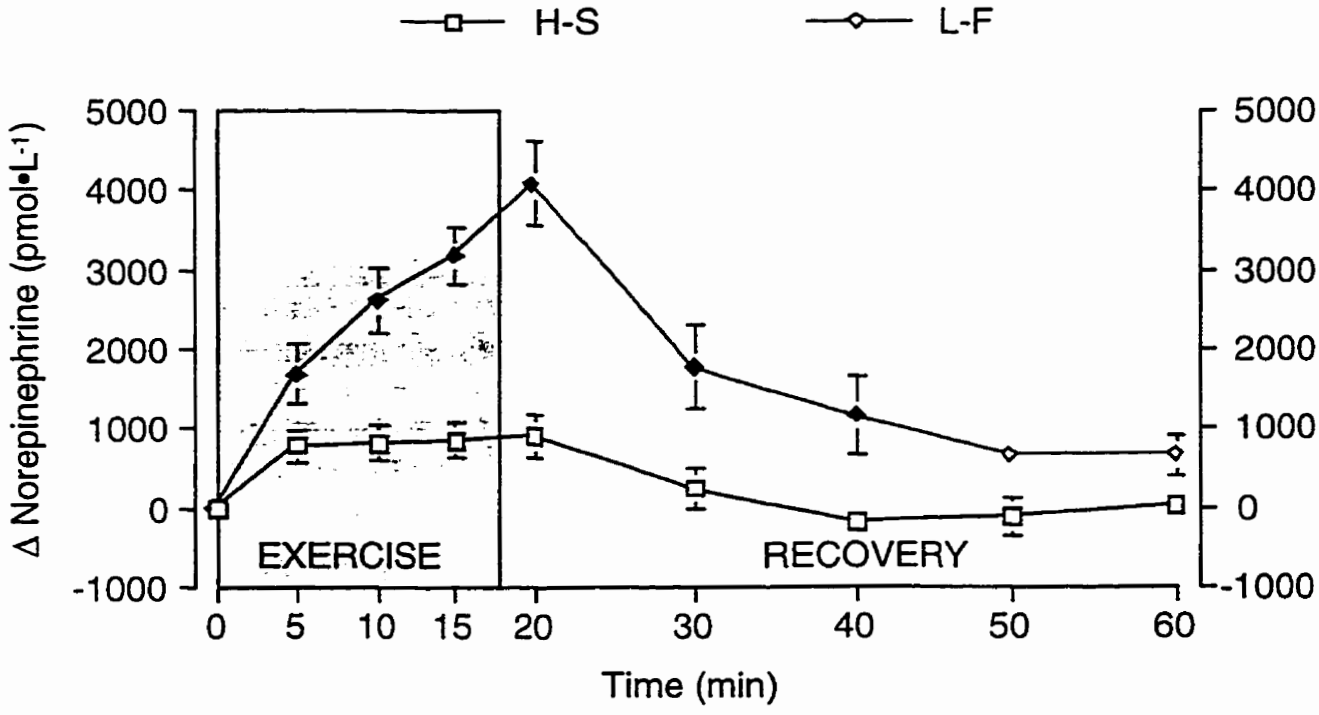

***Figure 3-6**: Mean Δ NE responses in H-S and L-F protocols. Solid symbols indicate significant responses between the H-S and L-F time pairs ($p<0.05$).*

Basal levels of NE were 2430.6 ± 139.9 pmol•L^{-1} and 2510.5 ± 165.7 pmol•L^{-1} in H-S and L-F (n.s.), respectively. The responses of Δ NE are demonstrated in Figure 3-6. Significantly different responses in NE during the exercise and recovery periods were found in the two protocols. No significant increases in NE occurred during the H-S protocol. In contrast, NE levels increased significantly during the exercise period in the L-F trial and peaked at the completion of the protocol (20 min) at 4080.2 ± 522.4 pmol•L^{-1}. Levels of NE decreased after the cessation of exercise to 658.7 ± 263.3 pmol•L^{-1} by 40 min of recovery.

The total area under the NE response curve (60 min) was 6-fold higher in the L-F protocol (101,625 ± 16,331.8 pmol•L^{-1}) than in the H-S protocol (23,980.8 ± 7978.1 pmol•L^{-1}) ($p<0.001$) (Figure 3-7).

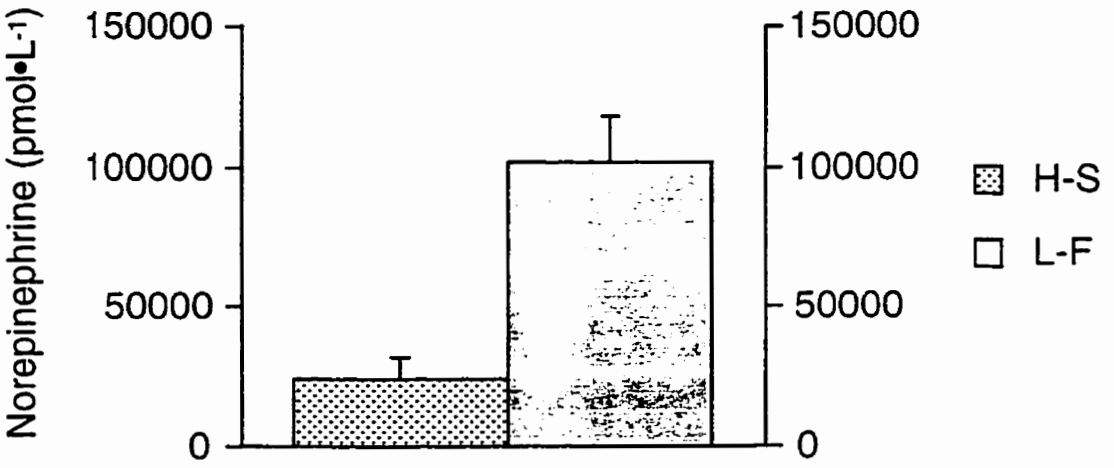

***Figure 3-7**: Incremental area of NE in H-S and L-F.*

vi. Epinephrine (EPI):

Resting basal levels for EPI were not significantly different between the two exercise

protocols (191.1 ± 28.9 and 210.0 ± 26.7 pmol•L^{-1} for the H-S and L-F, respectively). The Δ EPI responses are demonstrated in Figure 3-8. Epinephrine concentrations responded similarly as the responses of NE in both protocols. The EPI responses in the H-S protocol demonstrated little variation above resting basal levels. However, in the L-F protocol, EPI levels increased significantly during exercise and reached peak maximal levels of 283.5 ± 54.8 pmol•L^{-1} at the end of the L-F exercise period (20 min). The EPI levels then proceeded to decrease throughout the recovery stage to finish at ± pmol•L^{-1}.

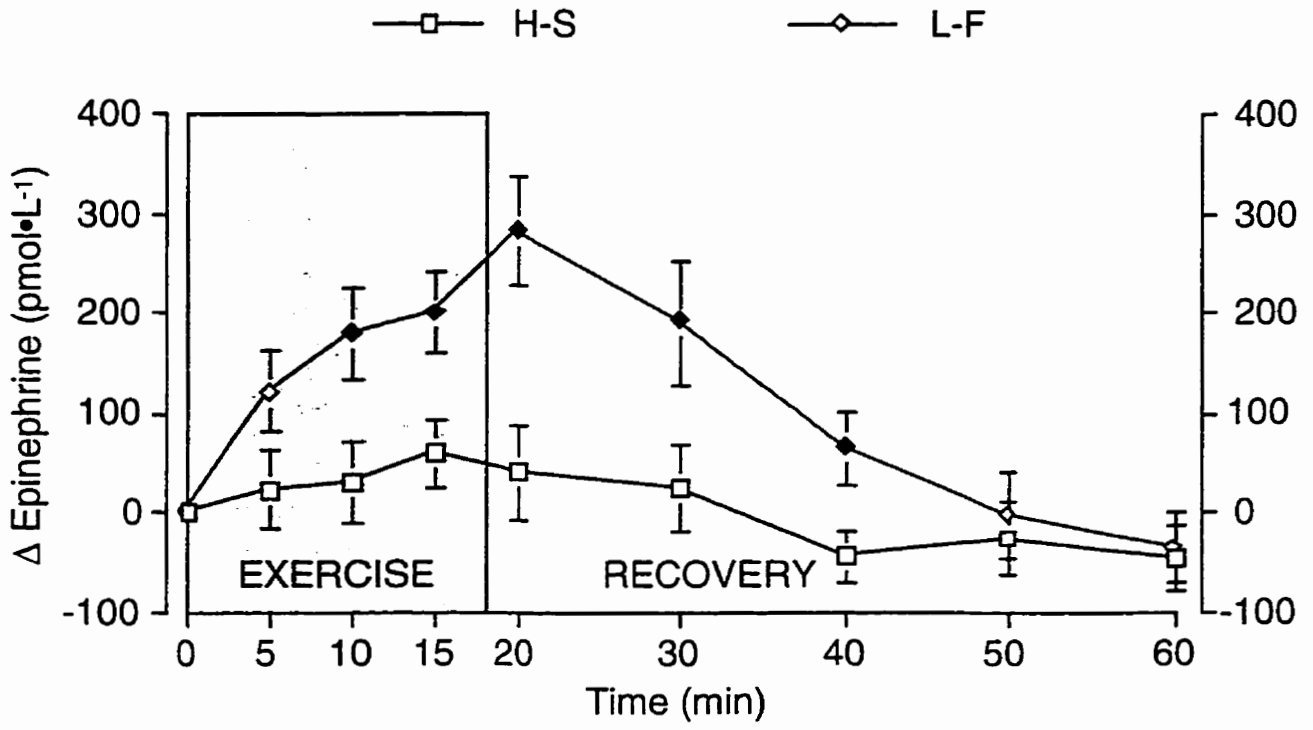

Figure 3-8: *Mean Δ EPI responses in H-S and L-F protocols. Solid symbols indicate statistically significant responses between the H-S and L-F time pairs ($p<0.05$).*

The total area under the Δ EPI response curve determined for the 60 min study period was 2107.5 ± 1320.7 pmol•L^{-1} in the H-S exercise protocol and 7225.0 ± 1634.2 pmol•L^{-1} in the L-F exercise protocol ($p<0.01$). Figure 3-9 demonstrates the total EPI area under the response curves

for this time period.

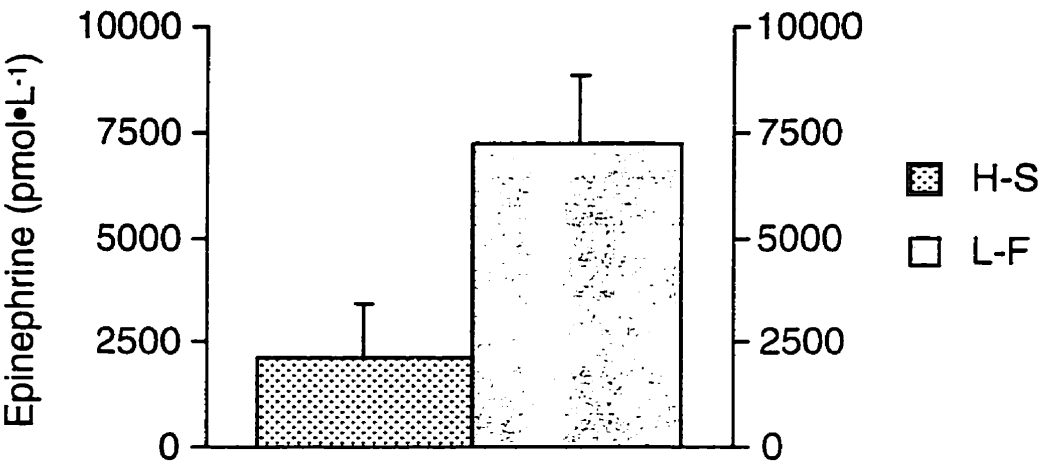

***Figure 3-9**: Incremental area of EPI in H-S and L-F.*

vii. Glucose:

Figure 3-10 demonstrates the responses of glucose concentrations in the H-S and L-F exercise protocols. The resting basal levels of glucose were not significantly different between the two exercise protocols. In the H-S protocol, the basal values were 4.73 $\pm$ 0.7 mmol•L^{-1} versus 4.7 $\pm$ 0.6 mmol•L^{-1} in the L-F protocol. While there were no statistically significant changes in glucose concentrations observed in the H-S exercise protocol, a significant increase in glucose levels occurred during the L-F protocol. Glucose responses increased from the start of the exercise period in L-F, peaking at the end of the exercise (0.47 $\pm$ 0.2 mmol•L^{-1}). After glucose levels peaked, there was a steady decrease to levels of 0.24 $\pm$ 0.2 mmol•L^{-1} by the end of recovery period in the L-F exercise trial.

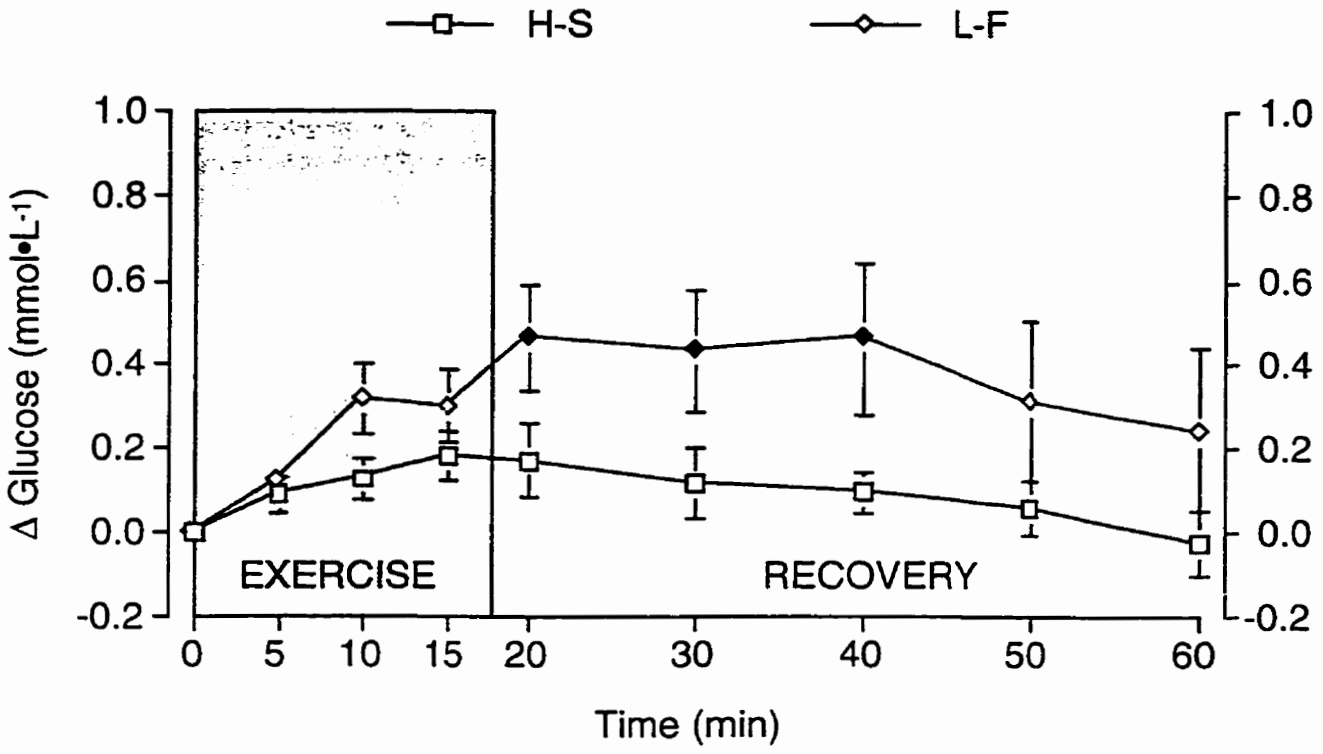

Figure 3-10: *Mean Δ glucose responses in H-S and L-F protocols. Solid symbols show significant responses between the H-S and L-F time pairs ($p<0.05$).*

The total under the Δ glucose response curve (60 min) are represented in Figure 3-11.

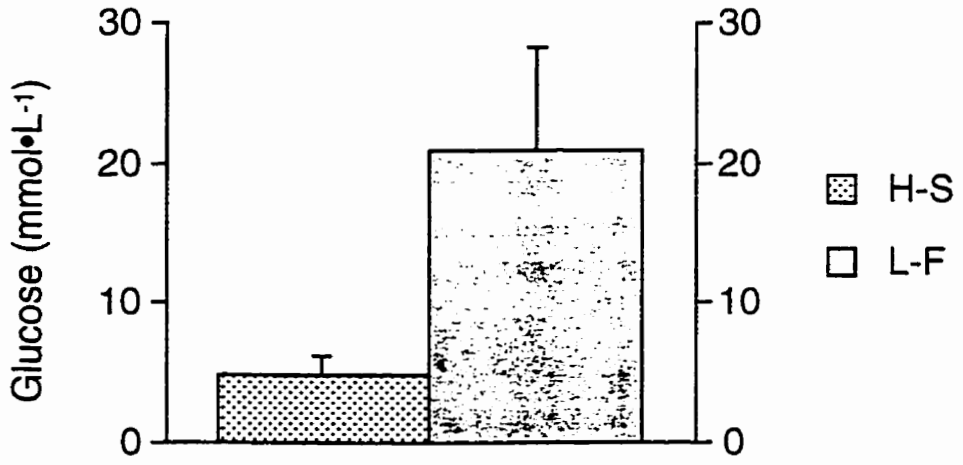

Figure 3-11: *Incremental area of glucose in H-S and L-F.*

The glucose area for the exercise and recovery periods was 4-fold higher in the L-F protocol than in the H-S protocol (7.8 ± 1.4 mmol•L^{-1} vs 21.1 ± 7.3 mmol•L^{-1}, respectively) ($p<0.001$).

viii. Lactate:

Resting basal levels of lactate were not statistically different between the two trials. No significant increase in lactate was observed in the H-S exercise protocol, but a significant increase in lactate occurred during the L-F protocol (Figure 3-12).

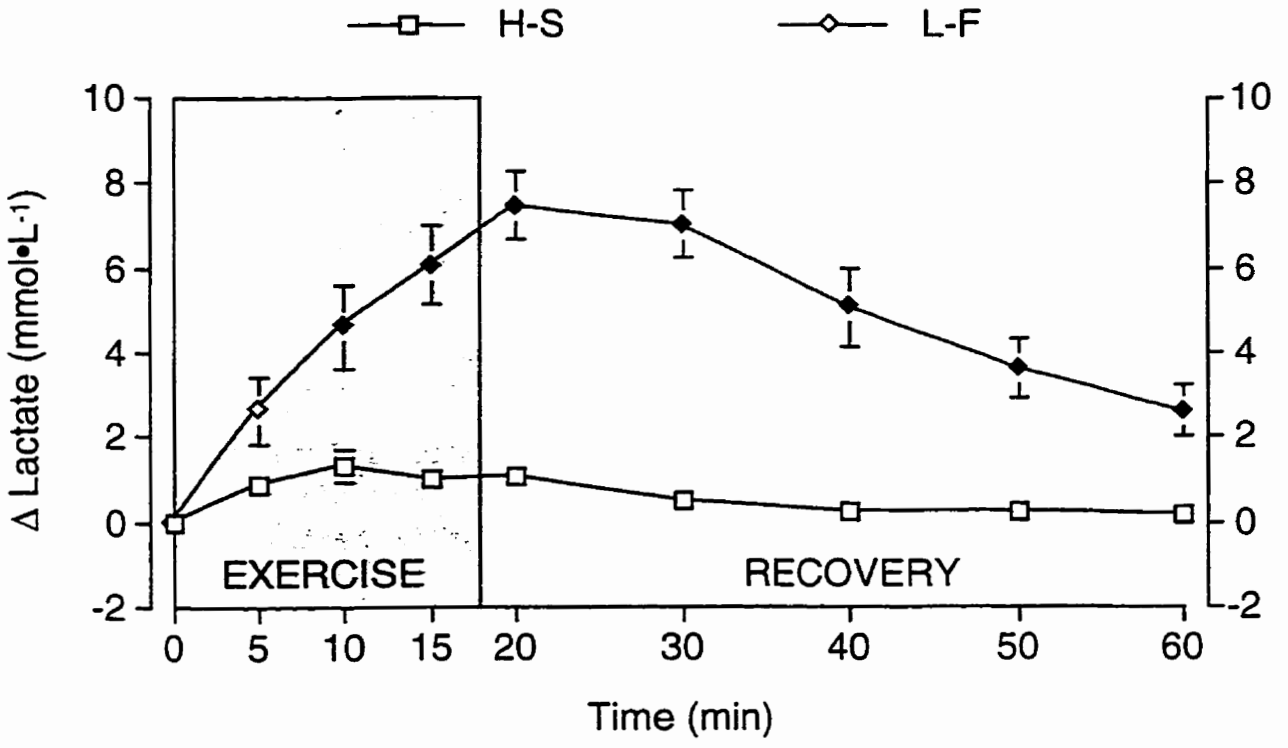

Figure 3-12: *Mean Δ lactate responses in H-S and L-F protocols. Solid symbols indicate statistically significant responses between the H-S and L-F time pairs ($p<0.05$).*

Lactate concentrations in the L-F exercise protocol peaked immediately at the end of the exercise period (7.46 ± 0.8 mmol•L^{-1}), followed by a decrease during recovery to 2.60 ± 0.6 mmol•L^{-1}.

The total area under the Δ lactate response curve (60 min) was 7-fold higher in the L-F protocol than in the H-S protocol (32.0 ± 7.9 mmol•L^{-1} vs 287.1 ± 40.8 mmol•L^{-1}, respectively) ($p<0.001$). Figure 3-1=3 demonstrates the lactate areas for the H-S and L-F protocols.

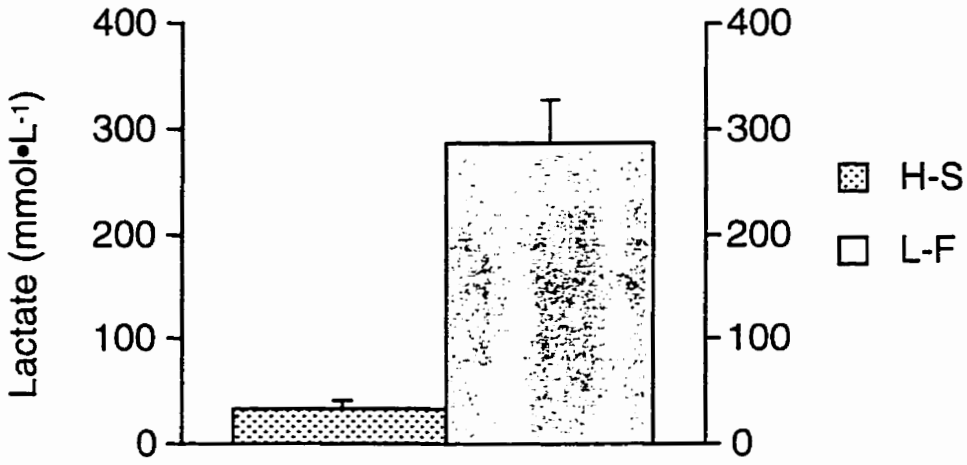

***Figure 3-13**: Incremental area of lactate in H-S and L-F.*

ix. Oxygen Consumption:

There were no significant differences in resting oxygen uptake found between the H-S protocols and L-F exercise protocols (Figure 3-14). The oxygen consumption during the exercise period was significantly higher in the L-F protocol than was obtained with the H-S exercise protocol. Inter-set (resting) oxygen consumption seemed to increase throughout the exercise periods of both H-S and L-F protocols. However, these changes were not statistically different. The oxygen uptake data were used to calculate the oxygen demand/availability ratio.

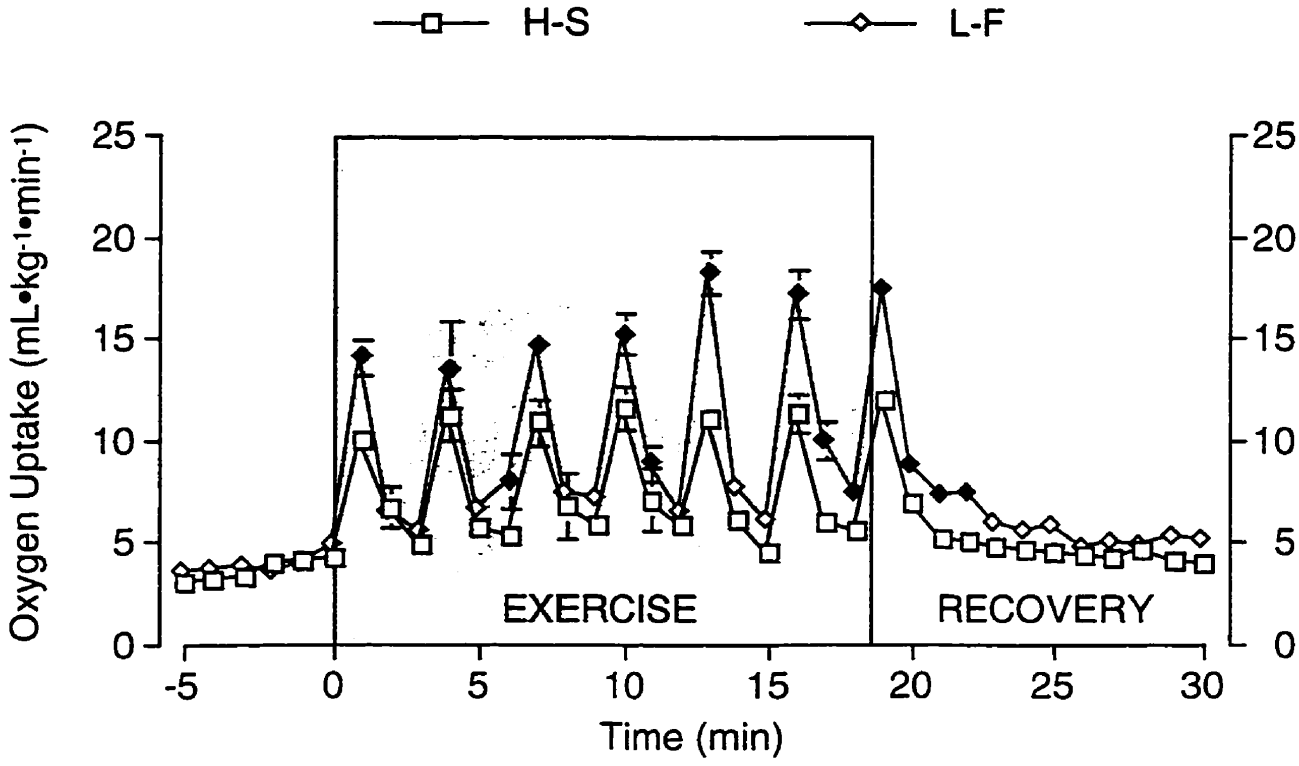

Figure 3-14: *Mean oxygen uptake in H-S and L-F protocols. Solid symbols indicate statistically significant responses between the H-S and L-F time pairs ($p<0.05$).*

x. Correlations:

A number of significant correlations were found in the L-F protocol during the exercise period (Table 3-3). The increases in circulating GH were significantly correlated with changes in cortisol, NE, lactate and the O_2 D/A ration during the exercise period of the L-F protocol. Significant correlations during exercise in the L-F protocol were also observed between lactate and cortisol, and NE and EPI.

Table 3-3:

Relevant hormonal & metabolic correlations during the L-F protocol.

Hormone or Metabolite:	r-value	significance
Growth Hormone & Cortisol	0.82	$p<0.05$
Growth Hormone & Norepinephrine	0.67	$p<0.05$
Growth Hormone & Lactate	0.64	$p<0.05$
Growth Hormone & O_2 D/A	0.89	$p<0.05$
Cortisol & Lactate	0.46	$p<0.05$
Norepinephrine & Epinephrine	0.69	$p<0.001$
Norepinephrine & Lactate	0.63	$p<0.001$
Epinephrine & Lactate	0.68	$p<0.001$

3-9. Discussion:

We hypothesized that the muscle contraction velocity and the work-rest intervals are important factors in the regulation of the GH response. By varying the load and frequency of the movement characteristics, we were able to design two heavy resistance exercise protocols, H-S and L-F, which maintained identical total external work output, exercise duration and work-rest intervals (30-150 s). Furthermore, through standardization of the length of time (30s) in which to complete the leg extension movements in each exercise set, we were able to investigate the innate differences between the two protocols.

In the present investigation, the speed of the concentric movement was recorded. In order to complete the prescribed 21 repetitions of the L-F protocol in the allotted time period (30 s), each of the repetitions had to be executed at a higher velocity of muscle contraction than the H-S protocol. The H-S protocol, with considerably more time allotted to complete the prescribed 7 repetitions, was completed at a significantly slower contraction velocity than in the L-F protocol. Furthermore, the resulting hormonal and metabolic responses to these two protocols were significantly different from each other.

Significant increases in several hormonal and metabolic variables occurred in the L-F protocol whereas the heavier loaded, less frequent exercise (H-S) failed to demonstrate any significant changes either during or after the leg extension exercises. Specifically, the GH levels in the L-F exercise were statistically more elevated over the responses observed during the H-S exercise protocol. Several factors could be responsible for the observed differences between the two protocols. The L-F protocol produced significantly elevated concentrations of blood lactate, while lactate in the H-S protocol did not increase beyond basal levels. It is obvious that the L-F protocol produced much greater demands on anaerobic glycolysis than the H-S protocol. The significant correlation of lactate levels with the responses of GH during exercise has been

demonstrated in a number of previous reports (Gordon et al., 1994; Lassare et al., 1974; VanHelder et al., 1984b; VanHelder et al., 1985; VanHelder et al., 1986). The infusion of lactate into resting subjects has produced contradicting responses of GH. Although lactate infusion failed to produce increases in GH (Sutton et al., 1976), this may be due to the existence of intact intracellular homeostasis, which was not affected by the infused substances, which might explain the lack of GH responses in the studies of Sutton et al. (1976) or Klimes et al. (1977). In a previous communication from our laboratory (VanHelder et al., 1987), it was suggested that intracellular lactate and/or pH or oxygen concentration may be responsible for the different levels of GH observed during the various kinds of exercise.

Evidence in support of this hypothesis has recently been provided by Gordon et al. (1994). They showed that increases in blood hydrogen ion [H^+] levels may be responsible for GH responses to an acute bout of high-intensity anaerobic cycling exercise. They combined exercise with the ingestion of either $NaHCO_3$ to induce an alkalotic condition, or a NaCl placebo. The concentration of [H^+] during the exercise was reduced during the alkalotic condition, but increased normally during the placebo condition. Exercise resulted in a significantly higher GH response during the placebo trial than in the alkalotic trial, showing that blood pH was a factor in the GH responses to exercise. The mechanism by which the status of blood [H^-] produced this effect was unknown.

In 1987, VanHelder et al. suggested that a combination of factors related to anaerobic metabolism, expressed as the ratio of oxygen demand to oxygen availability, was an important determinant of the GH response to exercise. Although we found a similar relationship in our study, our L-F protocol produced higher GH responses than the H-S protocol, findings which are in direct contrast to those of VanHelder et al. (1984b). In both studies, two types of resistance exercise were tested with identical total external work output and total average power output (i.e., work per exercising time period of 30 s). However, in our study, the non-contractile portion of

approximately 3 s between each repetition in the slower contracting, heavier loaded exercise (H-S) may be responsible for the different findings. In the present study, no significant eccentric component was performed during the exercise protocols. However, an eccentric component of exercise was present in the study of VanHelder et al. (1984b). In this case, the exercising muscles were subjected to both concentric and eccentric loading virtually continuously during each of the seven 30 s exercise periods. Thus, this different lag time between the repetitions may have resulted in reductions in local blood flow to the exercising muscles. In our study, the diminishing supply of blood flow and oxygen to the muscles during the 21 repetitions of the L-F protocol would have resulted in increased anaerobic glycolysis and subsequent lactic acid production. Our H-S protocol would have allowed for sufficient restoration of blood flow between repetitions, and thus, the removal of metabolic waste by-products and the re-supply of oxygen and other blood nutrients, in contrast to the H-S protocol of VanHelder et al. (1984b).

Our findings seem to agree with a more recent report by Kraemer et al. (1990) who showed that during heavy resistance exercise, the more glycolytic exercise with the highest lactate concentration, may have stimulated a greater increase in GH than the identical exercise type of similar total work output but higher intensity (as defined by "force production"). In the case of Kraemer et al. (1990), the hormonal responses to protocols of 5 RM and 10 RM were compared. Assuming that the 10 RM protocol would have taken longer to complete than the 5 RM protocol, different blood perfusion conditions in the muscles may have existed. During the 5 RM test, blood flow would have been occluded for a shorter time than during the 10 RM protocol. A greater anaerobic glycolysis was evident in the 10 RM protocol where lactate and GH were significantly elevated, with no changes in the 5 RM protocol.

The possibility of stimulation of the hypothalamic-pituitary axis through activation of chemoreceptors within the working muscles has been supported by a number of other laboratories (Buono et al., 1986; Coote et al., 1971; Farrel et al., 1983; Few et al., 1980). The work of Kjaer

et al. (1989) concluded that ACTH responses during exercise were the result of afferent nervous activity from within the working musculature. Farrel et al. (1983) and Few et al. (1980) suggested that ACTH secretion was stimulated during exercise via the by-products of anaerobic metabolism. Furthermore, Few et al. (1980) and Coote et al. (1971) also suggested that this stimulus resulted from the activation of chemoreceptors located within the active musculature. These ACTH changes also corresponded to the changes in cortisol.

Inherent in the activation of an intracellular detector, and its subsequent sympathetic activation, the present findings also demonstrated a strong correlation between NE and GH during the exercise period. When the exercise was insufficient to cause an increase in the activation of NE, GH levels failed to respond. Conversely, when the exercise was adequate to activate anaerobic glycolysis, lactate production increased substantially. Subsequently, such a change in internal homeostasis might activate an intracellular detector(s), eventually leading to an increase in the adrenergic activation of the hypothalamic releasing hormones and an increase in pituitary GH secretion.

In this study, GH release was examined during various types of exercise, along with its relationship to the responses of lactate, catecholamines (specifically NE), and the oxygen demand/availability ratio. The effects of the velocity of muscle contraction on hormonal responses was also documented in this study. The higher velocity exercise protocol was associated with the highest elevations in GH levels. Although the GH response was associated with a higher peripheral concentration of the neurotransmitter, NE, one cannot exclude the possibility that the higher NE level played a role in the GH regulation via a "central command" hypothesis (Kjaer et al., 1987). Kjaer et al. (1987) have proposed that impulses originating from motor centers can stimulate the neuroendocrine centers, via the central nervous system, to release their hormonal stores. However, the full extent of the central command hypothesis needs to be addressed before its relevance in the regulation of GH during exercise can be clearly described.

3-10. Conclusions:

Although increases in certain stress and growth-promoting hormones have been reported during exercises of different types, durations, intensities and total work output (Karagiorgos et al., 1979; Kindermann et al., 1982; Kuoppasalmi et al., 1976; Lukaszewska et al., 1976, Shephard and Sidney, 1975), the findings of VanHelder et al. (1984a; 1984b; 1985; 1986; 1987) and Kraemer et al. (1990; 1991; 1993) have shown that several specific variables actually lead to increases in plasma GH concentrations during exercise. Our data extend the results of VanHelder et al. (1984b) and Kraemer et al. (1990), and support the belief that the exercise producing the greatest demands on anaerobic glycolysis also stimulates the greatest increases in plasma GH level.

In conclusion, the higher levels of GH were associated with elevations in lactate concentration and the anaerobic component of the resistance exercise protocol. Furthermore, the response of the adrenergic nervous system, as indicated by the levels of NE, correlated significantly with the time-matched GH response during each exercise period. The persistent relationship between the GH responses and the oxygen demand/availability ratio during this experiment, and other investigations, suggests that the difference between the oxygen requirement of the exercise and the ability of the oxygen transport system to meet that supply, are important regulators of GH secretion during exercise. Indeed, the factors associated with the oxygen demand/availability ratio may directly activate the adrenergic activity to increase pituitary GH secretion.

The hypotheses tested in this investigation were that:

i. The heavier load, slower repetition (H-S) protocol, with a lower velocity of muscle contraction would produce greater GH responses than a lighter load, faster repetition (L-F) protocol, with a higher velocity of muscle contraction.

This hypothesis was rejected. The H-S protocol, completed at a significantly slower speed of movement, failed to produce a greater GH response. The L-F exercise protocol, completed at a much faster speed of movement, resulted in the greatest increases in GH during both the exercise and recovery periods.

ii. NE responses are not related to the GH responses.

This hypothesis was rejected. The responses of GH and NE were highly correlated during the L-F exercise protocol.

iii. The oxygen demand/availability ratio is not correlated with the response of GH during either resistance exercise protocol.

This hypothesis was rejected. Significant correlations between the oxygen demand/availability ratio and the GH responses were observed during the L-F exercise.

3-11. Suggestions for Future Study:

Given that in our present investigation, a significant relationship was observed between the responses of GH and NE, it seems advisable to repeat the experimental protocol, but under the influence of α-adrenergic receptor blockade with an α-antagonist, such as phentolamine. This would clarify the contribution of the adrenergic nervous system to GH regulation during resistance exercise.

CHAPTER 4

Growth Hormone Responses During Arm Flexion Exercises of Varying Load and Frequency Characteristics

4-1. Abstract:

Objectives: The objectives of this study were to examine the hormonal and metabolic responses to two arm flexion (biceps curls) exercise protocols of equal total external work output, duration and work-rest intervals while varying the load and frequency characteristics of these protocols. It was hypothesized that the volume of muscle involved in the exercise was an important factor in the amplitude of the GH response.

Design of the Study: A controlled, cross-over design was designed. Six healthy men, mean age 27.0 $\pm$ 1.4 years, randomly completed two intermittent resistance training exercise protocols of arm biceps flexion. Each session lasted 18.5 min. The heavier load-lower repetition protocol (H-S) consisted of 7 sets of 7 repetitions at 75 % of each subject's 7 repetition maximum (7 RM). The lighter load-higher repetition protocol (L-F) involved 7 sets of 21 repetitions at one-third of the load used in the H-S. All exercise sessions were conducted on a calibrated Ariel computerized ergometer, which allowed for the acceleration and velocity characteristics of each lift to be determined.

Analyses: Peripheral venous blood samples were collected every 5 min during the exercise period (5 samples), every 10 min throughout 40 min of recovery (5 samples) and a final sample was taken at 70 min of recovery, and analyzed for growth hormone (GH), cortisol, glucose, and lactate. All values were adjusted for changes in plasma volume by the method of Dill and Costill (1974).

Results: Total external work for the 7 sets was 7.5 ± 1.1 kJ and 7.4 ± 1.1 kJ for the H-S and L-F protocols, respectively. The speed of movement during the flexion phase (concentric) of the arm curl movements averaged 74.7 ± 10.4 deg•s^{-1} in the H-S protocol and 129.4 ± 17.8 deg•s^{-1} in the L-F exercise. Plasma GH levels changed very little during the H-S exercise but increased significantly in the L-F protocol after 15 min of exercise and remained elevated throughout the 70 min period of recovery ($p<0.05$). Cortisol followed a similar pattern as that of GH in the two protocols. Glucose levels increased in both protocols, but the response was higher in the L-F testing. The lactate response was significantly higher throughout the exercise and recovery periods of L-F than during the H-S protocol ($p<0.05$). A significant correlation was found between the lactate and GH responses during the exercise period ($r=0.79$, $P<0.05$).

Conclusions: The techniques developed during this experiment allowed for the comparison of the hormonal and metabolic responses to two resistance protocols that were equal in total external work output, duration and work-rest intervals, but utilizing varying load and frequency of movement characteristics. The plasma GH response during exercise was related to the speed with which the flexion movement was completed. The high correlation between the lactate and GH responses during the exercise period suggested a possible regulatory mechanism for the hormonal response observed during the resistance exercise. Furthermore, the results of this investigation were compared to the findings in the same group of subjects performing 7 sets of concentric-only leg extension exercises. In this case, the arm exercise, with the identical exercise type, duration and work-rest interval (30-150 s), produced a significantly higher GH response than the leg extension exercise.

4-2. Introduction:

The enhancement of GH secretion during physical exercise is a well known phenomenon, but the regulatory mechanism behind its control is less well understood. A number of humoral and neural stimuli have been implicated as the signals for increased release of GH during exercise. Most studies have focused on the influence of insulin availability (Galbo, 1983), increases in blood lactate (Galbo, 1983; VanHelder et al., 1984; Vigas et al., 1974) and [H^+] (Gordon et al., 1994), decreases in blood glucose levels (Glick et al., 1965), changes in free fatty acids (Casanueva et al., 1981), acid-base shifts (Luger et al., 1994; Sutton et al., 1976) or oxygen deficit (Lassare et al., 1974). Our previous investigations have shown that exercise characteristics, such as load, frequency, and the speed of muscle movement are important factors in the regulation of the GH response during exercise. We have also speculated that metabolic factors dependent on these characteristics, as represented by the oxygen demand/availability ratio, are responsible for the regulation of GH during exercise.

A small number of investigations have compared the contributions of activated muscle volume to the subsequent GH response. While the majority of these investigations have focused on the use of aerobic exercise activities, with a limited number of studies of anaerobic activities, a study of the GH response to a resistance-type of exercise, utilizing varying amounts of activated muscle volume has not been previously reported in the literature.

4-3. Growth Hormone Responses to Exercises of Varying Muscle Volumes:

Several studies of the physiologic consequences of exercise have compared the responses of small muscle groups to larger muscle groups (Astrand & Saltin, 1961; Bobert, 1960). From these, and other investigations, it was concluded that physical work with small muscle groups produced a greater physiologic strain and an increase in the anaerobic component of exercise, than

when the same intensity of exercise was completed with larger muscle volumes (Davies et al., 1974). Unfortunately, the comparison of the GH response to exercise utilizing different muscle volumes has not been well studied, and the few investigations found in the literature have concentrated on the GH response to cycle ergometer exercise of equal oxygen uptake (Kozlowski et al., 1983). The study of resistance type exercise has not been employed to date.

Although the response of GH was not examined, an early study by Davies et al. (1974) compared the catecholamine responses of arm, one leg and two leg exercise during an identical 60 min cycling ergometer exercise. Blood catecholamine and lactate responses were determined in response to arm, one-leg and two leg cycling with a range of intensities that progressed from 15 to 30, 45, 60, 70 to 100 % of $\dot{V}O_2$ max. The results consistently demonstrated that, for a given relative workload (% of $\dot{V}O_2$ max), the physiologic responses were greater when the smaller muscle groups were employed. At all workload intensities, catecholamine levels were inversely related to both the effective muscle (plus bone) volume used to perform the work and the corresponding lactate responses. These results were supported by a number of other investigations (Bevegard et al., 1968; Freyschuss & Strandell, 1963). Davies et al. (1974) concluded that the differences in adrenergic and lactate responses were the result of the system's attempt to maintain blood flow and pressure to the cardiovascular system during the exercise stimulus.

Few et al. (1980) investigated the hormonal and metabolic responses to moderate endurance exercise on a cycle ergometer involving different muscle volumes. They compared two lower limb cycling protocols which differed in the amount of muscle mass utilized. In this case, subjects exercised for 30 min on the ergometer using just one leg. The hormone results of this exercise stimulus were then compared to the observations made when the identical exercise type and work load was completed with two legs. Cycling workloads averaged 1.93 $L \cdot min^{-1}$ and were not significantly different between one and two-leg exercise. However, power output per unit volume of the muscle was greater in the one-leg than the two-leg exercise. Few et al. (1980)

reported that the differences in power output per unit volume of the muscle resulted in the significantly different hormonal and metabolic responses. Increases in cortisol, aldosterone and blood lactate were greater in the one leg exercise than the two leg cycling. The authors proposed that the activation of the hypothalamic-pituitary-adrenal system during exercise was mediated through the stimulation of chemoreceptors in the exercising muscles. While the authors acknowledged the possible contribution of a substance produced by the exercising muscles and detected directly by the hypothalamus, they believed that this substance would be too diluted by the central blood volume to be detected centrally. Its detection was more likely at the local muscle site, rather than the hypothalamus. In this case, the only metabolic by-product of exercise measured was whole blood lactate concentration. The one-legged exercise, completed at the identical exercise intensity of $\dot{V}O_2$ max as the two-legged cycling, produced significantly higher lactate responses (Few et al., 1980). Few et al. (1980) speculated that their results were an indication of the activation of the hypothalamic-pituitary-adrenal axis which was mediated through the stimulation of chemoreceptors in the exercising muscles.

While investigating the possible role of muscle metabolic receptors in the regulation of GH secretion during exercise, Kozlowski et al. (1983) compared the hormonal responses of arm and leg exercise performed at an equivalent oxygen uptake. They assumed that the stimulation of muscle chemoreceptors during exercise would be proportional to the workload per unit of muscle mass utilized during the exercise session and that this difference in stimulation would then result in different GH responses. They compared the effect of an arm cranking exercise on a cycle ergometer at a workload of 0.75 $W \cdot kg^{-1}$, a leg cycle ergometer at an equivalent oxygen uptake, and treadmill running at the identical oxygen uptake. The design of the investigation produced arm cycle, leg cycle and treadmill running workloads of 49 W, 75 W and 1.6 $m \cdot s^{-1}$, and oxygen uptakes of 1.22, 1.38 and 1.36 $L \cdot min^{-1}$, respectively. Despite the higher exercising workload and oxygen consumption in the leg cranking and treadmill running protocols, the arm cranking exercise activity produced the highest GH response. This specific exercise type also utilized the smallest

muscle mass. Interestingly, the greatest responses of lactate and norepinephrine (NE) concentrations also occurred in the arm cranking activity. The GH and NE responses during this exercise were linearly correlated ($r=0.52$; $p<0.05$), while GH was also correlated with the lactate response ($r=0.56$; $p<0.05$). Furthermore, Kozlowski et al. (1983) discounted the possibility that increased GH response in the arm exercise was the result of an increased local release from binding sites within the active muscle by using three different blood sampling sites. In each case, the GH level was significantly greater in the arm exercise than in either of the two leg exercise protocols. Kozlowski et al. (1983) suggested that neural afferent signals, generated by muscle metabolic receptors participated in the activation of GH release during this particular type of physical exercise.

4-4. Literature Conclusions:

The lack of sufficient scientific studies on the hormonal responses to exercise of equal intensity, but varying muscle volume, needs to be addressed further. The literature suggests that exercise with smaller muscle volumes produces greater metabolic responses and both adrenergic and hypothalamic-pituitary-adrenal activation (Davies et al., 1974; Few et al., 1980; Kozlowski et al., 1983). It has been suggested that the observed responses are the result of an increased activation of chemoreceptors, possibly in the central nervous system, but more probably in the local active muscle tissues (Kozlowski et al., 1983). In addition, a less substantiated hypothesis has been proposed suggesting that the responses of GH and cortisol during exercise may be the result of influences in the motor centres of the central nervous system acting as feed-forward stimuli for the increased secretion of these hormones (Galbo, 1983; Kjaer et al., 1987). At present, this issue remains to be settled.

While a small number of studies on the contribution of muscle volume to hormonal and metabolic responses have been completed, these studies have focused on moderate, aerobic

exercise performed on a modified cycle ergometer. Information on GH responses to resistance exercise of equal intensity, duration and work-rest intervals (30-150 s) using varying muscle volumes are lacking.

4-5. Objectives:

The objectives of this investigation were to examine the:

i. Hormonal and metabolic responses to two resistance exercise protocols of equal total external work output, duration and work-rest intervals while varying the load and frequency characteristics of arm flexion biceps curls.

ii. Contribution of muscle volume to the exercise-induced increases of GH.

iii. Role of lactate in the regulation of GH secretion during resistance exercise protocols.

4-6. Hypotheses:

The hypotheses tested in this investigation were that:

i. The exercise protocol with the higher load and a less frequent movement (H-S) will produce a greater GH response compared to that seen in other protocols with lower load and a higher frequency of movement (L-F).

ii. The use of a smaller volume of muscle during the arm flexion exercise will result in significantly less GH being secreted than during leg extensions.

4-7. Materials and Methods:

This investigation was completed at the Immunology and Physiology Laboratories of the Defence and Civil Institute of Environmental Medicine at the Canadian Forces Base Toronto. Some additional testing and analysis were conducted at various departments within the University of Toronto and the Mount Sinai Hospital.

i. Subjects: Six males, not currently involved in any resistance exercise training, were selected as subjects for this experiment from a pool of university and military volunteers. The same subjects had previously completed the investigation involving the leg extension protocols (Chapter 3). Each subject was informed of all risks associated with the experiment and signed an informed consent form which was previously approved by the Ethics' Committees of the University of Toronto and the Defence and Civil Institute of Environmental Medicine. Subjects were requested to abstain from eating, smoking, caffeine and sexual activity for 12 h prior to the start of any of the testing procedures. Each of the subjects visited the laboratory on five different occasions, three visits for physical tests and familiarization, and two visits for protocol testing.

ii. Physical Tests, Familiarization and Protocol Design: On the first visit, two weeks before beginning the experiment, each subject completed a medical questionnaire and was examined by a staff physician. Medical screening excluded any subject with acute or chronic medical conditions. Physical characteristics, including percentage body fat assessed by skinfold calipers (Durin & Womersley, 1974), were determined and the $\dot{V}O_2$ max was predicted using a Monark cycle ergometer (Jack Watson Sports, Toronto, Canada) using the method of Astrand (Astrand & Rodahl, 1977) (Table 4-1).

On the second visit, an incremental strength test was performed. This test consisted of a seven repetition maximum (7 RM) test, similar to that of VanHelder et al. (1984b) and the leg

extension experiment described in Chapter 3. The test was performed by each subject using the Biceps Curl Station of the Global Gym apparatus (Global Gym & Fitness Equipment, Weston ON, Canada). As in the previous experiment, the strength testing was continued until the subject could no longer perform the required 7 repetitions. Thus, the penultimate load was considered the 7 RM. A 1 RM test was also performed during this occasion and was identified as the last single repetition load that the subject could perform before failure. To establish that no strength adaptation had occurred during the experimental period, the testing procedures were also conducted at the end of each subjects' participation in the experiment. No differences were found between the pre and post testing results. The results of these testing procedures are displayed in Table 4-2.

After the strength testing, each subject was familiarized with the arm ergometer (Ariel Computerized Exerciser, Ariel Dynamics Inc. Model # M-4000, Trabuco Canyon, California, USA), which was based on the ergometer exercise system that was used in the leg extension experiment. The range of flexion that the Ariel controlled was preset at 70°, and the velocity and acceleration characteristics were automatically calculated for each lift. Personal settings for seat height, seat incline, and arm length were recorded for each subject and kept constant during the protocol. The Ariel was calibrated before each testing session as per the manufacturers' specifications. As the Ariel suspended workloads during the relaxation phase of the movement, no data were collected during the eccentric phase of the arm extension, when the ergometer was returned to the starting position. To standardize the total external work, a technician applied an external force to reset the apparatus to the starting position after each contraction. Total external work output was calculated as the product of the load, the number of lifts and the distance of the flexion movement (Table 4-2).

Each of the two arm curl exercise protocols was specifically designed based on the results of each subject's 7 RM testing, but the overall total external work output was identical. The heavier load-lower repetition protocol, (H-S), consisted of 7 sets of 7 arm flexion movements

using 75 % of the 7 RM results. The lighter load-higher repetition exercise protocol (L-F) consisted of 7 sets of 21 extensions at one-third of the load in the H-S protocol. The workload on the Ariel was determined by using either 75 % or 25 % of each subject's 7 RM workload.

Acting as their own controls, all subjects performed both protocols, with completion of each protocol separated by at least 7 days. The tests were administered randomly at 0900 h each day following an overnight fast. Each of the 7 exercise sets began at 0, 3, 6, 9, 12, 15 and 18 min (Figure 4-1). The subjects performed either 7 or 21 arm flexions in 30 s, followed by 150 s of rest before beginning the next exercise set. This work-rest interval arrangement was continued for the entire 7 sets. The two protocols were of equal total external work output and exercise duration, with identical work-rest intervals (30-150 s), but different load and frequency characteristics.

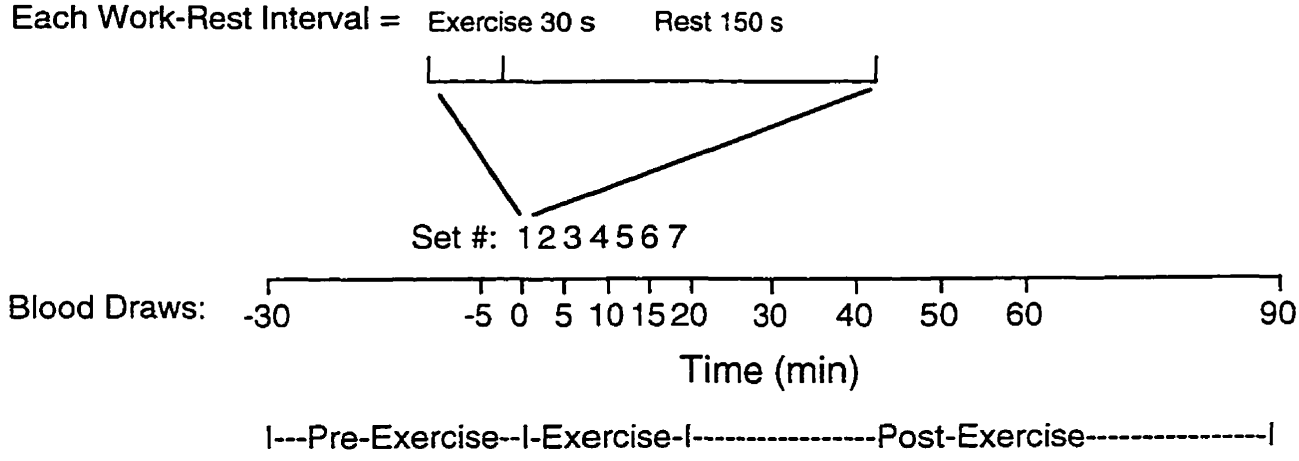

Figure 4-1: Time sequences for exercise sets and blood sampling.

iii. Measures: An I.V. catheter (Insyte 20G, 1") was inserted into the cephalic vein 35 min before the protocol testing experiment was to commence. The catheter was fixed with an injection adapter M.L. lock (Medex 1 7/16") and remained patent throughout the testing procedures with the use of an inter-sampling 0.6 cc heparin-saline locking solution (100 units•mL^{-1}) after each sample. The

lock solution was removed prior to withdrawing each of the samples and quickly re-administered following the specific sampling. Resting blood samples were taken in seated subjects at 30 and 5 min prior to the start of the exercise testing. After the 5 min pre-exercise blood sample, each subject was positioned on the Ariel arm ergometer. Blood sampling continued during the exercise period at 0, 5, 10, 15 and 20 min and at 10, 20, 30, 40 and 70 min of recovery.

The contents of each vacutainer were gently mixed by repeated inversion. Blood samples for GH and cortisol were collected in 10 mL vacutainer tubes containing 0.4 mL EDTA acid (15%) solution (#6456, Becton Dickinson, Rutherford NJ, USA). Blood samples for glucose and lactate were collected in 3 mL vacutainer tubes containing 30 mg sodium fluoride (NaF) powder (#6383, Becton Dickinson, Rutherford NJ, USA). For lactate analysis, a 25 μL sample from the NaF tube was mixed with 200 μL of ice-cold perchloric acid ($HClO_4$) and immediately frozen. Another 25 μL sample was used to determine the hematocrit and hemoglobin levels for calculation of plasma volume changes. After sampling, each vacutainer was promptly centrifuged for 15 min at 2500 RPM and 4° C. The separated plasma was immediately frozen at -80° C until analysis. A total of 156 mL of blood was obtained from each subject during each protocol testing period (12 samples). After the last blood sample was obtained, the I.V. catheter was removed from the cephalic vein and discarded.

iv. Analysis: All subsequent biochemical analyses were performed in duplicate, except for GH, which was performed in triplicate. The plasma GH and cortisol levels were analyzed using radioimmunoassay kits, (Pharmacia, Uppsala, Sweden and Farmosgiagn Diagnostica, Espoo, Finland, respectively). The standard curve for GH was modified with the addition of three extra standards of low GH levels (0.25, 0.0125 and 0.00625 $\mu g \cdot L^{-1}$), in order to allow for better detection of extremely low resting levels. Radioactivity was measured using a calibrated Cobra Auto-gamma Counter (Packard Model 5002, Meriden CT, USA). Glucose levels were determined with a glucose enzyme kit (Boehringer Mannheim, Mannheim Germany) as described by Trinder

(1969). Lactate levels were determined with a lactate enzyme kit (Boehringer Mannheim, Mannheim, Germany) as described by Maughan (1982). Hematocrit and hemoglobin levels were determined with the use of microhematocrit capillary tubes (Autocrit Ultra-3, Franklin NJ, U.S.A.) and the Hemocue β-Hemoglobinometer (Hemocue Photometer, Helsingborg, Sweden), respectively. The responses observed in each protocol were corrected for percent changes in plasma volume by the method of Dill & Costill (1974).

v. Statistical Analysis: As no significant differences were found in any of the measured resting blood factors between -30, -5 and 0 min, all exercise and recovery levels were normalized with respect to the average of the three resting levels and compared using a repeated measures ANOVA (Superanova, Abacus Concepts Inc., Berkeley CA, U.S.A.). Post-hoc analysis was also completed using a means comparison. The integrated area under the response curve was calculated using the trapezoidal rule for unequally space X values. In addition, relevant correlations for each hormone were also calculated and compared between the H-S and L-F protocols (Statview, Abacus Concepts Inc., Berkeley CA, U.S.A.). The level of statistical significance accepted was $p<0.05$. Mean data in figures are presented with SE, but SD is used in the table presentations. Where no error bars are evident in the figures, the particular error for that value was too small to be detected in the figure.

4-8. Results:

i. Subject Characteristics: The data collection for this experiment began in early April and was completed by the end of May of that same year. All six subjects were able to complete all phases of the experimental design, including the arm flexion tests of the current investigation and the leg extension protocols of the previous chapter. The physical characteristics for each subject completing both studies are presented in Table 4-1. The calibration of Ariel arm ergometer was completed before and after each testing session and demonstrated no significant variation throughout the length of the experiment.

TABLE 4-1:

Physical characteristics of subjects (n=6).

Characteristic (unit):	Mean ± SD	Range
Age (y)	27.0 ± 1.4	25-29
Body Mass (kg)	83.9 ± 6.7	74.2-93.6
Height (m)	1.8 ± 0.1	1.7-1.8
Body Mass Index (kg/m^2)	26.3 ± 1.5	24.0-27.9
Body Fat (%)	18.9 ± 2.8	16.5-23.5
Maximal $\dot{V}O_2$ (Absolute) ($L \cdot min^{-1}$)	3.7 ± 0.4	3.4-4.2
Maximal $\dot{V}O_2$ (Relative) ($mL \cdot kg^{-1} \cdot min^{-1}$)	43.8 ± 3.4	38.4-46.4

Over the course of the experiment, there were no significant changes in the subjects' fitness levels or medical conditions (Table 4-2). The Ariel apparatus did not report any data during the

eccentric or relaxation phase of the movements as the work load was suspended by the ergometer during this period.

TABLE 4-2:

Strength testing and workload determinations.

Characteristic (unit):	Mean ± SD	Range
Seven Repetition Maximum - 7 RM (kg)	39.2 ± 5.9	31.8-47.7
One Repetition Maximum - 1 RM (kg)	52.6 ± 9.8	38.6-59.1
Workload for H-S (kg)	29.3 ± 4.2	23.9-35.5
Workload for L-F (kg)	9.7 ± 1.4 *	6.8-11.8
Total Work for H-S (kJ)	7.5 ± 1.1	6.1-9.1
Total Work for L-F (kJ)	7.4 ± 1.1	6.1-9.1
Speed of Movement in H-S ($deg \cdot s^{-1}$)	74.7 ± 10.4	56-91
Speed of Movement in L-F ($deg \cdot s^{-1}$)	129.4 ± 17.8 **	95-176

* $p<0.05$ significance from H-S trial

** $p<0.005$ significance from H-S trial

ii. Speed of Concentric Movement:

The total work completed during each of the two protocols was not significantly different from each other (Table 4-2). Total work was 7.5 ± 1.1 kJ and 7.4 ± 1.1 kJ for the H-S and L-F protocols, respectively. The average speed of the flexion movement was significantly higher in the L-F protocol than in the H-S protocol ($p<0.05$). The speed of movement computed by the Ariel

was 129.4 ± 17.8 deg•s^{-1} in the L-F exercise protocol and 74.7 ± 10.4 deg•s^{-1} in the H-S protocol (Table 4-2).

iii. Growth Hormone (GH):

The resting GH concentrations were not significantly different between the two resistance exercise protocols with 2.65 ± 0.9 µg•L^{-1} in the H-S and 3.12 ± 0.5 µg•L^{-1} in the L-F protocol. Figure 4-2 describes the averaged plasma Δ GH responses during the exercise and recovery periods of the H-S and L-F protocols.

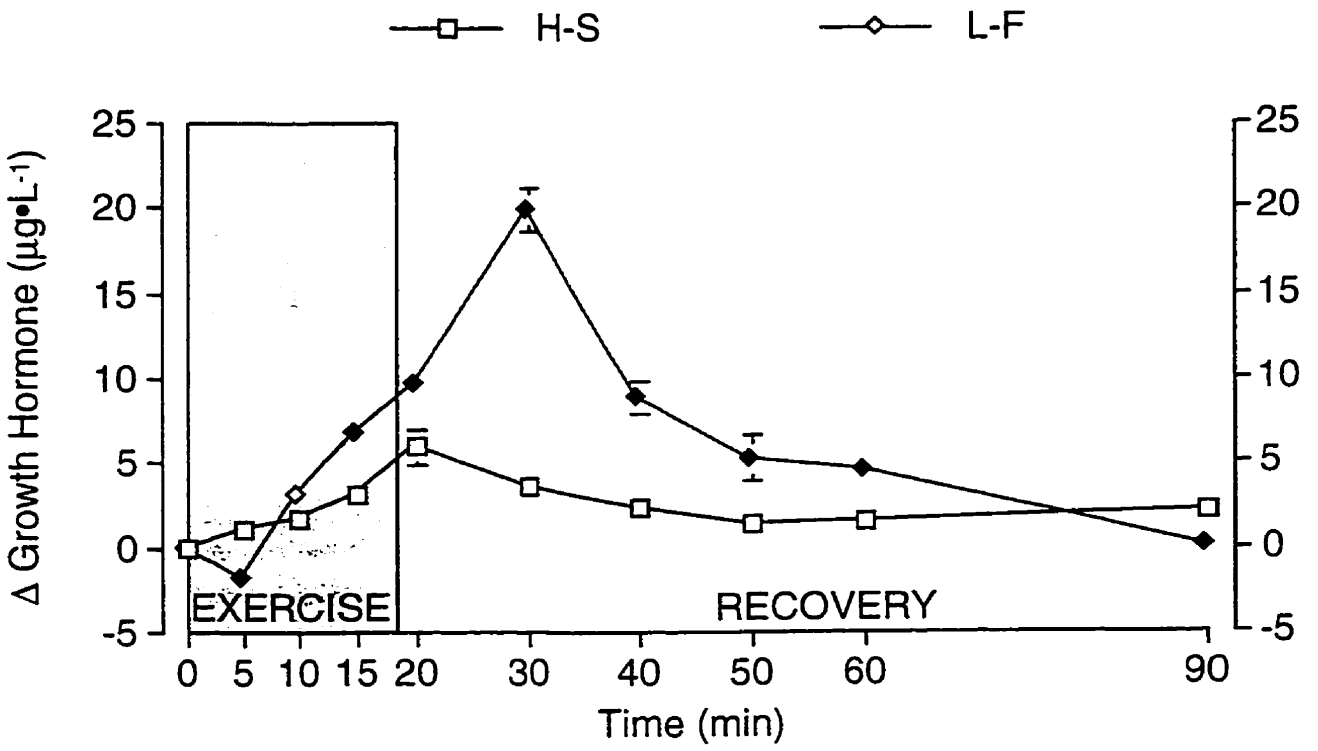

Figure 4-2: *Mean Δ GH responses in H-S and L-F protocols. Solid symbols indicate statistically significant responses between H-S and L-F time pairs ($p<0.05$).*

While the GH changes in the two protocols closely resembled each other in shape, significantly higher responses of GH occurred in the L-F exercise protocol. Plasma GH failed to respond substantially above the resting values in the H-S testing, but increased significantly in the L-F protocol ($p<0.05$). The GH levels in the H-S protocol responded only slightly, peaking at 5.9 $\pm$ 1.0 $\mu g \cdot L^{-1}$ at the end of the exercise period ($p<0.05$), and declined throughout the recovery period. The peak GH response of 19.9 $\pm$ 1.3 $\mu g \cdot L^{-1}$ in the L-F occurred after 10 min of recovery. The values of GH decreased immediately after peaking and continued to decline throughout the entire period of recovery to a low of 0.2 $\pm$ 0.8 $\mu g \cdot L^{-1}$. The total area under the GH response curve during the exercise and recovery periods (90 min) was significantly greater in the L-F protocol (493.3 $\pm$ 42.5 $\mu g \cdot L^{-1}$) than the GH responses in the H-S exercise (201.4 $\pm$ 53.5 $\mu g \cdot L^{-1}$). Figure 4-3 demonstrates the total GH areas for the H-S and L-F protocols.

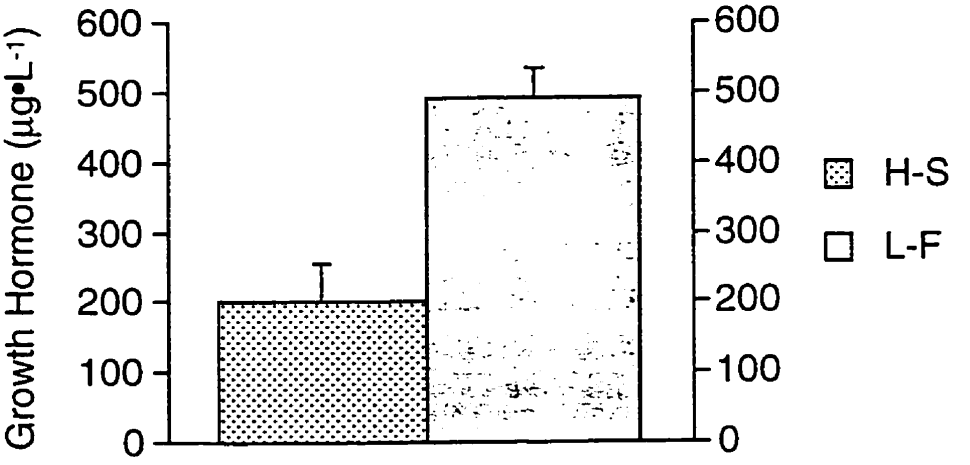

***Figure 4-3**: Incremental area of GH in H-S and L-F.*

iv. Cortisol:

There were no statistical differences in the initial cortisol concentrations of the H-S and L-F exercise protocols (203.5 $\pm$ 4.4 $nmol \cdot L^{-1}$ and 211.3 $\pm$ 3.1 $nmol \cdot L^{-1}$, respectively). The plasma

cortisol responses are described in Figure 4-4. Cortisol responses in the H-S and L-F protocols closely resembled each other in shape, but not amplitude. The cortisol responses in the H-S protocol increased from the start of exercise, peaked (55.3 ± 8.7 nmol•L^{-1}) at the end of the 20 min exercise period ($p<0.05$), and decreased throughout the recovery period to resting levels. The cortisol response of the L-F protocol followed the same pattern, but peaked at much higher levels of 90.8 ± 8.1 nmol•L^{-1} ($p<0.05$) at the end of the exercise period (20 min), and then decreased to 9.7 ± nmol•L^{-1} at the end of 70 min of recovery.

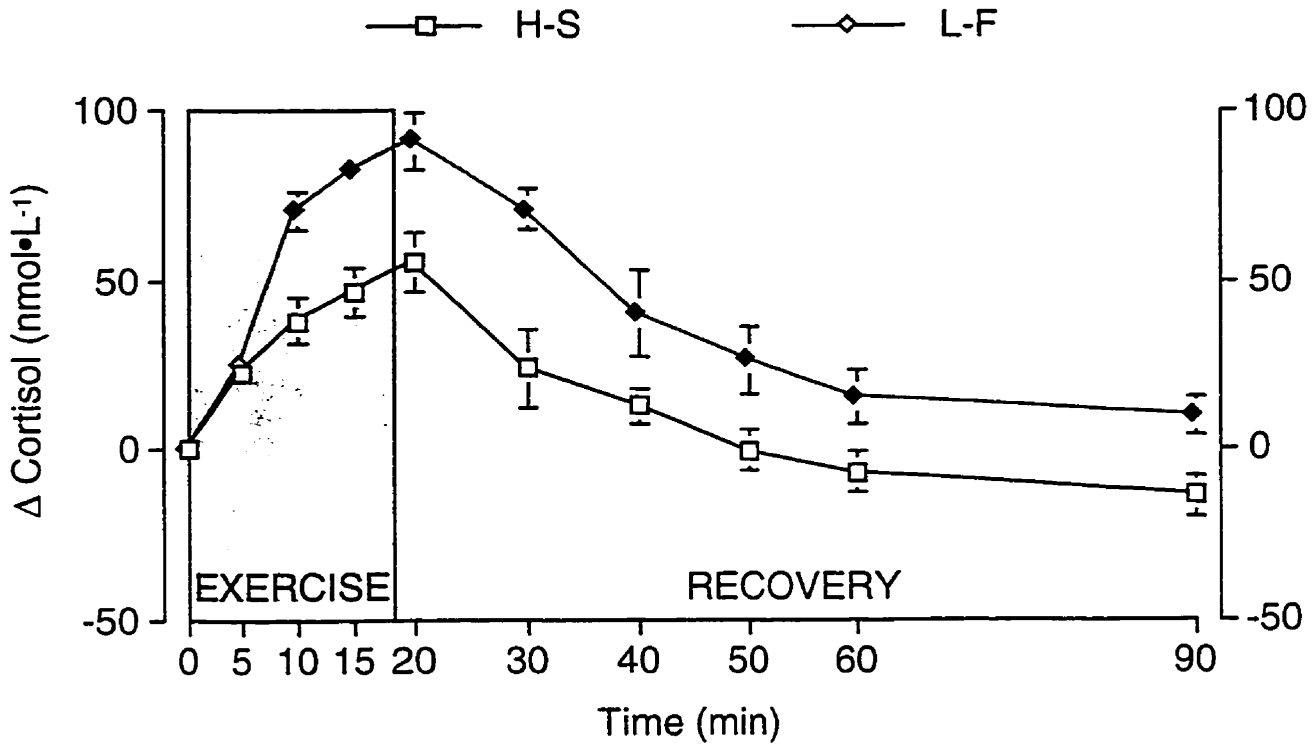

Figure 4-4: *Mean Δ cortisol responses in H-S and L-F protocols. Solid symbols indicate statistically significant responses between H-S and L-F time pairs ($p<0.05$).*

The total area under the Δ cortisol response curve during the exercise and recovery periods (90 min) was significantly greater ($p<0.05$) in the L-F protocol (3149.2 ± 565.0 nmol•L^{-1}) than the Δ cortisol response in the H-S exercise (1261.7 ± 303.8 nmol•L^{-1}). Figure 4-5 demonstrates the total cortisol areas during the exercise and recovery periods of the H-S and L-F protocols.

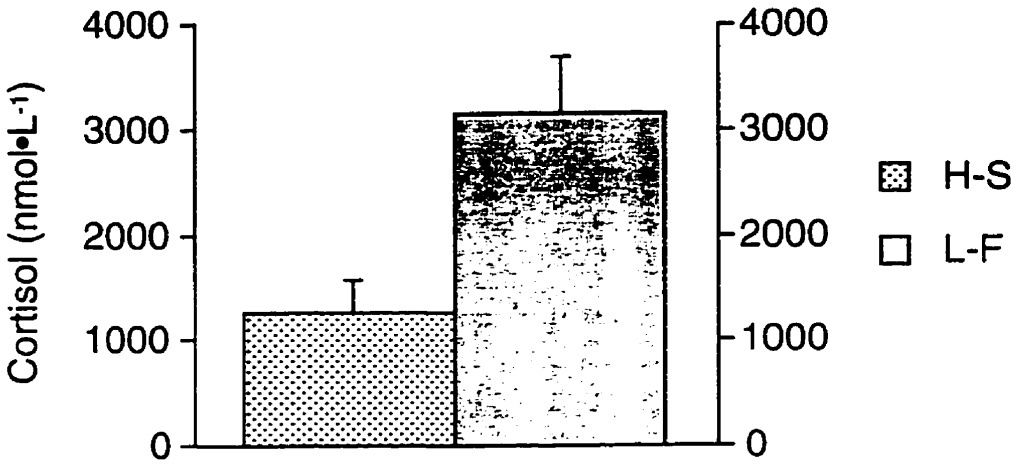

Figure 4-5: Incremental area of cortisol in H-S and L-F

v. Glucose:

Figure 4-6 describes the mean Δ glucose response for the two exercise protocols.

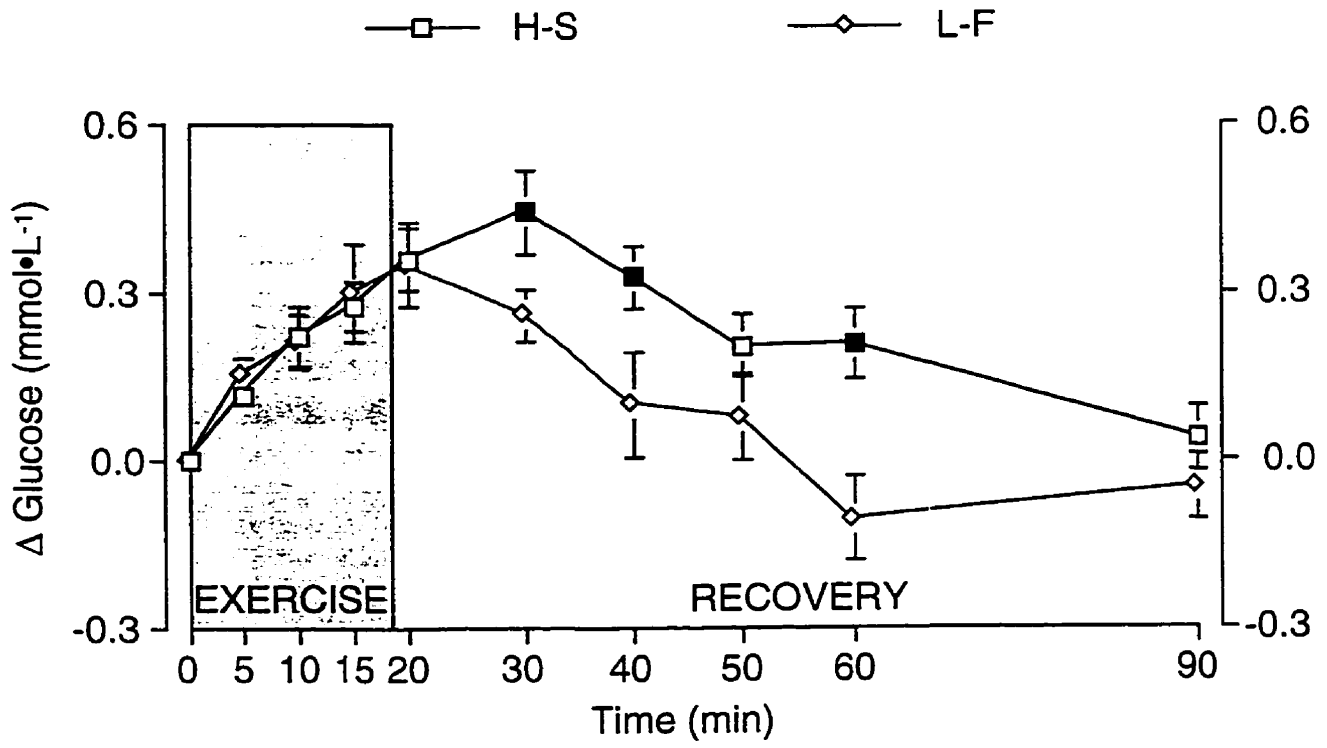

Figure 4-6: Mean Δ glucose responses in H-S and L-F protocols. Solid symbols indicate significant responses between H-S and L-F ($p<0.05$).

There were no significant differences in the resting glucose levels between the two protocols. Although there were no statistical differences in the Δ glucose levels between the H-S and L-F protocols during the exercise period, glucose response of the H-S protocol was significantly elevated over the L-F during the recovery period.

vi. Lactate:

The Δ lactate responses to the H-S and L-F exercise protocols are shown in Figure 4-7.

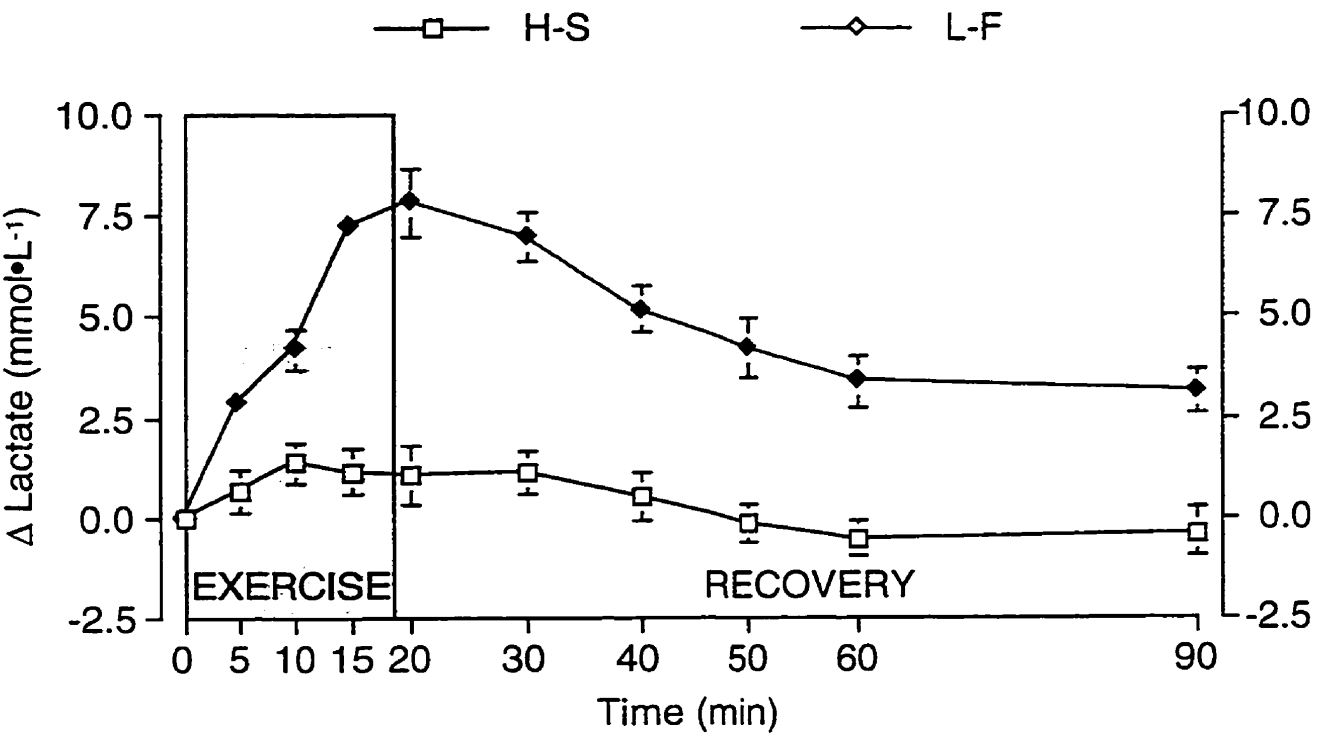

Figure 4-7: *Mean Δ lactate responses in H-S and L-F protocols. Solid symbols indicate significant responses between H-S and L-F ($p<0.05$).*

There were no significant differences in the resting basal lactate levels between the two exercise protocols. No significant changes in plasma lactate were found during the H-S exercise. In contrast, the lactate responses in the L-F activity increased immediately following the start of the

biceps flexion activity, becoming significantly different from the H-S protocol after 5 min of exercise ($p<0.05$). In the L-F exercise, lactate levels peaked at the end of the exercise period at 7.8 $\pm$ 0.9 mmol•L^{-1} and then proceeded to decrease throughout the recovery process to 3.1 $\pm$ 0.5 mmol•L^{-1}.

The calculated total area under the Δ lactate response curve during the exercise and recovery periods (90 min) was significantly greater in the L-F protocol (379.5 $\pm$ 55.7 mmol•L^{-1}) than the Δ lactate response in the H-S exercise (62.2 $\pm$ 19.8 mmol•L^{-1}). Figure 4-8 demonstrates the total lactate area for the H-S and L-F exercise protocols.

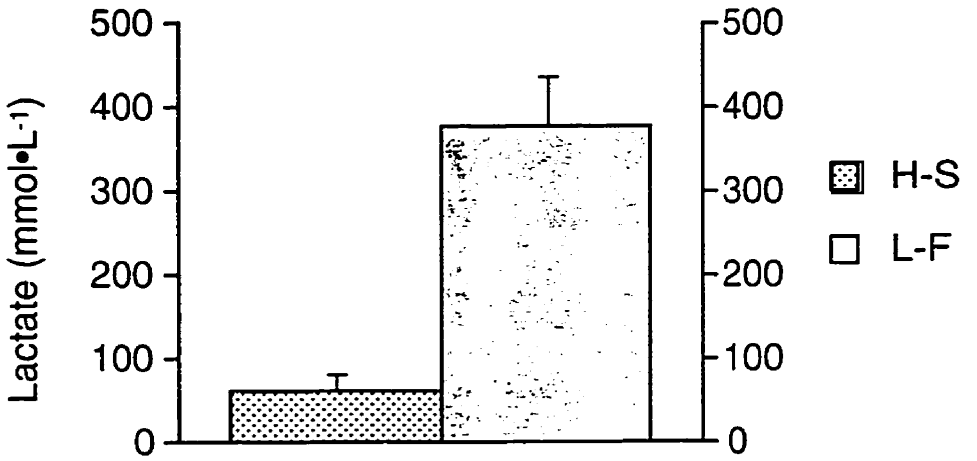

Figure 4-8: Incremental area of lactate in H-S and L-F

vii. Correlations:

The response of GH during the exercise period of the L-F protocol was also significantly correlated (linearly) with the corresponding lactate concentration ($r=0.79$; $p<0.05$) as shown in Figure 4-9. Also a significant linear correlation was found between the Δ GH and Δ cortisol concentrations during the exercise period of the L-F protocol ($r=0.86$; $p<0.05$) (Figure 4-10).

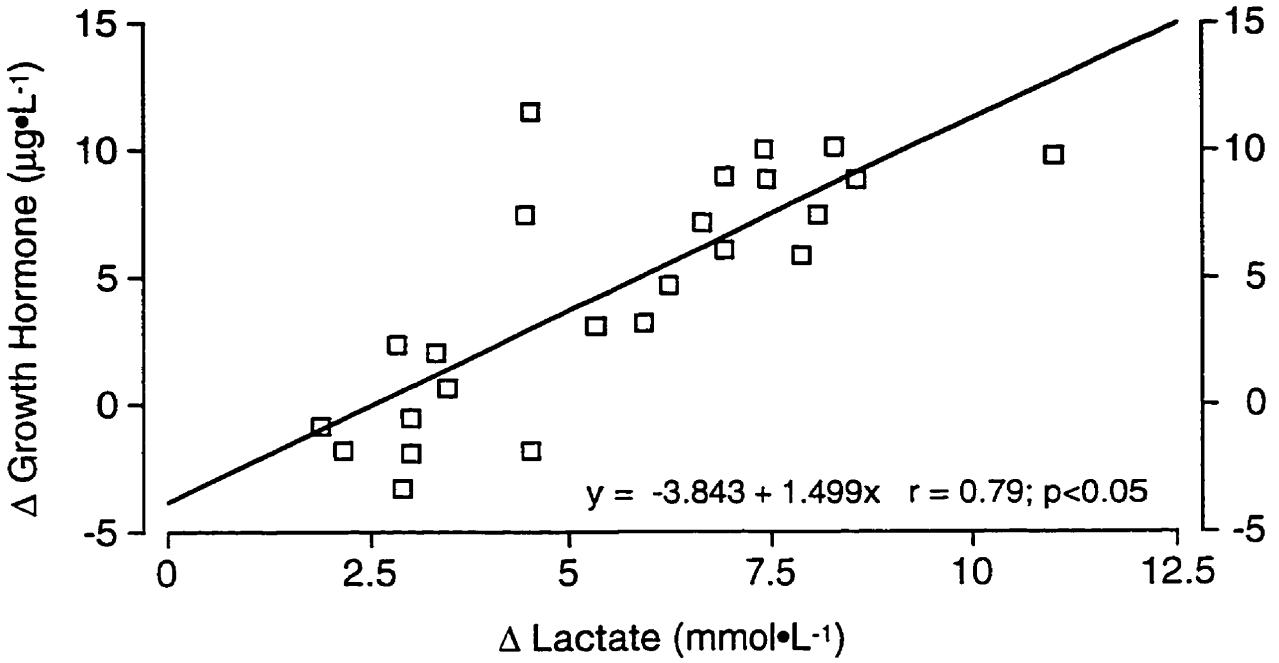

Figure 4-9: Correlation of Δ GH and Δ lactate responses during L-F.

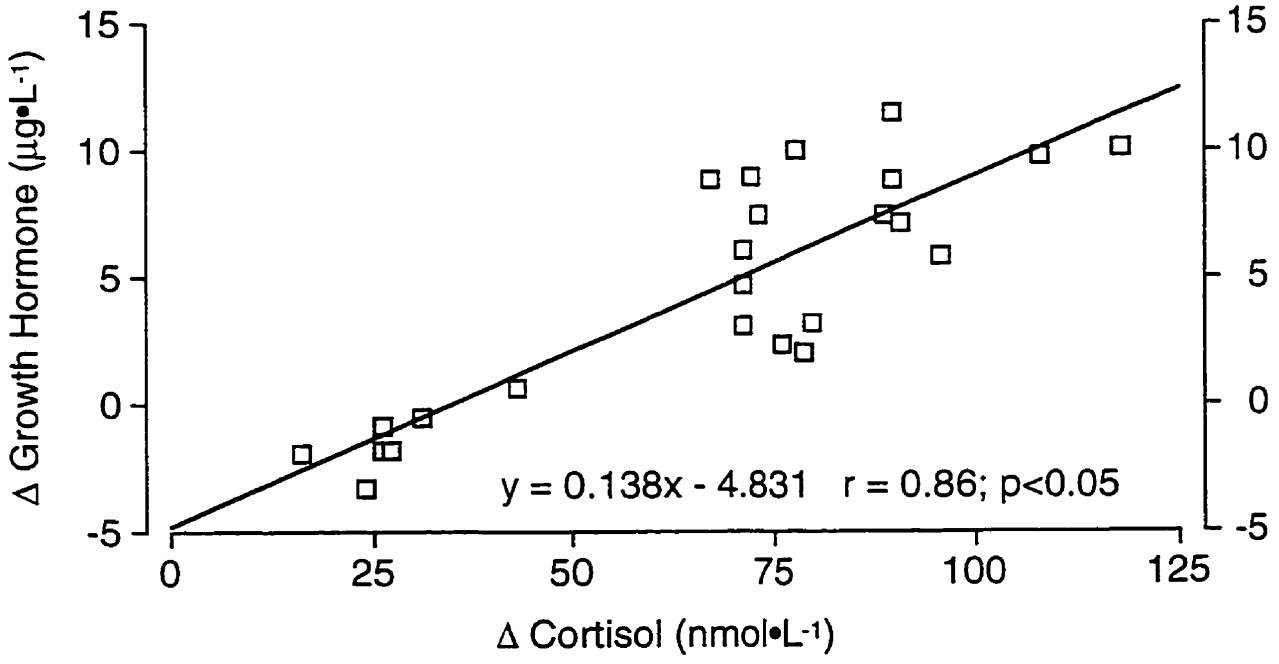

Figure 4-10: Correlation of Δ GH and Δ cortisol responses in L-F.

A significant linear relationship was also observed between the Δ cortisol responses of the L-F protocol and the corresponding Δ lactate concentrations ($r=0.78$; $p<0.05$) (Figure 4-11).

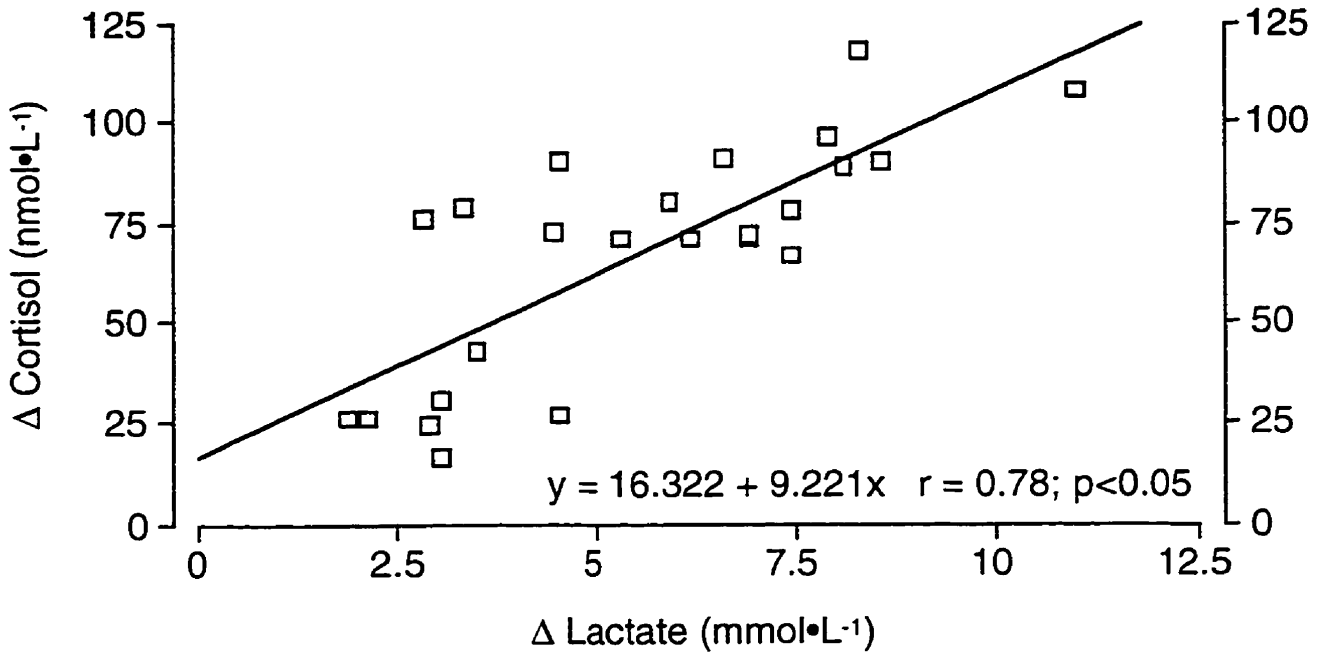

Figure 4-11: Correlation of mean Δ cortisol and Δ lactate responses in the L-F protocol.

4-9. Discussion:

The findings of this investigation clearly demonstrated significant differences in the hormonal and metabolic responses to two biceps flexion protocols of equal total external work output, duration and work-rest intervals (30-150 s), but varying in the load and frequency characteristics. Whereas the H-S protocol was conducted at a significantly slower speed of concentric movement, the more frequent repetitions of the L-F protocol were conducted at a much faster speed of concentric movement, which resulted in the greater hormone response of the two protocols. In addition, a highly significant correlation was found between the Δ GH and Δ lactate responses during the resistance exercise in the L-F protocol.

We have previously postulated that neural signals initiated by chemo-metabolic receptors within the working muscles may be responsible for the activation of the hypothalamic-pituitary axis, and therefore, the subsequent responses of GH (VanHelder et al., 1984b; VanHelder et al., 1987). One of the original aims of this project was to investigate the contribution of different muscle volumes, and hypothetically, the contribution of contrasting quantities of muscle chemo-metabolic receptors to the resulting GH response. This investigation was designed, in part, to allow for the comparison of the hormonal and metabolic responses of two arm flexion exercise protocols to the same measured responses during the leg extension experiment of Chapter 3. This was done by only including those subjects who completed both the leg extension project and the arm flexion series of experiments.

We hypothesized that the strength testing of the quadriceps leg muscles would result in significantly higher values, for both the 7 RM and the maximal 1 RM strength tests than the arm strength tests. As demonstrated in Table 4-3, the leg extension exercise produced significantly higher maximal strength results ($p<0.05$). Furthermore, the two exercise protocols, heavy-slow (H-S) and light-fast (L-F), based on the leg 7 RM, produced much greater total external work than

their equivalent arm flexion protocols (H-S and L-F, respectively). It is interesting that both of the arm exercise protocols were conducted at significantly slower speeds of concentric movement than the leg extension exercises. Furthermore, although the leg exercise resulted in much greater total work being completed, the highest hormonal and metabolic responses were found during the arm flexion protocols.

TABLE 4-3:

Strength and protocol testing during arm and leg resistance exercise.

(Mean ± SD)

	Arm Exercise:	Leg Exercise:
Factor (unit):		
7 RM (kg)	39.2 ± 5.9 *	59.5 ± 11.6
1 RM (kg)	52.6 ± 9.8 *	75.7 ± 18.5

Breakdown of Protocol Workloads

	Arm Exercise:		Leg Exercise:	
	H-S	L-F	H-S	L-F
Protocol Workload (kg)	29.3 ± 4.2	9.7 ± 1.4 **	44.7 ± 8.7	15.0 ± 2.7 **
Total Protocol Work (kJ)	7.5 ± 1.1 *	7.4 ± 1.1 *	11.4 ± 2.2	11.3 ± 2.2

* $p<0.05$ significance from leg study
** $p<0.005$ significance from H-S trial

The speed of concentric movement was significantly different between the two exercise experiments. In the H-S exercise trial, the 7 leg extension movements were completed at 141.4 ± 15.9 $deg \cdot s^{-1}$, while the arm flexions in the H-S protocol were completed at 74.7 ± 10.4 $deg \cdot s^{-1}$ ($p<0.05$). During the 21 repetitions of the L-F leg protocol, the speed of movement was 178.0 ±

17.3 deg•s^{-1}, while the equivalent protocol of the arm flexion exercise, L-F, was completed at 129.4 ± 17.8 deg•s^{-1} ($p<0.05$). Thus, both exercise protocols of the leg extension project were completed at faster speeds than the matched protocols during the arm flexion exercise tests.

When the data were adjusted to allow for the comparison of the hormonal responses during equal time periods, both of the arm flexion protocols, H-S and L-F, resulted in significantly greater total areas under the GH curves than was observed with the leg extension protocols (Figure 4-12). The H-S leg extension exercise produced a total GH response of only 80 µg•L^{-1}, while the identical exercise intensity (i.e. 75 % of the 7 RM) during the H-S arm flexion protocol resulted in a significantly higher total GH response (340 µg•L^{-1}). The L-F leg extensions produced a GH response of 341 µg•L^{-1} compared to a level of GH of 1036 µg•L^{-1}, for the L-F arm flexion exercise.

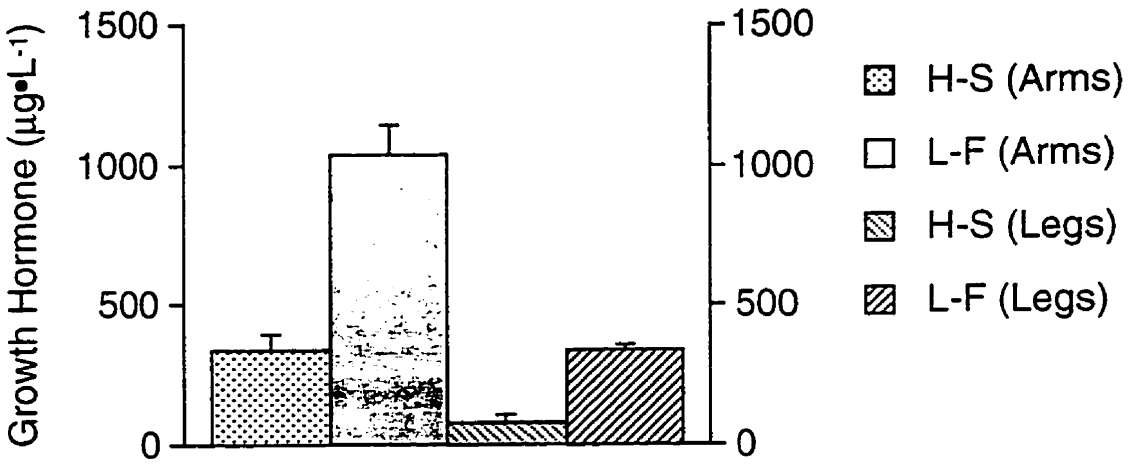

Figure 4-12: Incremental grea of GH in arm and leg exercise.

A similar pattern of responses in lactate was found. The lactate levels of the arm flexion exercises were significantly greater than the lactate concentrations observed during both of the leg extension protocols. The results of this study show that, for identical exercise intensities per unit

of muscle volume, the arm exercise produced significantly higher hormonal and metabolic responses than the leg exercise (Figure 4-13).

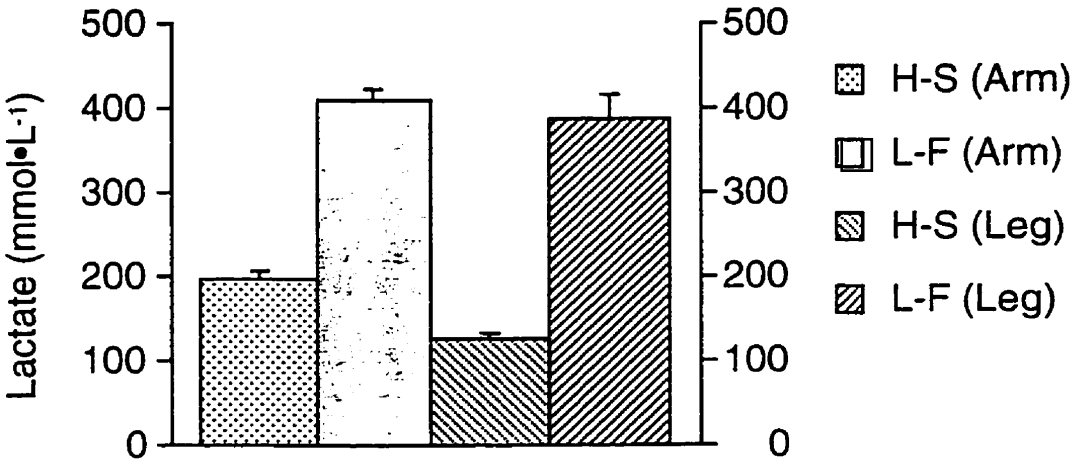

***Figure 4-13**: Incremental area of lactate in H-S and L-F.*

Given that both experiments were completed with the identical work-rest interval schedule (30-150 s) and pre-programmed range of movement on the ergometer (70 °), the speed of the extension movement during the leg exercise experiment should have allowed for more time to rest between repetitions. Our current understanding of muscle blood flow both during and following muscular contractions, and the enhanced hormonal responses experienced with ischemic exercise, may help explain the observed differences between the two experiments. The arm exercise repetitions, either 7 or 21, were completed at a significantly slower velocity during the prescribed 30 s of exercise than the leg exercises, thereby reducing the capacity of blood flow to remove and prevent the accumulation of metabolic waste by-products. This was evident by the enhanced lactate concentrations observed during both protocols of the arm exercise experiment. It is the accumulation of such metabolic waste products, be it lactate, $[H^+]$, or some other consequence of accelerated anaerobic glycolysis, that may be responsible for the observed changes in GH during exercise.

Interestingly enough, other factors may have played crucial roles in the blood flow and perfusion circumstances of the exercising muscles. It has been previously demonstrated that the identical exercise, conducted above or below the level of the heart, produces significantly different blood flow rates, perfusion pressures and lactate responses (Folkow et al., 1971). The nature of the Ariel ergometer made it necessary that the arm flexion movement be completed above, or at least at equal level to the heart itself. Furthermore, the Ariel leg station dictated that subjects' exercising legs be stationed below the level of the heart. According to the work of Folkow et al. (1971), and supported by the findings of Eiken & Bjurstedt (1987), these conditions should have resulted in significantly different blood flow and lactate conditions between the two experimental conditions. While blood perfusion was not measured, the concentrations of lactate, a valid indicator of ischemic exercise, were significantly more elevated during the arm exercise experiment. We believe that it is this factor, blood perfusion/flow, that may explain the observed differences in circulating GH concentrations between both the two experiments and the two protocols within each of the experiments. We also believe that the metabolic consequences of this factor are reflected in the oxygen demand/availability ratio.

While our data tend to support the thesis that a by-product(s) of rapid anaerobic glycolysis are responsible for the regulation of GH secretion during exercise, an alternative hypothesis has been suggested by Kozlowski et al. (1983). These investigators focused on the existence of a central command system and its contribution to the observed GH responses during identical aerobic intensity exercise while utilizing different muscle volumes. Kozlowski et al. (1983) speculated that the hormonal and metabolic responses of arm and leg exercise would be the result of stimulation of hypothalamic neurosecretory cells by neural efferent impulses arising from the motor centers of the cerebral cortex. In this investigation, while the power output was identical, a greater number of neural units must have been recruited during the arm exercise protocols than in the stronger and larger leg muscles (Kozlowski et al., 1983). It was believed that these differences

in the central neural contribution to GH secretion accounted for the greater GH responses in the arm exercise performed at the identical exercise intensity.

However, in the present investigation, with the use of a muscle specific intensity indicator, the 7 RM for the specific muscle tissues, we have been able to separate these specific effects and the contribution of the central nervous system. By using the limb specific 7 RM for the arms and the legs in our protocol determinations, we applied a stimulus that was muscle volume specific. With this in mind, the contribution of the central nervous system to the secretion of GH, and its feed-forward capability, would have been equal in our investigation. Our results imply that the central nervous system was not involved in the feed-forward regulation of GH secretion during the resistance exercise. Rather, with the higher levels of GH and lactate detected during the arm exercise, and not the leg exercise experiment, we can speculate that the detection of the metabolic alterations in the internal milieu within the exercising tissue lead to the differences in the GH responses.

From the results of our present investigation, and the observations of Kozlowski et al. (1983), it can be suggested that exercise conducted above the level of the heart greatly enhances the production of lactate, and the corresponding GH responses, in comparison to the identical exercise performed below the level of the heart. Previously, we have demonstrated significantly different responses of GH and lactate to resistance protocols where the repetitions were completed consecutively, as in the L-F protocol of this study or the L-F protocol of the leg extension experiment. Furthermore, we have shown opposite results when the exercise was conducted with some pause or hesitation between the repetitions, as was the case with the H-S protocol of the present study or the H-S exercise of the previous experiment. With these factors in mind, one cannot discount the probability that muscle blood flow and perfusion were quite different under these exercise conditions. Thus, it appears that the exercise-induced increases in GH secretion may be the result of local changes in tissue metabolism which are enhanced by the changes in

blood flow and perfusion, and not the result of some feed-forward, efferent neuroendocrine regulatory system.

In this study, we investigated the contribution of muscle mass to GH responses during heavy resistance exercise, and observed a higher GH response when smaller muscle groups were used in comparison to a larger muscle mass. Upper arm flexion exercise produced a greater GH response than the identical muscle specific intensity utilized during leg extension movements.

4-10. Conclusions:

A number of investigators have speculated that the participation of peripheral neural afferent signals may be responsible for the stimulation of GH release during different types of exercise (Kozlowski et al., 1983; Few et al., 1980). In this case, the existence of afferent muscle chemo-metabolic receptors, specifically activated by an increase in lactate concentration in the environment surrounding the contracting muscle cells, would have initiated events leading to the stimulation of the hypothalamic-pituitary axis, and subsequently, the secretion of GH (Stegman & Kenner, 1971). Other local muscular changes, such as an increase in PCO_2 (McCloskey & Mitchell, 1972), a decrease in pH (Longhurst & Zelis, 1979) or a change in $[H^+]$ concentration (Gordon et al., 1994) may have also been responsible for the observed differences. Thus, it is plausible that the alterations in these factors, in response to changes in blood flow, were greater in a smaller group of muscles, such as the arm biceps, compared to larger muscles, such as the quadriceps of the legs. Furthermore, the upper arm may also have had a greater density of sensory innervation for the detection of these regulators, than that found in leg muscles.

The data presented in this investigation demonstrate that, for a given work load based on the 7 RM, independent of the load and frequency of movements utilized, the resistance exercise with the arm biceps curl is a more potent stimulus for the elevation of GH than the same intensity

of resistance exercise performed with leg extensions. Our data are supported by the previously published observations of Kozlowski et al. (1983) utilizing moderate aerobic exercise. However, this investigation used heavy resistance exercise protocols and a different indicator of intensity based on the results of the 7 RM strength test. We can speculate that a feed-forward system may not be responsible for the regulation of GH during exercise. Rather, the detection of specific metabolic by-products appears to be involved in the regulation of GH during resistance exercise.

The hypotheses tested in this investigation were that:

i. The H-S protocol, with higher loads and less frequent movements, would produce a greater GH response compared to that of the L-F protocol, where lower loads and a higher frequency of movements are used.

This hypothesis was rejected. Repetitions in the H-S exercise protocol were completed at a significantly slower speed of muscle contraction, but failed to alter the GH response. In all subjects, the L-F protocol, completed at a much faster speed of movement, resulted in the greatest increases in Δ GH during both the exercise and recovery periods.

ii. The use of a smaller volume of muscle during the arm flexion exercises would result in significantly less GH being secreted than the levels observed in the leg extensions of the previous experiments.

This hypothesis was rejected. The use of a smaller volume of muscle during the arm flexion exercise resulted in significantly more GH being secreted than the levels observed in the leg extension protocols of the previous experiments.

iii. The responses of lactate and GH during the two exercise protocols are not correlated significantly.

This hypothesis was rejected. The GH and lactate responses were significantly correlated during the exercise.

4-11. Suggestions for future study:

Given the results of the present investigation, it would seem appropriate to repeat this investigation with the addition of catecholamine determination and expired gas analysis. By analyzing the catecholamine levels, the activation of the sympathetic nervous system and the contribution of adrenergic mechanisms to the GH response could be determined. The various responses could then be compared to the hormonal and metabolic responses of the leg exercise protocols. Furthermore, by utilizing breath-by-breath analysis of the expired gases, the oxygen demand/availability ratio could be determined and related to the responses of GH during the exercise period.

CHAPTER 5

The Influence of Alpha(α)-Adrenergic Blockade On The Growth Hormone Responses To Resistance Exercise

5-1. Abstract:

Objectives: The objectives of this investigation were to examine the role of alpha(α)-adrenergic mechanisms in the secretion of growth hormone (GH) during different resistance exercise protocols. By employing an α-adrenergic receptor block, Phentolamine, the hormonal responses to two resistance exercise protocols of equal total external work output and work-rest intervals were compared. The two resistance exercise protocols differed only in the load and frequency characteristics. We hypothesized that the use of the α-adrenergic blockade would not change the pattern of the GH response to the two exercise protocols.

Design of the Study: A controlled, cross-over study was designed. Eight healthy men, aged 26.4 ± 1.6 years, randomly completed two intermittent resistance training exercise protocols, a heavy load-slow repetition protocol (H-S) and a lighter load-faster repetition (L-F) protocol, of equal total duration (18.5 min). Each protocol was performed twice, once under phentolamine block and once under saline infusion (control). The H-S protocol consisted of 7 sets of 7 repetitions of knee extensions at 75 % of each subject's 7 repetition maximum (7 RM). The L-F protocol included 7 sets of 21 repetitions of similar knee extensions, but at one-third of the load used in the H-S protocol. All exercise sessions were conducted on a calibrated Ariel computerized ergometer. Each subject completed all of the four exercise trials with and without the blocker, designated H-S, L-F, H-S (block) and L-F (block).

Methods: Peripheral venous blood was collected every 5 min during the exercise period (5 samples) and every 10 min throughout the 70 min of recovery (5 samples). Subsequently, all plasma samples were analyzed for GH, cortisol, norepinephrine (NE), epinephrine (EPI), glucose, free fatty acids (FFA) and lactate before, during and after each of the knee extension protocols. Hormone analysis was conducted using radioimmunoassay techniques. Catecholamine analysis was conducted using high performance liquid chromatography. Glucose, FFA and lactate analyses were completed using colorimetric analysis techniques. All values were adjusted for changes in plasma volume by the method of Dill and Costill (1974).

Results: The speed of concentric movement was 3.6-fold faster in the L-F exercise than in the H-S exercise. Without the blocker, the GH levels increased significantly in both exercise protocols from the beginning of exercise, peaking at 20 min into the recovery period. The total GH response was 192 % higher in the L-F trial than in the H-S trial (833.9 $\pm$ 113.4 $\mu g \cdot L^{-1}$ vs. 285.4 $\pm$ 58.9 $\mu g \cdot L^{-1}$, respectively). Phentolamine blockade of α-adrenergic receptors during the exercise and recovery periods significantly inhibited the GH responses to the two exercise protocols as well as eliminating any differences in GH-responses between the two exercises. A different pattern of response was observed with respect to NE levels during exercise. Although NE increased significantly during both the H-S and L-F protocols, no differences were found between the total NE responses. Phentolamine blockade elevated significantly the resting and exercise-induced elevations in NE, but with no differences between H-S and L-F. The L-F exercise induced a peak increase in EPI that was 275 % higher than the H-S protocol. Phentolamine induced a partial inhibition of the EPI increase, resulting in a decrement in the peak level of 25 % in both protocols. Glucose levels increased significantly in both exercises, with the L-F producing a higher level than the H-S in both the control and block studies.

Conclusions: The experimental design allowed us to compare two resistance exercise protocols of equal total external work output, duration and work-rest intervals, but with varying load and

frequency of movement characteristics. The differences in plasma GH response between the H-S and L-F protocols were eliminated by the blockade of the α-adrenergic receptors via the infusion of phentolamine, suggesting that the GH response to heavy resistance exercise is related to the activation of α-adrenergic receptors, rather than to circulating levels of NE, EPI or glucose.

5-2. Introduction:

The control of pituitary growth hormone (GH) secretion is regulated by two hypothalamic factors, somatostatin (SS) which inhibits secretion, and growth hormone releasing hormone (GHRH) which stimulates secretion. These hypothalamic factors are released from the median eminence of the hypothalamus where they are transported via the hypothalamic-pituitary portal system to interact with receptors on the somatotrophes of the anterior pituitary. The regulatory control of these secretory factors, SS and GHRH, has been attributed to a number of biogenic amines, including norepinephrine (NE), dopamine, serotonin, acetylcholine, and gamma-aminobutyric acid, which have excitatory or inhibitory effects at brain sites modulating hypothalamic control (Muller, 1967). The α-adrenergic mechanisms are known to play a key role in the regulation of physiologic GH secretion. With respect to physical exercise, increases in plasma GH secretion can be inhibited by the administration of α-adrenergic, cholinergic, dopaminergic and serotonergic blocking agents and enhanced by α-adrenergic agonists and ß-adrenergic receptor blocking agents (Galbo, 1983). In order to elucidate further the regulatory control of GH secretion during heavy resistance exercise, it was our objective to examine the role of catecholamines, specifically α-adrenergic receptors, in the response of GH to different resistance exercises.

It has been postulated that central nervous system catecholamines play a major role in the neural-humoral regulation of GH secretion. Early evidence supporting the contribution of the adrenergic nervous system to the pituitary secretion of GH was found by Muller et al. (1967), who demonstrated that the depletion of brain NE completely suppressed the GH secretion response during hypoglycemia induced by insulin, an accepted and potent stimulus of GH secretion. The investigators postulated that the lack of the central sympathetic tone resulted in the inhibition of GH secretion through the blockade of NE-stimulated GHRH secretion.

A number of other investigations have clearly demonstrated the GH stimulatory effect of α_2-adrenoceptor agonists, such as clonidine and guanfacine (Lancranjan & Marbach, 1977). It has also been well established that blockade of the β-adrenergic receptors with propranolol leads to a significant increase in the secretion of pituitary GH (Blackard & Heidingsfelder, 1968; Mauras et al., 1987), suggesting that the role of the adrenergic nervous system in GH secretion is both stimulatory and inhibitory and based on the interaction of specific receptor types (Mazza et al., 1990).

We have previously investigated the responses of GH, NE and EPI to two resistance exercise protocols of equal total external work output, duration and work-rest intervals but differing in load, i.e., heavy vs. light, and frequency of repetition, i.e., high vs. low, characteristics (Chapter 3). We reported that both NE and GH concentrations increased similarly in the L-F (light & fast) protocol immediately after the onset of exercise and peaked at the end of the resistance exercise period. A significant correlation ($r=0.67$; $p<0.05$) was found between the NE and GH levels during the exercise period. In contrast, the GH and NE responses in the H-S (heavy & slow) protocol failed to change significantly during both the exercise or recovery periods.

The association between GH and catecholamines, specifically NE, observed during our previous investigations led to the present study. Although a number of different neurotransmitter systems regulate GHRH and SS secretion from the hypothalamus during various physiologic states, it is postulated that NE exerts a powerful influence over the responses of these peptides during exercise. It is also believed that the exercise-induced secretion of GH is due, in whole or in part, to the α-adrenergic stimulation of these hypothalamic hormones. It is our intention to investigate the contribution of catecholamines to GH secretion during various resistance exercise protocols.

5-3. The Neural Regulation of Growth Hormone Secretion:

Early investigations into the regulation of GH secretion have shown that plasma GH levels responded to several different factors, including rapid changes in blood glucose concentrations, amino acid ingestion, exercise, heat and stress (Devesa et al., 1992). These stimuli appeared to influence the pituitary GH secretion through activation of hypothalamic releasing factors (Imura et al., 1971). It was also discovered that the catecholamine composition of the hypothalamus is greater than in any other central nervous system structure (Carlsson, 1959; Shute & Lewis, 1966). Since adrenergic mechanisms in the hypothalamus are concentrated in the median eminence (Carlsson et al., 1962), and patients suffering from phenochromocytoma also display elevated resting GH levels, it was suggested that catecholamines might play a major role in the regulation of GH secretion via the hypothalamic releasing hormones, GHRH and SS.

As it was well established that the hypothalamus was rich in monoaminergic fibers (Shute & Lewis, 1966), Muller et al. (1967) investigated the effects of injecting a number of different catecholamine depletor chemicals (reserpine, α-methyldopa, α-methyl-m-tyrosine and tetrabenzamine) on the subsequent responses of GH to insulin-induced hypoglycemia. From this series of investigations, Muller et al. (1967) were able to conclude that the reduction in brain NE stores impaired the release of GH and, that the brain amines played a significant role in the release of hypothalamic neuro-humoral transmitters, GHRH and SS.

Blackard & Heidingsfelder (1968) demonstrated the influence of both α-adrenergic and β-adrenergic blockers on the subsequent GH response to different physiologic stimuli. The infusion of 0.5 $mg \cdot min^{-1}$ of phentolamine, an α-adrenergic antagonist, inhibited the increase in GH by 30 to 50 % of the values obtained during the identical insulin-induced hypoglycemia without an α-adrenergic blocker. Subsequently, the infusion of the β-adrenergic blocker propranolol amplified the GH response to the insulin-induced hypoglycemia. The authors concluded that phentolamine

infusion did not eliminate the increase in GH secretion, but served only to diminish it, whereas β-adrenergic blockers greatly enhanced the GH increase to the insulin-induced hypoglycemic challenge. Thus, the existence of more than one regulatory neural system for GH secretion was put forward by these investigators.

Subsequently, Imura et al. (1971) studied the effects of a number of adrenergic stimulating and blocking agents on the responses of plasma GH level to insulin-induced hypoglycemia and arginine infusion. In this case, propranolol infusion resulted in an increase in GH, a transient decrease in free fatty acids (FFA), no effect on insulin concentration, and a significant enhancement of the plasma GH response to an insulin-induced hypoglycemia or the ingestion of the arginine. Conversely, the infusion of the α-adrenergic stimulating agents, phenylephrine and methoxamine, increased GH levels and decreased FFA concentrations slightly. The β-adrenergic stimulant isoproterenol suppressed the propranolol-induced GH secretion. These investigators concluded that either β-adrenergic blockade or α-adrenergic stimulation could enhance the secretion of GH, whereas either an α-adrenergic antagonist or a β-adrenergic agonist suppressed the secretion of GH (Imura et al., 1971).

In an early series of experiments, Hansen (1971) investigated the plasma GH response to exercise on a Monark cycle ergometer in normal and diabetic males during the infusion of either the α-adrenergic antagonist, phentolamine, or the β-adrenergic antagonist, propranolol. After an initial 10 min of phentolamine block, infused at a rate of 0.5 $mg \cdot min^{-1}$, each subject exercised at a very light workload intensity of 450 $kg \cdot min^{-1}$ for 40 min. The phentolamine infusion was discontinued after 10 min into the recovery period. By repeated blood sampling, Hansen (1971) found an almost total disappearance of the exercise-induced GH response during the phentolamine infusion. Furthermore, GH levels failed to increase after the infusion was discontinued. Conversely, the infusion of propranolol during the identical exercise stressor produced peak GH levels that were 2-fold higher than in the controls. The authors concluded that the phentolamine blockage of the α-

receptors inhibited the secretion of GH while the blocking of β-receptors dramatically stimulated the increase in GH secretion in response to the exercise stimulus.

Hansen (1971) postulated that the adrenergic control of GH secretion was mediated through catecholamines that were released locally from the monoaminergic fibers within the hypothalamus, as opposed to peripherally released catecholamines. This hypothesis was supported by Muller et al. (1967) who demonstrated that the insulin-induced GH release could be blocked by medications that depleted the brain reserves of catecholamines, but not by drugs that depleted the peripheral stores of catecholamines. While Hansen (1971) could only speculate that the stimulus for GH secretion during exercise was the result of some neural-humoral signal from within the working musculature, he was able to demonstrate that the exercise-induced GH secretion was modified by the use of adrenergic blocking agents, either positively with the application of ß-blockers, or negatively with the use of α-blockers.

In apparent contrast to Hansen (1971), Sutton & Lazarus (1974) demonstrated an effect of phentolamine that was intensity-dependent when they repeated the investigation of Hansen (1971). In this case, the authors investigated the GH response to various exercise intensities during the use of various adrenergic blocking agents. As before, the phentolamine infusion, consisting of 0.5 $mg \cdot min^{-1}$ of Rogitine, was initiated 10 min before the exercise period and continued for an additional 60 min at the identical infusion rate. The results demonstrated that phentolamine was able to suppress the GH response when the 20 min exercise period was conducted at either 450 $kpm \cdot min^{-1}$ or 600 $kpm \cdot min^{-1}$ compared to the same exercise intensities in the placebo-control. Average peak GH levels of 16.9 ± 14.3 $\mu g \cdot L^{-1}$ and 18.2 ± 15.0 $\mu g \cdot L^{-1}$ occurred during the 450 $kpm \cdot min^{-1}$ exercise test in the control and phentolamine infusions, respectively. During the 600 $kpm \cdot min^{-1}$ exercise test, peak GH values of 10.7 ± 5.4 $\mu g \cdot L^{-1}$ and 10.9 ± 7.5 $\mu g \cdot L^{-1}$ were detected in the control and phentolamine trials, respectively. While the phentolamine infusion was able to prevent the increase in GH when the exercise intensity was fixed at 900 $kpm \cdot min^{-1}$, the responses

between the phentolamine and placebo were not statistically different from each other. During the 900 kpm•min^{-1} exercise test, peak GH levels of 37.6 ± 10.3 μg•L^{-1} and 31.1 ± 7.4 μg•L^{-1} occurred during the control and phentolamine infusions, respectively. Interestingly enough, the infusion of propranolol enhanced the exercise-induced GH response at all levels of work output intensity. Sutton & Lazarus (1974) attributed the differences between these results and the earlier findings of Hansen (1971) to the small number of subjects used in their investigation and the fact that the statistical significance of Hansen (1971) was only apparent during one sampling time. Sutton & Lazarus (1974) concluded that it was unlikely that an α-adrenergic mechanism plays a major role in the mediation of GH secretion due to physiological stimuli.

A closer inspection of the data presented by Sutton & Lazarus (1974) revealed that only 4 subjects were tested in the high intensity group (900 kpm•min^{-1}). This would tend to minimize any statistical effects that the infusion of phentolamine may have had on the secretion of GH during the exercise trials. It is also difficult to accept the report that the very light work load (450 kpm•min^{-1}) resulted in a significantly greater GH response than the heavier exercise stress of 600 kpm•min^{-1}. Furthermore, Sutton & Lazarus (1974) have attempted to compare the results of 20 min exercise periods in their investigation and those of Hansen (1971) whose subjects were exercising for 40 min. These factors make the results of Sutton & Lazarus (1974) suspect, and should provide reason for caution when interpreting their findings and conclusions.

Berger et al. (1980) also investigated the effect of the infusion of either phentolamine or propranolol on the GH response to a graded submaximal exercise test. The progressive incremental exercise test at approximately 85 % of the subjects' $\dot{V}O_2$ max was 27 min in finishing. During the control experiment, the peak GH level of 13 ± 4.0 μg•L^{-1} occurred by 3 min of recovery. While Berger et al. (1980) reported that there was no significant response of GH to the exercise conducted during the infusion of phentolamine (0.5 mg•min^{-1}), the use of a propranolol infusion (0.08 mg•min^{-1}) produced a peak GH level of 23 ± 7.0 μg•L^{-1} by the end of the exercise

period (27 min). The experiment with the phentolamine infusion produced areas under the GH response curve that were 18 % (0-27 min) and 25 % (0-40 min) of the identical exercise completed during saline infusion trial. Conversely, the propranolol infusion produced GH areas that were 2.5 and 2.0 fold greater than observed during the saline trials, and 13.0 and 8.0 fold higher than with phentolamine infusion. These findings support Hansen's proposal (1971) that GH secretion during exercise is regulated by the α-adrenergic system.

A closer inspection of the data of Berger et al. (1980) revealed that the resting basal GH levels between the three conditions were not identical. Thus, the specific conditions that started out with the highest resting GH levels also resulted in the greatest peak GH increases during the exercise and recovery periods. Furthermore, while the initial infusion of phentolamine from -10 to 27 min suppressed the GH response to the exercise stimulus (0-27 min), the GH level began to increase after 21 min of exercise, and became significantly elevated (30 min) over the baseline value while the infusion continued for another 13 min of recovery (40 min). Thus, as the other two conditions (saline and propranolol) demonstrated GH levels that fell by the 35 min blood sample (8 min into recovery), the phentolamine-exercise trial still demonstrated an increasing response of GH secretion. It is interesting to speculate that there may actually be more than one system involved in the release of GH during exercise, as there appears to be two distinct patterns, an initial suppression due to the adrenergic blockade and a later period of enhancement, despite the continued infusion of phentolamine. This would allow for an acute or short-acting stimulus mediated by the adrenergic neural transmitters and a chronic or longer-acting stimulus arising possibly from the exercising tissues.

In a recent series of experiments, Struthers et al. (1986) investigated the contribution of a specific adrenergic receptor subtype, the α_2-adrenoceptors, to the neural regulation of GH secretion during various physiologic stimuli. This objective was accomplished with the administration of idazoxan, an α_2-adrenoceptor antagonist, during both the infusion of GHRH and the stress of 25

min of intermittent cycle ergometer exercise. Since the GHRH-induced increase in GH was unaffected by the administration of idazoxan, the investigators concluded that the α_2-adrenoceptors were not involved in GH regulation at the level of the pituitary. However, the findings of Struthers et al. (1986) contradicted the previously accepted opinion that the α_2-adrenoceptors were involved in the increased secretion of GH during physical exercise (Hansen 1971; U'Prichard et al., 1977) by virtue that the pretreatment with idazoxan significantly augmented the plasma GH response to the exercise stress. From the results of this investigation, Struthers et al. (1986) concluded that the α_2-adrenergic receptors were capable of exerting a dual effect on GH release during exercise, and that the contribution of the α_2-adrenoceptors to GH regulation did not occur at the level of the pituitary.

5-4. Literature Conclusions:

In conclusion, it appears from the published literature that both the α-adrenergic and the β-adrenergic receptors are involved in the regulation of GH secretion during exercise. There have been a number of different investigations that have demonstrated that activation of α-receptors resulted in an increased secretion of plasma GH. while stimulation of the β-adrenergic receptors produced a decrease in the release of GH from the anterior pituitary. The use of both α-adrenergic blockers and β-adrenergic stimulators has resulted in the suppression of the GH response induced by exercise, by insulin-induced hypoglycemia, and by the ingestion of the amino acid arginine. While a number of difficulties are apparent in the findings and conclusions of the literature on the regulation of GH secretion, especially during exercise, to our knowledge there have not been any investigations focusing on the α-adrenergic contribution to GH secretion during various resistance exercise training protocols. We speculate that the resistance exercise-induced secretion of GH is due, in whole or in part, to the α-adrenergic stimulation of the hypothalamic hormones, GHRH and SS. It is our intention to investigate the contribution of catecholamines to GH secretion during

various resistance exercise protocols by using the infusion of the α-adrenergic antagonist, phentolamine.

5-5. Phentolamine Use, Pharmacology and Administration:

Pharmacologic agents that inhibit the interaction of catecholamines, NE and EPI, with their specific adrenergic receptors are known as adrenergic receptor antagonists. Phentolamine, specifically the CIBA-Geigy brand Rogitine, is from the family of analogs referred to as imidazolines that produce a moderately effective competitive α-adrenergic receptor blockade of fairly short duration. Of the two main sub-populations of α-adrenergic receptors, α_1 and α_2, phentolamine has an equal affinity for each, and therefore, is known as a non-selective α-adrenergic antagonist. Clinically, phentolamine has been employed in the prevention and control of both the hypertensive episodes in patients with pheochromocytoma and dermal necrosis and sloughing, and in the diagnosis of pheochromocytoma. Phentolamine has also been utilized during investigations where the α-adrenergic contribution to a physiologic stimulus or process is under study.

Phentolamine can be administered in a number of different fashions. During extended α-adrenergic blockade, intravenous infusion is usually carried out with doses of 0.5 $mg \cdot min^{-1}$, while shorter acting dosages of 2-5 mg of phentolamine can be injected either intramuscularly or intravenously. Unfortunately, there is very little known about the fate of phentolamine in the body. Not more than 10 % of the infused dose is recovered during the analysis of urine.

When phentolamine is given to patients/subjects, it quickly produces vasodilation and cardiac stimulation; the resulting blood pressure response varies according to the relative contributions of the two effects. There is usually a fall in blood pressure with the use of phentolamine, which is caused by a direct vasodilatory action on smooth muscle, including the

vasculature and the gastro-intestinal tract. Phentolamine also has direct, but less noticeable, positive inotrophic and chronotrophic effects on cardiac muscle. The receptor-blockade produced by phentolamine also causes an increase in the synthesis and release of NE (Gould & Reddy, 1976). Side-effects of phentolamine are attributable to cardiac and gastrointestinal stimulation, such as tachycardia, cardiac arrhythmias and anginal and abdominal pain, nausea, vomiting, diarrhea, and accerbation of peptic ulcer (Hoffman & Lefkowitz, 1990).

5-6. Objectives:

The objectives of this investigation were to examine the:

i. Role of adrenergic stimulation during exercise on subsequent exercise-induced increases in GH secretion. Two resistance exercise protocols were designed that were equal in total external work output, duration and work-rest intervals, but different in load and frequency characteristics.

ii. Effect of α-adrenergic blockade using phentolamine infusion on the responses of GH and catecholamines during the above resistance exercise protocols.

5-7. Hypotheses:

The hypotheses tested in this investigation were that:

i. Adrenergic stimulation and catecholamine changes during resistance exercise are not related to the stimulation of GH secretion.

ii. The differences in GH response between the two exercise protocols will not be affected by α-adrenergic blockade by phentolamine.

5-8. Materials and Methods:

This study was completed at the Immunology and Physiology Laboratories of the Defence and Civil Institute of Environmental Medicine at the Canadian Forces Base Toronto. Some additional testing and analysis were also conducted at various departments within facilities at the Mount Sinai Hospital and the University of Toronto.

i. Subjects: Eight healthy males from the university and military populations, that were not currently involved in any resistance training program, volunteered as subjects for this investigation. Each participant was informed of all of the risks associated with the experiment and signed an informed consent which was previously approved by the Ethics' Committees of the University of Toronto and the Defence and Civil Institute of Environmental Medicine. Medical screening excluded any subject with acute or chronic medical conditions, including a known hypersensitivity to phentolamine or saline infusion. Subjects with medical findings were directed to further health service organizations and consequently dismissed from participation in the study. The testing team included the principle investigator, one physician and two research technicians. Emergency resuscitation equipment, including a crash cart and a phentolamine pharmacologic antidote kit (Norepinephrine & Ephedrine), were located in the laboratory. Subjects were asked to abstain from eating, smoking, caffeine ingestion and sexual activity for 12 h before the start of any of the testing procedures. Each subject visited the laboratory on seven separate occasions, three visits for physical tests and familiarization and four visits for protocol testing purposes.

ii. Physical Tests and Familiarization: On the first laboratory visit, two weeks prior to beginning the experiment, the physical characteristics for each subject were established. The percentage of body fat of each subject was determined through the use of skin-fold thickness measurements according to the method of Durin & Womersley (1974). In addition, the predicted $\dot{V}O_2$ max was determined using a cycle ergometer exercise test (Astrand & Rodahl, 1977) (Table 5-1).

One week before the experiment, a seven repetition maximum (7 RM) strength test, similar to the strength test used in Chapters 3 and 4 and by VanHelder et al (1984b), was performed by each subject using the Global Gym Quadriceps Extension apparatus (Global Gym & Fitness Equipment, Weston ON, Canada). The quadriceps strength testing was also measured by a 1 RM test. Each subject returned to the laboratory at the end of participation in the experiment to perform a re-test of both the 7 RM and 1 RM determinations. No significant differences were found between the pre and post testing results. The results of these testing procedures are presented in Table 5-2.

The 7 RM was employed as a bench mark for establishing the protocol characteristics, i.e., load and frequency manipulations. Seventy-five percent of the 7 RM was used to establish the work load for the two protocols utilized during the actual testing. The heavier load-slow repetition exercise protocol, designated H-S, consisted of 7 sets of 7 leg extensions using 75 % of the 7 RM. The lighter load-faster repetition exercise protocol, assigned L-F, consisted of 7 sets of 21 extensions, but with one-third of the load used in the H-S test.

After the strength testing, each subject was familiarized with the leg ergometer (Ariel Computerized Exerciser, Ariel Dynamics Inc. Model # M-4000, Trabuco Canyon, California, U.S.A.). The Ariel controlled work loads throughout the range of extension (preset at 70°) and calculated the velocity and acceleration characteristics of each concentric lift. As the Ariel suspended workloads during the relaxation phase of the movement, no data were collected during the eccentric phase of the knee extension when the ergometer was returned to the starting position. To standardize the total external work, a laboratory technician applied an external force after each contraction to reset the apparatus to the starting position. Total external work output for the two exercise protocols, was calculated as the product of the load, the number of lifts and the distance of the extension movement (Table 5-2).

iii. Protocol Design and Execution: Each of the H-S and L-F exercise protocols was completed on two separate occasions. In a random fashion design, each protocol was tested with the infusion of either: phentolamine or placebo (Figure 5-1).

	Heavy-Slow	Light-Fast
Control (saline)	H - S	L - F
Phentolamine	H-S (block)	L-F (block)

Figure 5-1: Randomized block for experimental design.

The timing sequence for each of the protocol testing is shown in Figure 5-2. Testing of the four protocols was conducted randomly, but separated by at least 7 days. Acting as their own controls, each subject performed both protocols, with protocol testing commencing at 0900 h and following a 12 h fast.

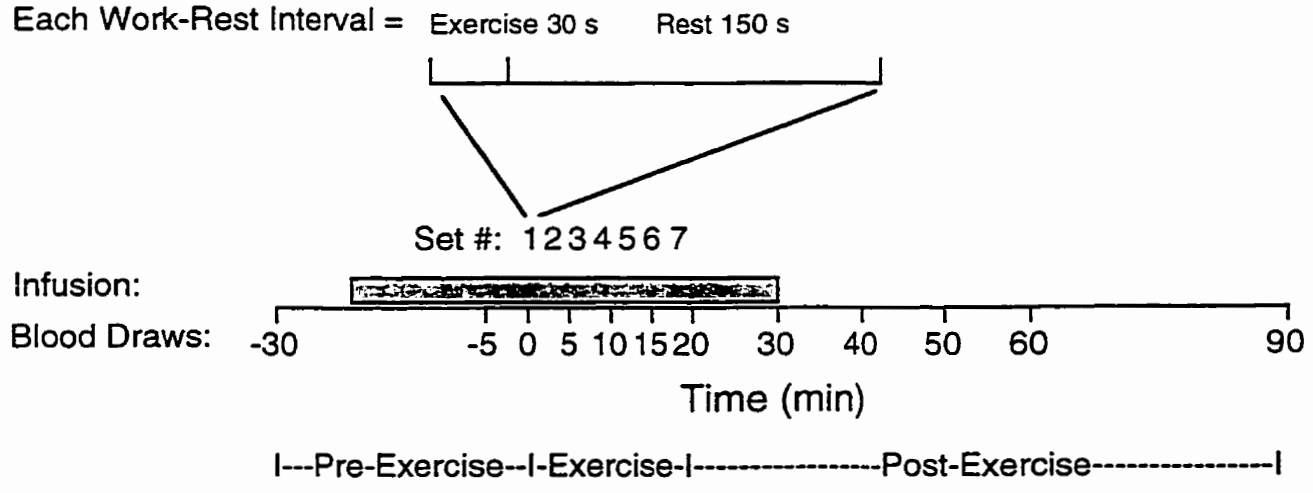

Figure 5-2: Time sequences for exercise sets, infusion and blood sampling in H-S and L-F protocols.

The exercise sets began at 0, 3, 6, 9, 12, 15 and 18 min. The subjects performed either 7 or 21 leg extensions in 30 s, then rested for another 150 s before beginning the next exercise set. An identical work-rest interval arrangement was repeated for the remainder of the 7 sets. The four protocols were of equal total external work output and exercise duration with identical work-rest intervals (30-150 s), but had different repetition and load characteristics (Table 5-2).

iv. Infusion: While each subject was in the recumbent position, a sterile I.V. catheter (Insyte, 20G, 2.5 cm), outfitted with a 3/4 ' HepLok extension, was aseptically inserted into the anticubital vein of the left forearm at -30 min before the start of the exercise test. Due to necessities within the laboratory setup, a site on the left arm was selected for use in the infusion, allowing the right arm to be used for the repeated blood sampling. The infusion pump (IMED 960 Volumetric Infusion Pump, IMED Corporation, San Diego CA, USA) was connected to a non-vented primary administration set (Accuset, IMED Corp., San Diego CA, USA) which had been outfitted with a three-way stopcock w/6" (Medex Inc., Hilliard OH, USA) and connected to the infusion bag. The I.V. administration line was then connected to the Heplock extension-catheter of left arm.

The intravenous infusion of either Rogitine (Phentolamine Mesylate, CIBA Pharmaceuticals, Mississauga, ONT.), or placebo (0.9 % sodium chloride, Baxter Medical, Toronto ON, CA) was started exactly 20 min before the testing of the specific exercise protocol was to take place. Phentolamine mesylate was freshly reconstituted on the morning of the testing by thoroughly mixing it with 100 mL of normal saline. At the end of the infusion period, the IMED pump was disconnected and the infusion solution was discarded appropriately. The infused solution was administered in a single blind format with either: 1. phentolamine; or 2. saline acting as single blind placebo.

After the subjects were attached to the infusion pump, they were moved into position on the ARIEL and the infusion of phentolamine or placebo was initiated at 0.25 $mg \cdot min^{-1}$ at a flow rate of

1.0 mL•min^{-1} in order to familiarize each subject with the infusion procedure and, in the case of the phentolamine infusion, to allow the subject's blood pressure to stabilize before the protocol testing was to begin. Blood pressure (auscultation method) and heart rhythm and rate were continually monitored (every min) throughout the length of each experiment, the latter with an EKG strip recorder (Litton Medical, Des Plains, IL, USA). After 10 min of infusion, the dosage was increased to 0.5 mg•min^{-1} by increasing the flow rate to 2.0 mL•min^{-1}. The infusion remained at this fixed amount until 10 min after the exercise period had finished. During the specific test, the total infused phentolamine was 45 mg while total infused saline was approximately 90 mL. Each subject remained in the laboratory for 1 hr after the end of the recovery in order to fully recuperate.

Data collection for this series of experiments began in the early May and was completed by the end of October for that same year. Each subject's specific time commitment was not more than 8 weeks. Over the duration of the experiment, there were no significant changes in the subject's aerobic or anaerobic fitness level, or medical condition.

v. Measures: At the same time that the infusion catheter was introduced into the left arm (-30 min), another sterile I.V. catheter (Insyte 20G, 2.5 cm), fixed with an injection adapter (Medex 1 7/16"), was inserted into the antecubital vein of the right arm. This blood sampling catheter was maintained patent with the use of a 0.6 cc heparin-saline locking solution (100 units•mL^{-1}) which was administered after each sample and removed before the next blood sample was obtained.

Resting blood samples were obtained in seated subjects at 30 and 5 min prior to each start of the protocol testing. Venous blood sampling continued during the exercise period at 0, 5, 10, 15 and 20 min and throughout recovery at 10, 20, 30, 40 and 70 min. Immediately after filling each vacutainer tube, the contents were mixed by gentle repeated inversion. Blood samples for GH were collected in 10 mL tubes containing 0.4 mL EDTA acid (15%) solution. Blood samples for catecholamine analysis were collected in 10 mL tubes containing 143 USP units heparin and 60

mg glutathione while blood samples for glucose were collected in 3 mL tubes containing 30 mg sodium fluoride (NaF) powder. A 25 µL sample from this tube was mixed with 200 µL of ice-cold perchloric acid ($HClO_4$) for lactate analysis. A 25 µL sample was also used to determine the hematocrit and hemoglobin levels for the calculation of blood and plasma volume changes. Each vacutainer was immediately centrifuged at 4° C and 2500 G for 15 min and the separated plasma frozen at -70° C until analysis. As each blood sampling interval consisted of a 23 mL volume, the total blood volume obtained during the sampling in each exercise protocol test was 276 mL.

vi. Analyses: All subsequent biochemical analyses were performed in duplicate, except for GH which was performed in triplicate. Plasma GH levels were analyzed using double-antibody radioimmunoassay kits, (Pharmacia, Uppsala, Sweden). The standard curve for GH was modified with the addition of three extra standards of low GH levels (0.25, 0.0125 and 0.00625 $\mu g \cdot L^{-1}$), in order to extend the curve to detect the existence of extremely low resting levels. Radioactivity was determined using a calibrated Cobra Auto-gamma Counter (Packard Model 5002, Meriden CT, U.S.A.). Catecholamine levels were analyzed on 5 mL plasma samples using high performance liquid chromatography and electrochemical detection (ESA HPLC, Coulochem II, Bedford MA, U.S.A.) according to the methods of Munoz et al. (1989). Glucose levels were determined with a glucose enzyme kit (Boehringer Mannheim, Mannheim Germany) as described by Trinder (1969). Lactate levels were determined with the use of a lactate enzyme kit (Boehringer Mannheim, Mannheim, Germany) as described by Maughan (1982). Colorimetric analysis for both glucose and lactate was completed on the Gilford Stasar III Spectrophotometer (Gilford Laboratories INC., Oberlin OH, U.S.A.) and the Perkin-Elmer 650-10M Fluorescence Spectrophotometer (Hitachi Ltd., Tokyo, Japan), respectively. Hematocrit and hemoglobin levels were determined with the use of microhematocrit capillary tubes (Autocrit Ultra-3, Franklin NJ, U.S.A.) and the Hemocue ß-Hemoglobinometer (Hemocue Photometer, Helsingborg, Sweden), respectively. The responses observed in each protocol were corrected for percent changes in

plasma volume by the method of Dill & Costill (1974) in order to eliminate the influence of plasma volume shifts during the exercise and recovery periods.

vii. Statistical Analysis: As no significant differences were found in any of the measured resting blood factors between -30, -5 and 0 min, all exercise and recovery levels were normalized with respect to the average of the three resting levels and compared using a repeated measures ANOVA (Superanova, Abacus Concepts Inc., Berkeley CA, U.S.A.). Post-hoc analysis was also completed using a means comparisons. The integrated area under the response curve was calculated using the trapezoidal rule for unequally space X values. In addition, relevant correlations for each hormone were also calculated and compared between the H-S and L-F protocols (Statview, Abacus Concepts Inc., Berkeley CA, U.S.A.). The level of statistical significance accepted was $p<0.05$. The data are presented as the mean data $\pm$ standard error (SE) in the figures and $\pm$ standard deviation (SD) in the tables. Where error bars do not appear to be presented, the particular error bar for particular data point was too small to be represented for the specific point.

5-9. Results:

i. Subject Characteristics:

All of the eight subjects were able to successfully complete all phases of the experimental design. On three separate occasions, a different subject was unable to tolerate the phentolamine infusion and complained of vertigo and nausea. In these instances, the experiment was immediately discontinued and appropriate treatment initiated. In such cases, the subject was re-scheduled for another testing opportunity. None of the trials were repeated more than twice. The physical characteristics for each of the subjects are presented in Table 5-1.

TABLE 5-1:

Physical characteristics of subjects (n=8).

Characteristic (unit):	Mean + SD:	Range:
Age (y)	26.4 ± 1.6	25 - 29
Body Mass (kg)	78.4 ± 7.3	67.6 - 88.9
Height (m)	1.8 ± 0.1	1.7 - 1.9
Body Mass Index (kg/m^2)	24.7 ± 2.5	20.3-27.9
Maximal $\dot{V}O_2$ (Absolute) ($L \cdot min^{-1}$)	3.7 ± 0.4	3.1 - 4.3
Maximal $\dot{V}O_2$ (Relative) ($mL \cdot kg^{-1} \cdot min^{-1}$)	45.4 ± 6.0	38.4 - 58.3
Body Fat (%)	16.6 ± 3.3	10.2 - 21.1

ii. Strength Testing and Workload Determinations:

As shown in Table 5-2, the total work outputs for the two resistance exercise protocols were not significantly different between the H-S (12.8 ± 1.9 kJ) and the L-F protocols (12.7 ± 1.9 kJ). Similarly, there were no statistical differences in the total work output between the phentolamine and placebo conditions within each protocol.

TABLE 5-2:

Strength testing, workload determinations and Ariel results (B = block).

Characteristic (unit):	Mean ± SD:	Range:
Seven Repetition Maximum - 7RM (kg)	66.5 ± 10.1	45.0 - 75.0
One Repetition Maximum - 1RM (kg)	86.2 ± 18.2	54.5 - 115.9
Workload for H-S (kg)	49.9 ± 7.4	34.1 - 56.3
Workload for L-F (kg)	16.5 ± 2.4	11.3 - 18.6
Total Work Output for H-S (kJ)	12.8 ± 1.9	8.8 - 14.4
Total Work Output for L-F (kJ)	12.7 ± 1.9	8.7 - 14.3
Speed of Movement for H-S ($deg \cdot s^{-1}$)	36.4 ± 19.7 *	24.1 - 49.5
Speed of Movement for H-S ($deg \cdot s^{-1}$) (B)	35.5 ± 18.1 **	21.3 - 48.8
Speed of Movement for L-F ($deg \cdot s^{-1}$)	126.4 ± 19.7 *	105.7 - 139.3
Speed of Movement for L-F ($deg \cdot s^{-1}$)(B)	136.2 ± 27.3 **	108.1 - 154.9

* Significantly different between H-S and L-F at $p<0.05$;

** Significantly different between H-S (B) and L-F (B) at $p<0.05$.

Although the concentric speed of contraction was significantly different between the H-S and L-F protocols (3.6-fold; $p<0.01$), there were no significant differences between phentolamine

and saline infusions within each protocol (Table 5-2). During the eccentric or relaxation phase of the movements on the Ariel, the ergometer suspended workloads and was reset manually to the starting position.

iii. Growth Hormone (GH):

Figure 5-3 shows the response of GH to each exercise protocol. The resting (0 min) GH levels were in the normal range, and were not significantly different between the H-S, L-F, H-S (block) and L-F (block) trials, averaging 5.0 ± 0.5 $\mu g \cdot L^{-1}$, 3.4 ± 0.4 $\mu g \cdot L^{-1}$, 3.5 ± 1.1 $\mu g \cdot L^{-1}$ and 4.2 ± 0.5 $\mu g \cdot L^{-1}$ (n.s.), respectively. There were, however, significant differences in the GH responses during the exercise and recovery periods between the four exercise trials.

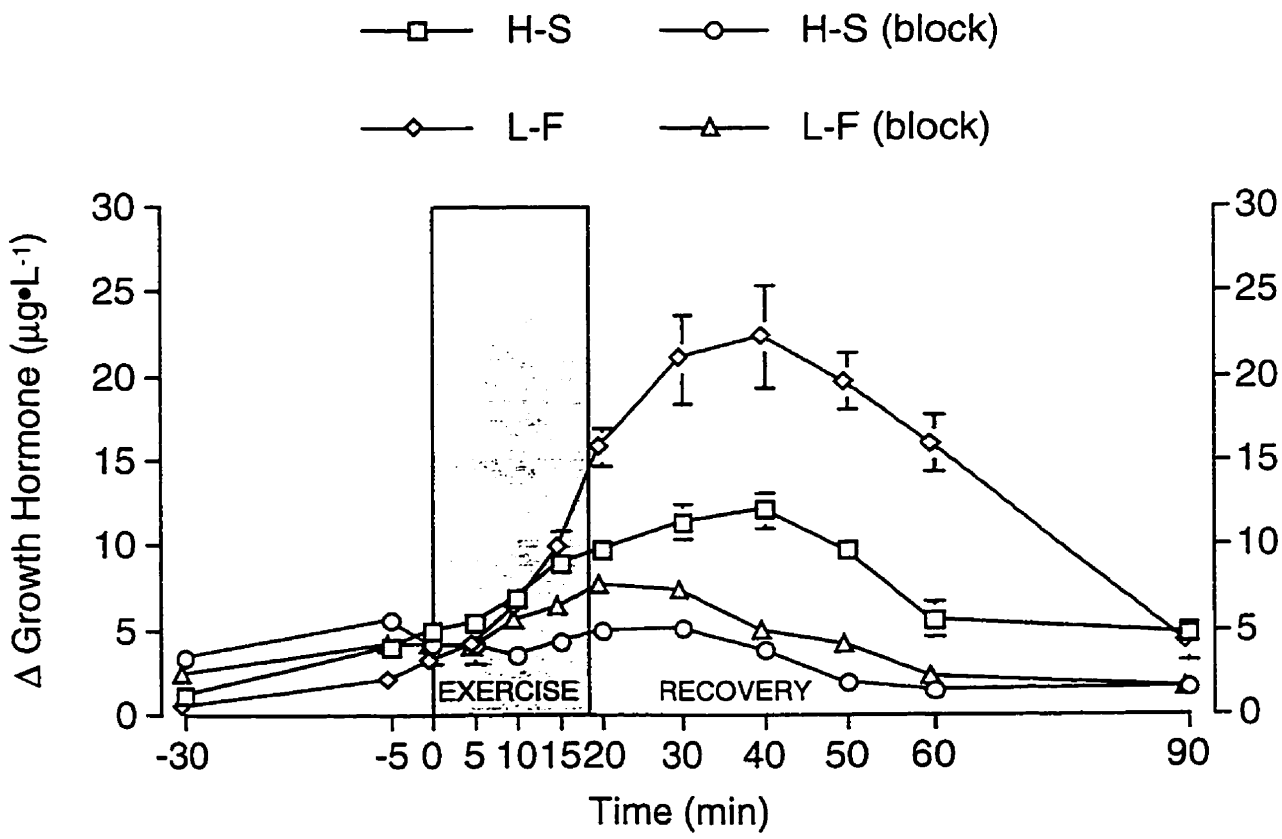

Figure 5-3: *Growth hormone responses during the four resistance exercise protocols with and without α-adrenergic blockade.*

It is obvious from Figure 5-3 that the greatest GH responses occurred during the exercise experiments that were completed with the infusion of saline. The GH levels increased in both the H-S and L-F protocols from the start of the exercise period and were statistically significant from the resting values by 10 min and 15 min of exercise, respectively. The L-F protocol showed the greatest response in GH secretion with peak GH levels of 22.4 $\pm$ 3.0 $\mu g \cdot L^{-1}$ by 20 min into the recovery period, compared to the H-S trial, with peak levels of 12.1 $\pm$ 1.0 $\mu g \cdot L^{-1}$ at 20 min into the recovery period ($p<0.01$). The α-adrenergic blockade with phentolamine inhibited any significant increases in GH secretion above resting basal levels in both protocols. Peak GH in the H-S (block) trial were 5.1 $\pm$ 0.6 $\mu g \cdot L^{-1}$ and 7.6 $\pm$ 0.4 $\mu g \cdot L^{-1}$ in the L-F (block) (n.s.).

The total area under the GH curves (90 min) for each of the four conditions are shown in Figure 5-4. The total GH response over the exercise/recovery period was approximately 192 % greater in the L-F exercise than in the H-S exercise ($p<0.01$). Phentolamine inhibited the total GH response in both exercise protocols ($p<0.01$), and also eliminated significant differences between the H-S and L-F trials.

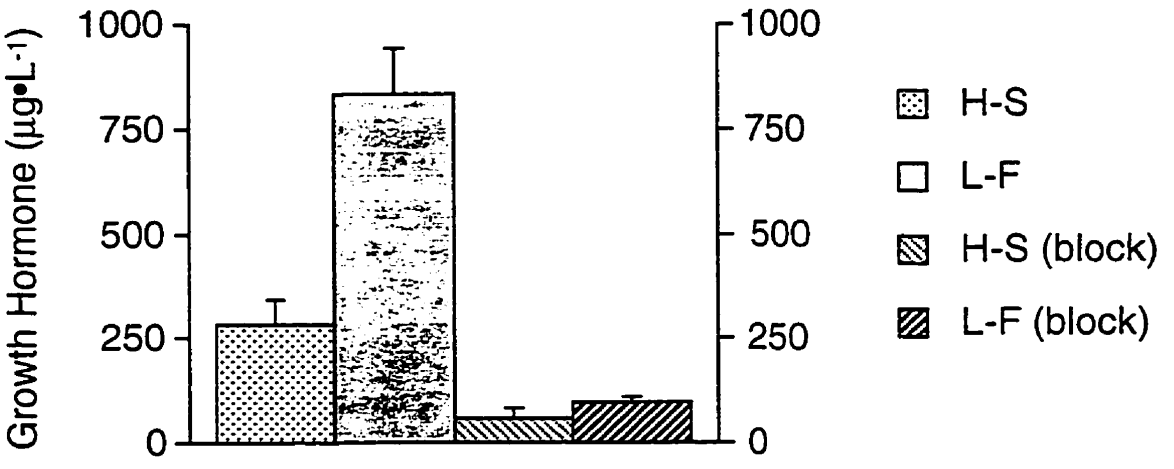

***Figure 5-4:** Incremental area of GH in H-S and L-F.*

iv. Norepinephrine (NE):

Figure 5-5 demonstrates the NE responses during the four exercise trials.

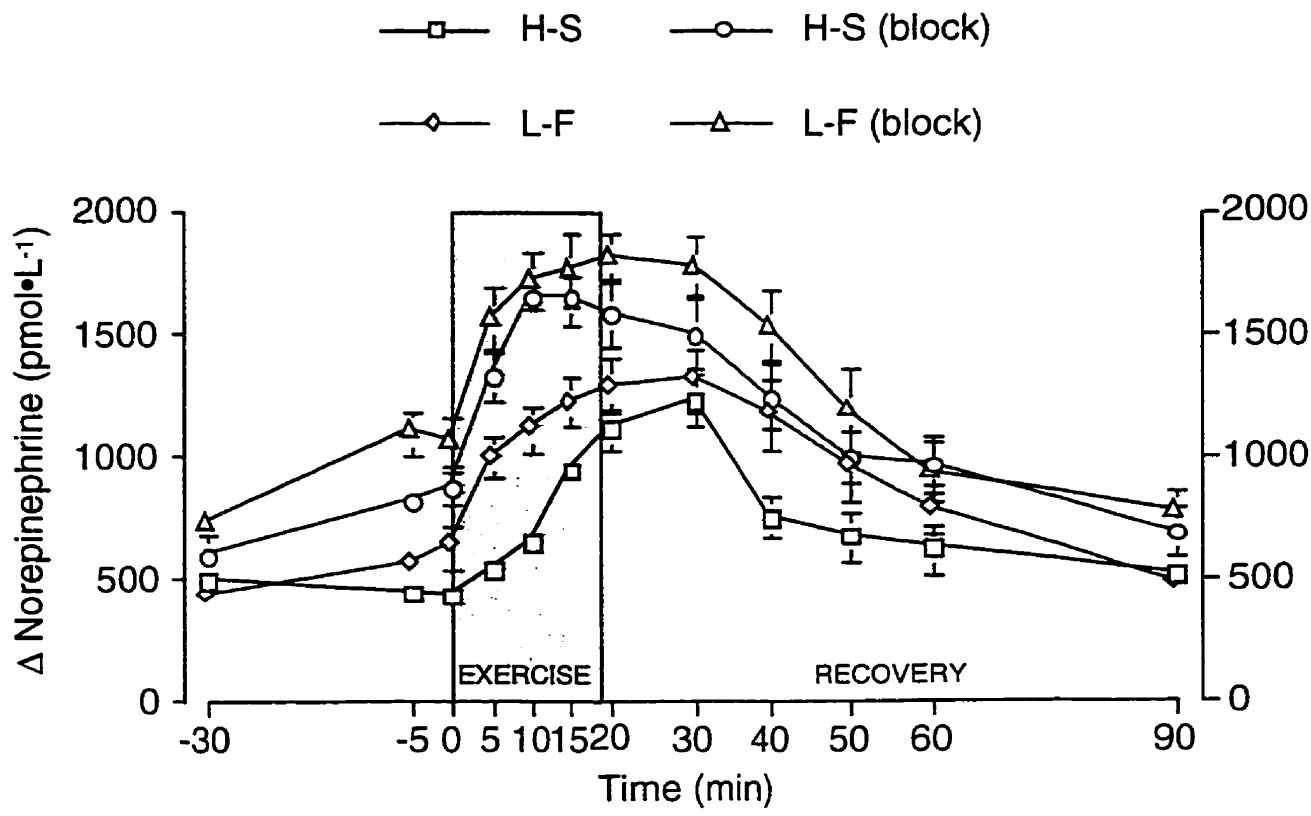

Figure 5-5: *Norepinephrine responses during resistance exercise protocols with and without α-adrenergic blockade.*

From the start of the blood sampling, there were significant differences in the NE levels between the four experimental trials. During the non-block protocols, resting basal levels (0 min) of NE were 430.0 ± 31.9 pmol•L^{-1} and 624.5 ± 88.3 pmol•L^{-1} in the L-F and H-S conditions (n.s.), respectively. The phentolamine infusion for the 20 min prior to the beginning of exercise significantly elevated resting (0 min) levels to 865.5 ± 71.4 pmol•L^{-1} and 1053.6 ± 94.6 pmol•L^{-1} in the H-S (block) and L-F (block) trials ($p<0.05$), respectively. Although NE levels increased more rapidly during the exercise period in the L-F protocol than in H-S, they peaked at 10 min into recovery at similar levels of 1221.4 ± 105.6 pmol•L^{-1} for the H-S and 1305.4 ± 130.3 pmol•L^{-1}

for the L-F trial (n.s.). In contrast, the NE levels during the phentolamine infusion peaked at 1635.9 ± 100.1 pmol•L^{-1} and 1814.9 ± 89.2 pmol•L^{-1} at 15 min and 20 min of the exercise in the H-S (block) and L-F (block) trials, respectively.

The total areas under the normalized NE curve (90 min) are demonstrated in Figure 5-6.

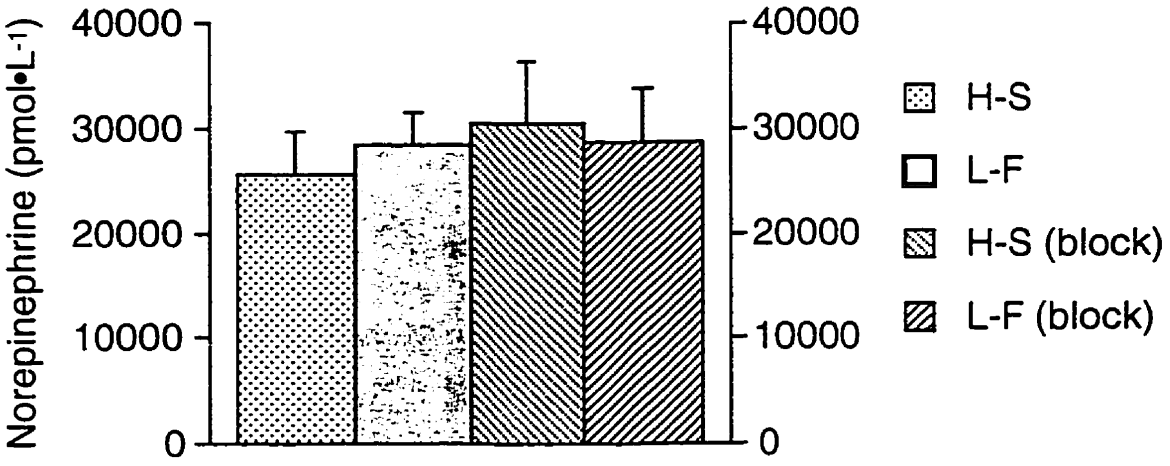

***Figure 5-6**: Incremental area of NE in H-S and L-F.*

No significant differences in total incremental NE response areas were found between any of the H-S and L-F protocols. Phentolamine had no effect on the total NE responses in the H-S trials and L-F trials that could not be accounted for by the initial level at time 0.

v. Epinephrine (EPI):

Figure 5-7 demonstrates the responses of EPI during the pre-exercise, exercise and recovery periods for the four exercise tests. Resting basal levels of EPI were closely matched at 180.1 ± 26.1 pmol•L^{-1}, 182.7 ± 8.6 pmol•L^{-1}, 170.7 ± 11.1 pmol•L^{-1} and 195.6 ± 22.9 pmol•L^{-1}

during the H-S, L-F, H-S (block) and L-F (block) trials (n.s.), respectively. The infusion of phentolamine had no significant effect on the resting levels of EPI.

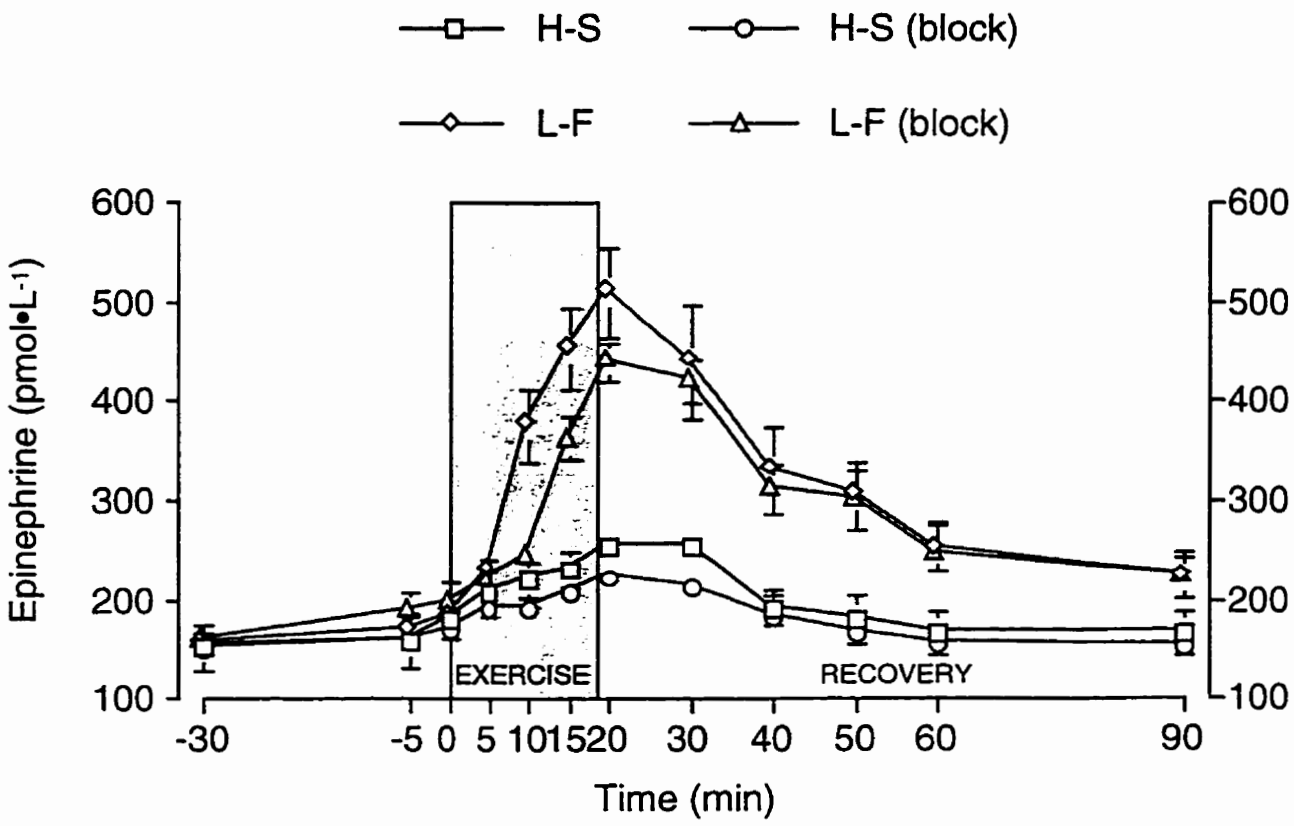

Figure 5-7: *Epinephrine responses during resistance exercise protocols with and without α-adrenergic blockade.*

In each case, placebo vs. phentolamine infusion, the responses of EPI during the exercise and recovery periods were statistically higher in the L-F protocols than they were in the H-S protocols. These differences were established as significant by the 10 min and 15 min point of exercise in the placebo and phentolamine infusions, respectively. Peak levels of 510.2 ± 44.7 pmol•L^{-1} and 440.6 ± 19.1 pmol•L^{-1} were achieved at the end of 20 min of exercise in the L-F and L-F (block) trials. The peak EPI responses in the H-S and H-S (block) protocols (254.9 ± 8.7 pmol•L^{-1} and 223.8 ± 12.7 pmol•L^{-1}, respectively) were significantly lower (50%) than in the L-F trials.

The area under the curve data for EPI (90 min) are demonstrated in Figure 5-8. In each case, block and control, the EPI responses of the L-F trials were higher than the H-S ($p<0.05$). However, phentolamine did not have any significant effect on the total EPI responses in the L-F or H-S trials, although the total areas were reduced by 25 % in each case (Figure 5-8).

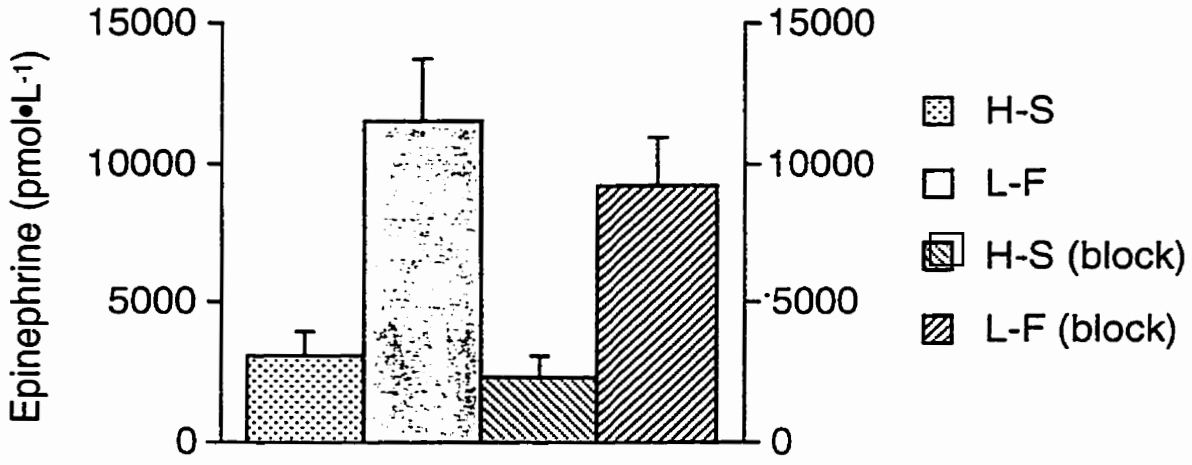

***Figure 5-8**: Incremental area of EPI in H-S and L-F.*

vi. Glucose:

Figure 5-9 illustrates the glucose responses during the pre-exercise, exercise and recovery periods of the four protocols. Resting basal levels (0 min) of glucose were not significantly different between each of the four trials. However, there were statistically different glucose responses during the exercise and recovery periods. Although glucose levels increased in all four trials during exercise, both of the L-F trials produced a greater glucose response during the last 15 min of exercise and the first 15 min of recovery than seen in the H-S trials. There were no significant effects of phentolamine within each exercise protocol.

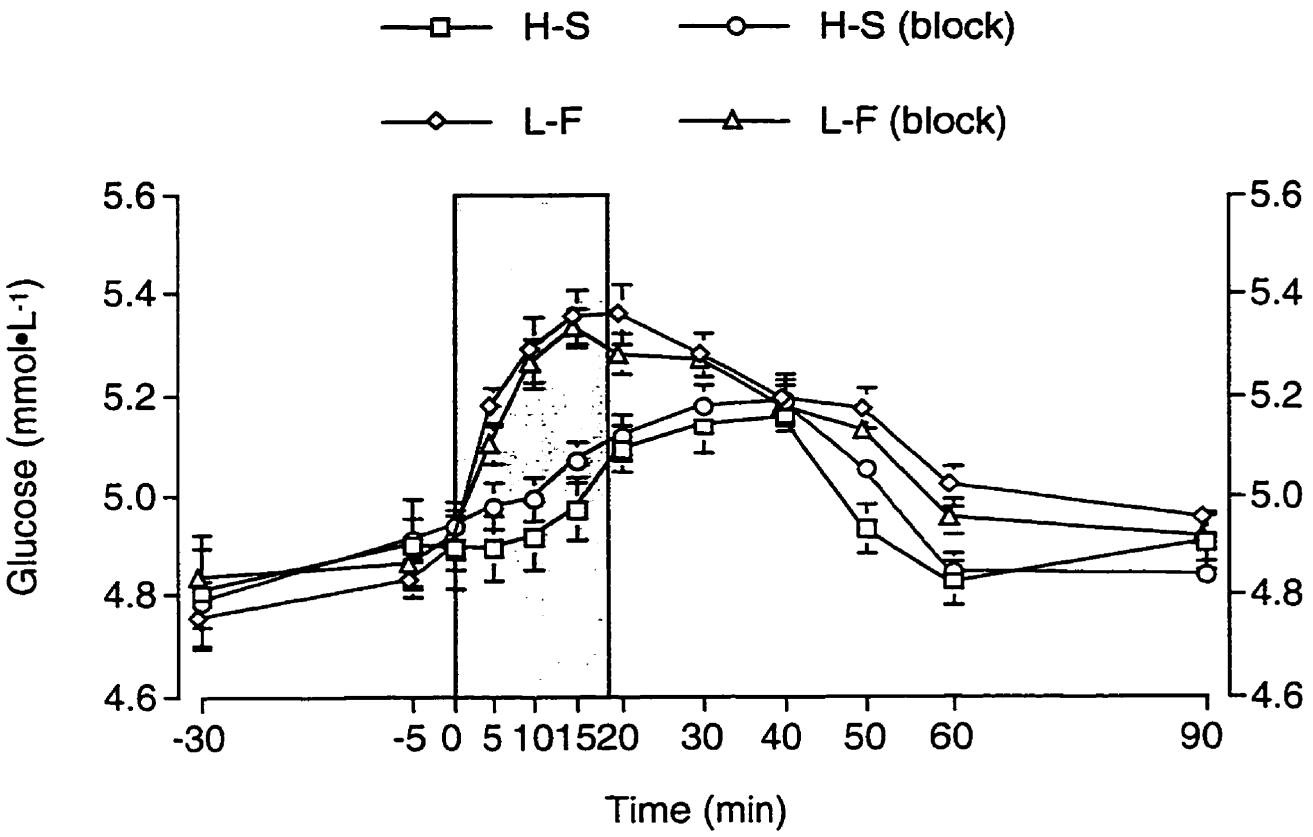

Figure 5-9: *Glucose responses during resistance exercise protocols with and without α-adrenergic blockade.*

Figure 5-10 demonstrates the total area of glucose responses (90 min).

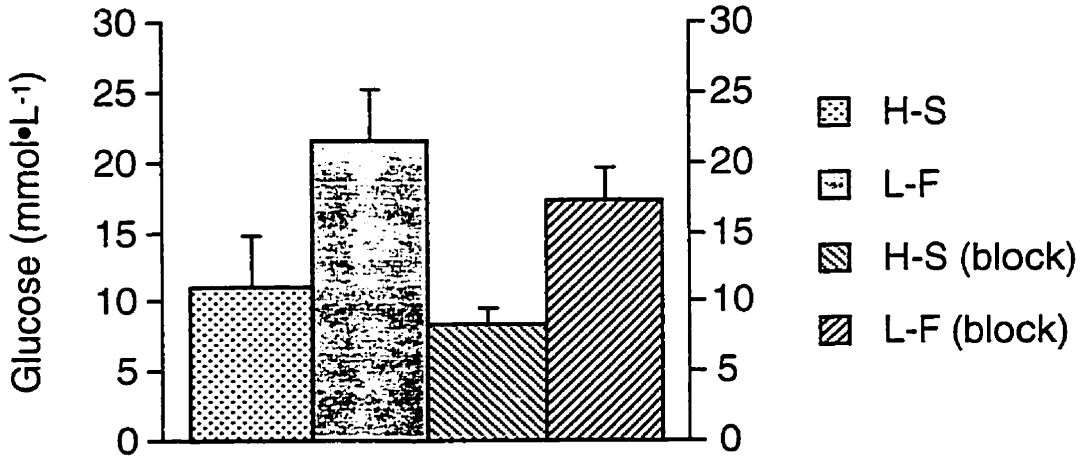

Figure 5-10: *Incremental area of glucose in H-S and L-F.*

Figure 5-10 confirms that the L-F exercise with and without blocker resulted in significantly higher total glucose responses than the H-S exercise. Phentolamine also seemed to reduce the total glucose response to the two exercise protocols, but this was not statistically significant.

vii. Lactate:

Figure 5-11 demonstrates the responses of lactate in each protocol during the phentolamine (block) and placebo infusions.

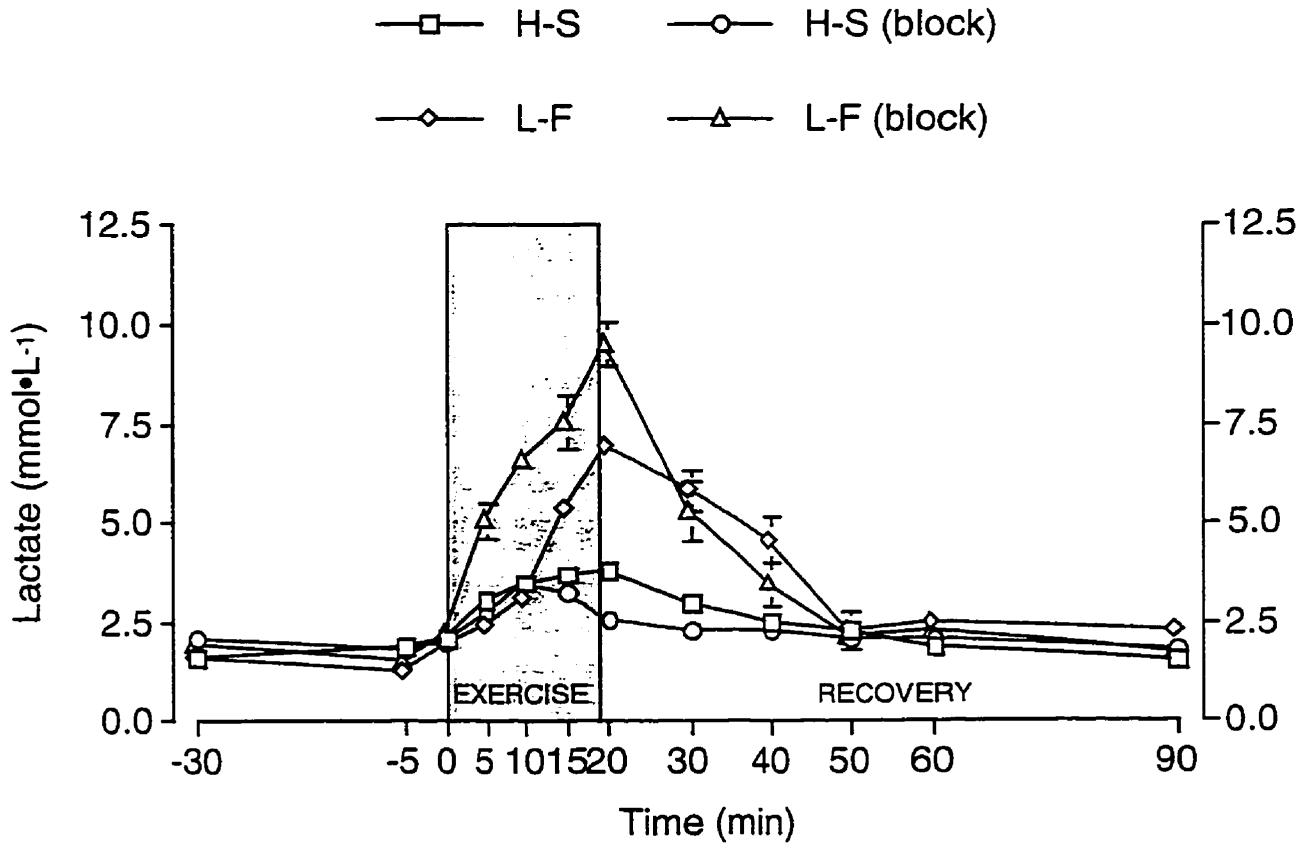

Figure 5-11: *Lactate responses during resistance exercise protocols with and without α-adrenergic blockade.*

There were no statistical differences between the basal levels of lactate in any of the four trials. The resting (0 min) lactate levels of the H-S, L-F, H-S (block) and L-F (block) trials averaged 2.1 ± 0.1 mmol•L^{-1}, 2.0 ± 0.1 mmol•L^{-1}, 2.0 ± 0.1 mmol•L^{-1} and 2.2 ± 0.2 mmol•L^{-1}, respectively. There were, however, dramatic differences in the lactate responses during the exercise and recovery periods between the four experimental trials.

In each case, phentolamine and placebo, the L-F protocols produced a greater lactate response than the H-S protocols. The blocker did not significantly affect the lactate levels during the H-S (block) or the L-F (block) exercise, when compared to their controls. During the control trials, the area under the lactate curves (Figure 5-12) were significantly greater (180%) in the L-F protocols than in the H-S trials (90 min). During the phentolamine trials, the L-F (block) protocols resulted in lactate increases that were 325% above the responses observed in the H-S.

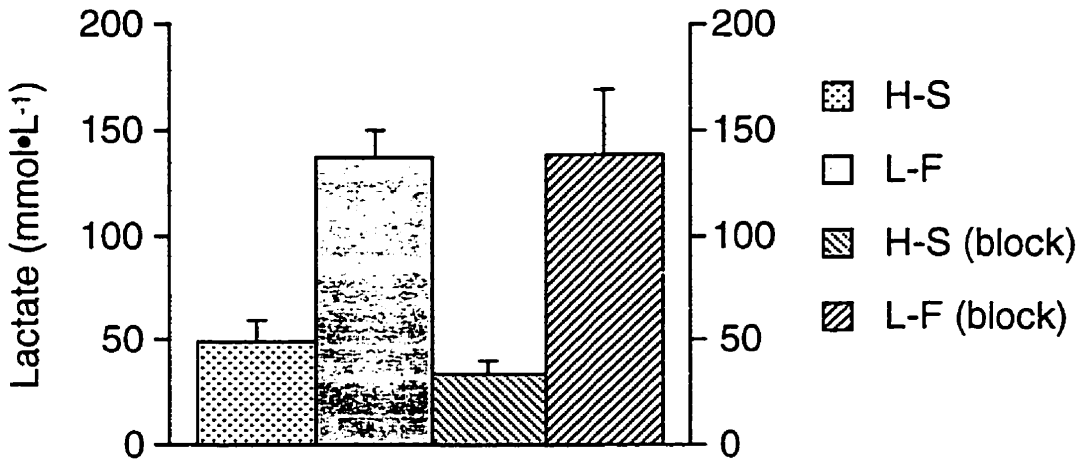

Figure 5-12: Incremental area of lactate in H-S and L-F.

5-10. Discussion:

The catecholaminergic system plays a dual role in the neural control of GH release, by having both a stimulatory and an inhibitory action that is mediated by different receptors (Figure 5-13).

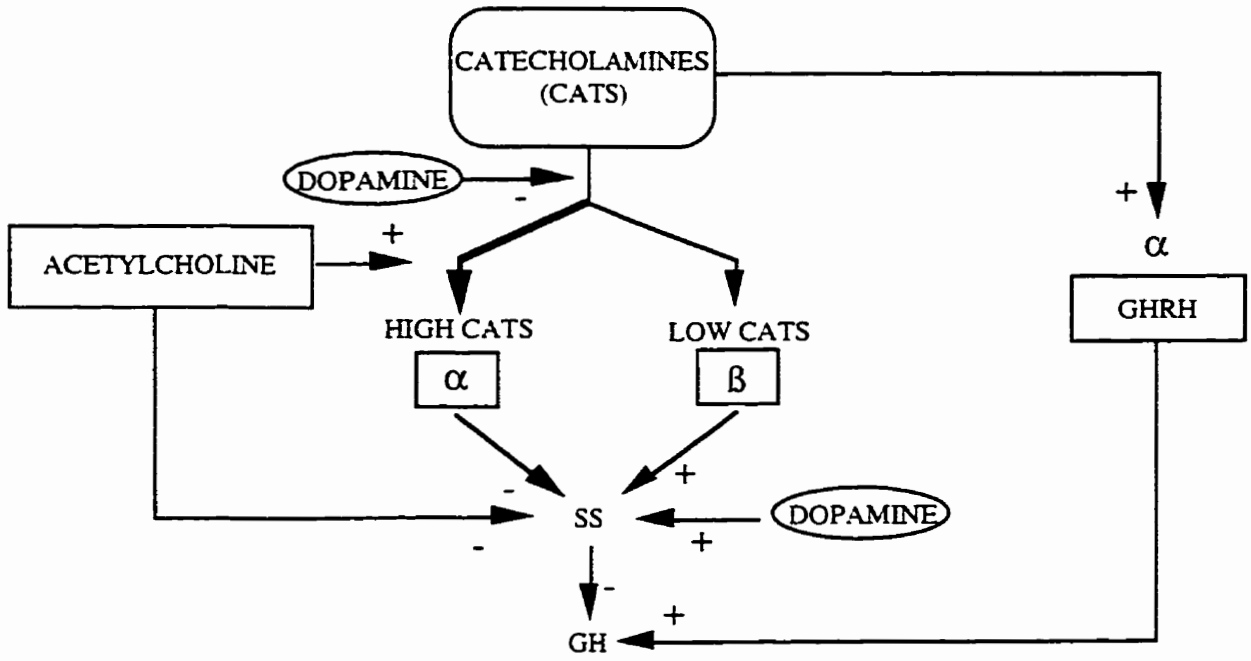

Figure 5-13: Neural control of GH secretion.

The stimulatory influence of catecholamines (CA) on pituitary GH secretion is mediated by activation of α_2-adrenergic receptors which appear to stimulate GHRH-secreting neurons positively, and concomitantly inhibit the SS neurons. It is also well known that β_2-adrenergic receptors mediate the inhibitory influences of CA on GH secretion dependent on the amount of CA released into the synaptic cleft. In this case, the SS neurons are stimulated in the presence of low levels of CA. Therefore, it appears that in man, a balance exists between α-(stimulatory/inhibitory) and β-(inhibitory) adrenergic influences on the hypothalamic GH-regulatory hormones, GHRH

and SS. Current evidence suggests that the inhibitory influence of β-adrenergic activation is due to the stimulation of hypothalamic somatostatinergic activity. Devesa et al. (1992) have shown in humans that the adrenergic system appears to play two antagonistic roles in human GH regulation. Its facilitatory effects are mediated by adrenoreceptors acting mainly on GH control by inhibiting SS release, rather than by stimulating GHRH secretion. Its inhibitory effects are dependent upon β-adrenergic activity which stimulates SS secretion. It is known that β-adrenergic antagonists enhance GH responses to GHRH due to the inhibition of hypothalamic SS release.

The increases in plasma catecholamines in response to various types of exercises have been well documented. Increases in plasma EPI levels during exercise most likely reflect true changes in hormonal release from the adrenal medulla. Norepinephrine, on the other hand, is released during exercise from the sympathetic nerve terminals, with active skeletal muscle being a major contributor to the increases in plasma NE during exercise in humans (Savard et al., 1988). Adrenergic neurons in the central nervous system, both in the paraventricular nucleus, as well as other areas in the hypothalamus, also release NE during exercise (Radosevich et al., 1989; Scheurink et al., 1990). Thus, the increases in both plasma and central nervous system catecholamines during exercise could exert a significant effect on the GH-response to exercise.

The aim of this current investigation was to study the effects of α-adrenergic blockade on the hormonal responses, specifically GH, to resistance exercise protocols differentiated as Light-Fast (L-F) and Heavy-Slow (H-S), and having identical total external work output, duration and work-rest intervals, but varying load and frequency characteristics. It has been shown in previous chapters that the L-F protocol induced a much greater GH-response to exercise than the H-S exercise protocol. The objectives of the study were to re-examine the relationship between CA and GH-responses to the two exercises, and to assess how this relationship would be affected by α-adrenergic blockade. We hypothesized that the use of an α-adrenergic blocker would not have any influence on the plasma GH response to the two resistance exercise protocols.

Table 5-3: Comparison of total hormonal and metabolic responses to the four exercise protocols.

	H-S	H-S(B)	L-F	L-F(B)
GH	++	+	++++	+
NE	++	++	++	++
EPI	+	+	+++	+++
GLUCOSE	++	++	++++	++++
LACTATE	++	++	++++	++++

The hormonal and metabolic responses to the exercise protocols found with and without blocker are summarized in Table 5-3 and show that:

1) L-F exercise induced a much greater GH, EPI, glucose, lactate responses than H-S;

2) Infusion of the α-adrenergic antagonist, phentolamine, prior to and during exercise significantly suppressed the GH responses to both of the resistance exercise protocols, eliminating the differences in GH secretion between the two resistance exercise protocols;

In Chapter 3, it was reported that there were significant differences in the GH and catecholamine responses between the H-S and L-F leg extension protocols. In the present study, plasma GH also differed significantly during both of the exercise protocols conducted without any α-adrenergic block. In this particular case, the L-F exercise trial was associated with significantly higher GH levels than the H-S exercise protocol that was of identical average power output, duration and work-rest intervals. However, the exercise-induced differences in plasma GH levels

between the H-S and L-F exercise protocols were eliminated by the use of the α-adrenergic receptor block. While the infusion of the phentolamine block was in effect, no statistically significant differences in plasma GH levels were detected between the two exercise protocols. Our findings support those of Hansen (1971) who was able to almost entirely block with phentolamine the GH response to a light workload (450 $kg \cdot min^{-1}$ for 40 min), and those of Berger et al (1980) who blocked the GH response to a graded submaximal exercise with phentolamine. However, these latter two studies are subject to criticism due to the small number of blood samplings, different initial basal levels in their groups and anomalous responses during the recovery period. Our data do not support the findings and conclusions of Sutton & Lazarus (1974), who found that phentolamine did not suppress the GH response to exercises at either 450 $kpm \cdot min^{-1}$ or 600 $kpm \cdot min^{-1}$ for 20 min. Sutton & Lazarus concluded that it was unlikely that α-adrenergic mechanisms played any major role in the mediation of GH secretion to exercise. However, their data are suspect as they found that the lighter workload induced a higher GH-response than the heavier workload, which is contrary to the current knowledge on workload intensity and GH responses.

The influence of the phentolamine-induced α-adrenergic block on the secretion of GH can occur at a number of receptor locations within the human nervous system. If we speculate that the action of phentolamine is either centrally mediated, in this case the hypothalamus, or peripherally mediated at α-adrenergic receptors outside of the central nervous system and at muscle sites, two lines of reasoning can be developed to explain our findings of the secretion of GH during our exercise stimulus.

It has been well established that a number of neurotransmitters are involved in the regulation of GH secretion via hypothalamic GHRH and SS secretion. These include NE, levodopa and dopamine, serotonin and acetylcholine (Martin, 1973). The sites of action of these neurotransmitters include the limbic system, and the arcuate and ventro-medial nuclei of the

hypothalamus. To this end, the hypothalamus has been found to be rich in NE-containing neurons (Shute & Lewis, 1966). These monoamine pathways are involved in the local secretion of NE, and therefore, the hypothalamic release of both GHRH and SS, and subsequently GH (Martin, 1978; Tuomisto & Mannisto, 1985). During our control trial, the higher peripheral levels of CA in the L-F versus the H-S protocols would influence the secretion of the GHRH and SS, with a resultant increase in pituitary GH secretion. Our findings of a positive relationship between plasma NE and GH in both exercise protocols support this hypothesis.

We have suggested previously that chemical-metabolic receptors within the active working tissues could be activated through the detection of by-products from the specific exercise (VanHelder et al., 1984b; VanHelder et al., 1987). Such an activation would be communicated to the central nervous system by specific changes in muscle homeostasis, initiating an increase in the secretion of GH. If catecholamines are the regulatory neurotransmitters involved in GH secretion during exercise, their effect on the hypothalamic hormones, GHRH and SS, would bring about the necessary changes in GH secretion (Figure 5-14). This then would explain the concurrent increases in GH and CA responses found during the control/placebo trials of this experiment and of our previous investigations (Chapter 3).

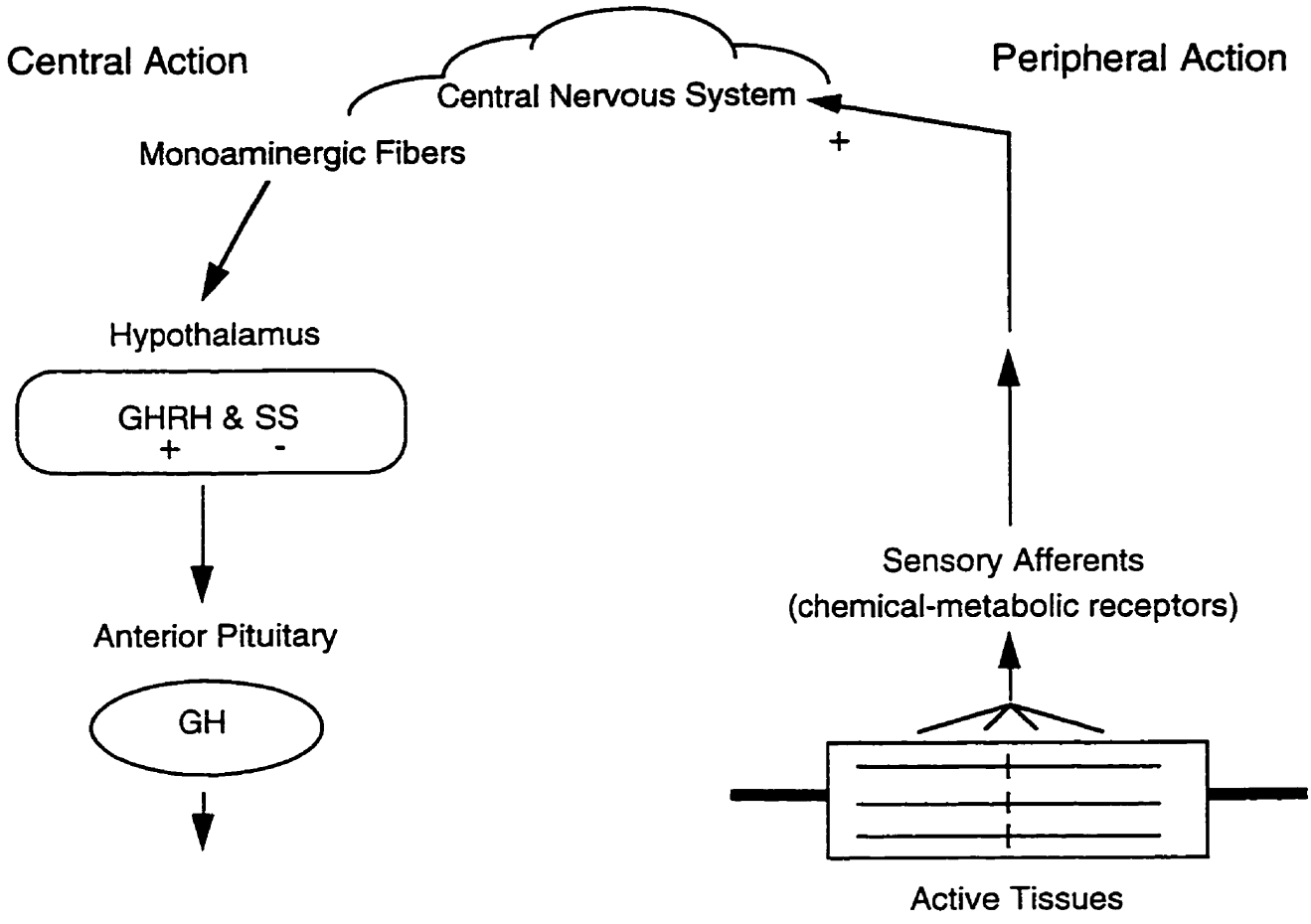

Figure 5-14: Central vs. Peripheral mechanisms in the regulation of GH secretion: actions of phentolamine.

However, this relationship broke down during α-adrenergic block, where both of the resistance exercise protocols were associated with significant increases in the plasma NE levels, but with inhibition of the GH responses, i.e., a "Central Action" (Figure 5-14). The α-adrenergic blocking agent, phentolamine, results in accelerated net NE release (Halter & Porte, 1977) and an increase in the synthesis and release of NE (Gould & Reddy, 1976). This could account for the elevated basal levels of NE in both block conditions, as well as the higher levels of NE during exercise in both the L-F (B) and H-S (B) exercise protocols. Such elevated levels would have no effect on GHRH as α-adrenergic receptor blockage was in effect. This would explain the dissociation between GH and NE levels under these two conditions.

It is unlikely that the complete α-adrenergic blockade of the peripheral receptor sites of the post-ganglionic sympathetic neurons would have any involvement with the secretion of pituitary GH. It is well known that the catecholamines are the main neurotransmitters of the post-ganglionic sympathetic autonomic nervous system. Indeed, the normal α-adrenergic activation due to NE or EPI would result in the vasoconstriction of blood vessels, a contraction of the GI tract and urinary sphincters and an inhibition or decrease in GI motility. With this in mind, the use of the phentolamine infusion in the resting subjects/patients would result in an assortment of physiologic changes at all smooth muscle sites, resulting in ureter and gastric sphincter relaxation or blood vessel vasodilation. Other changes as a result of the phentolamine infusion could include the inhibition of insulin secretion and an increase in hepatic glycogenolysis. These changes should have little to do with the secretion of GH during resistance exercise.

However, although little is known about the neurotransmitters of the afferent nervous system, an α-adrenergic receptor block of the chemical-metabolic receptors of the working muscle that we have previously discussed could also eliminate any potential stimulus for an increase in GH secretion, i.e., a "Peripheral Action" (Figure 5-14). Thus, if a peripheral view of phentolamine action is taken, we are led to speculate that the perceived changes in the internal milieu of the exercising tissues, be it changes in the levels of pH, lactic acid, PO_2 or some other remnant of accelerated glycolysis, are detected by the muscle receptors, but are unable to carry out their afferent actions due to the α-adrenergic blockade. In the H-S and L-F protocols completed under the infusion of placebo/control, these receptors should have been unobstructed to signal the changes in the homeostatic condition within the muscle cells, and therefore, bring about an increase in the secretion of GH.

The two exercise types also differed with respect to the responses of plasma glucose, EPI, and lactate. The L-F exercise with phentolamine block induced greater responses in these three factors than the H-S exercise. The association between GH and lactate has been reported by

several groups (Gordon et al., 1994; Luger et al., 1992). However, this relationship disappeared under α-adrenergic blockade as the lactate levels continued to increase under L-F (B) even though the GH level was suppressed. Thus, if lactate acts peripherally to stimulate GH centrally, the α-adrenergic receptors must be involved as the blockage of these receptors blocked the stimulatory action of lactate. Of course, plasma lactate levels may not mirror cellular levels of lactate, and the relationship observed between GH and lactate observed by others (VanHelder et al., 1984b) may not be a causal one.

There appeared to be an association between the EPI levels and those of glucose and lactate. An increased secretion of EPI is known to inhibit glucose-stimulated insulin secretion via an α-adrenergic effect, but it has been shown that α-adrenergic blockade with phentolamine results in an increase in basal insulin secretion with no change in the basal levels of glucose (Robertson & Porte, 1973). However, increments in plasma EPI which result in glucose intolerance have been shown to interfere with the action of insulin rather than inhibiting insulin secretion. Epinephrine also acts to raise blood sugar by stimulation of glycogenolysis and gluconeogenesis, inhibition of insulin-mediated glucose uptake, increased lipolysis and stimulation of glucagon secretion. The glycogenolytic effect of EPI is considerably greater than that of NE and occurs in liver and muscle tissue primarily through β-adrenergic receptors (Felig et al., 1981). This would explain the higher glucose responses found in the L-F protocols compared to the H-S protocols.

5-11. Conclusion:

In conclusion, the results of this investigation suggest that NE acting through the stimulation of α-adrenergic receptors, either centrally or peripherally (Figure 5-14), is involved in a cascade that regulates the release of pituitary GH during various types of resistance exercise. The use of the phentolamine block effectively suppressed the release of GH during both of the H-S and L-F exercise protocols. The findings also demonstrate that when the α-adrenergic block was in

effect, the ability of the GH regulating system to differentiate between the various types of resistance exercise was lost. Significant differences in plasma GH levels associated with various types of exercise of identical work output, duration and work-rest intervals, but varying load and frequency characteristics, are obliterated while the phentolamine block is in effect.

5-12. Suggestions for future study:

To substantiate the α-adrenergic regulation of GH secretion during resistance exercise, it would be necessary to reproduce this investigation with the use of phentolamine and control infusions during the different exercise protocols. However, to describe further the role of the central components, the analysis of the hypothalamic regulating hormones, GHRH and SS, would be necessary. This would conclusively demonstrate that the suppression of GH secretion during the different exercise protocols was the result of the α-adrenergic suppression of the GHRH and the accompanying changes to SS release.

Further experimentation could also help to determine the role of the "peripheral" adrenergic nervous system in the responses of GH during exercise. In this case, neural blockade of the sensory afferent impulses from the working tissues during the identical exercise protocols would provide insight into the existence and activation of the chemical-metabolic receptors within the active tissues. Thus, the suppression of the GH response during the sensory block would provide evidence of a peripheral adrenergic component involved in the regulation of GH.

CHAPTER 6

The Effects of Thermal Stress on the Endocrine and Metabolic Responses During Rest and Exercise

6-1. Abstract:

Objectives: The objective of this investigation was to examine if the increase in core temperature that accompanies physical activity was a factor responsible for stimulating the hormonal and metabolic responses observed during moderate aerobic exercise.

Design of the Study: A controlled, cross-over trial was designed. Eight healthy men aged 27.3 ± 6.0 years were exposed to four 80 min periods of water immersion to mid-chest level, two at a low water temperature of 23° C (C), and two at a high temperature of 39° C (H). During the first 40 min of two exposures, one at each temperature, subjects performed a 40 min period of cycle ergometer exercise at an oxygen consumption of approximately 2 $L \cdot min^{-1}$ (65 % of $\dot{V}O_2$ max). After the exercise period or throughout the control trials, the subjects remained sedentary in the immersion tank until the 80 min was over. Each subject performed one control (C) and one exercise (E) trial at each of the two temperature conditions, H and C. Therefore, the four experimental conditions were identified as Hot-Control (H-C), Hot-Exercise (H-E), Cold-Control (C-C), Cold-Exercise (C-E).

Analyses: Core temperatures were measured by thermal probe. Peripheral venous blood was collected every 5 min during the first 40 min of exercise or rest (9 samples) and then every 10 min for the remaining 40 min of recovery (4 samples), during which time the subjects remained immersed in the water. Oxygen consumption was continuously monitored using breath by breath analysis. All samples were analyzed for growth hormone (GH) and cortisol concentrations as

determined by radioimmunoassay. Lactate levels were measured by colorimetric analysis. In addition, hematocrit and hemoglobin values were determined by Coulter counter. All values were adjusted for changes in plasma volume by the method of Dill and Costill (1974).

Results: In the H-E trial, core temperature increased during the exercise period, peaking at 39.1 ± 0.4° C at 10 min post-exercise. In the C-E condition, the core temperature was clamped at 37.8 ± 0.3° C at the end of the exercise period, dropping to 37.5 ± 0.3° C during recovery. In the H-C trial, the temperature responses during the first 40 min closely matched the C-E trial, whereas resting in the C-C condition produced a steady decrease in core temperature to 36.4 ± 0.6° C. The increases in core temperature during the H-E, H-C and C-E corresponded with the observed increases in GH levels. The H-E trial resulted in a greater GH response than observed in the H-C and C-E trials, which were closely matched. Core temperature clamping reduced the exercise-induced increase in GH concentration by essentially half. Exercise in the H-E trial produced higher lactate levels that were significant from the C-E between the 5 min and 30 min of exercise and during the 50 min and 70 min samples of the recovery phase. The oxygen demand/availability ratio correlated with the corresponding GH response during the exercise segment of the H-E trial ($r=0.55$; $p<0.001$).

Conclusions: The technique of clamping core temperature during exercise used in this study allowed us to examine the independent effects of metabolic heat production, and of energy expenditure during exercise, upon the endocrine system. The release of GH during the exercise period was enhanced by an increase in core temperature, and was significantly reduced when core temperature was prevented from increasing. The results suggest that the rate of core temperature increase during exercise is an important factor in the regulation of GH secretion.

6-2. Introduction:

While physical exercise results in a number of hormonal changes, muscular activity also increases the metabolic rate which, in turn, produces heat that must be dissipated by mechanisms within the body and by thermal exchange with the ambient environment. A number of studies, (Christensen et al., 1984; Okada et al., 1972b) have reported a significant relationship between the exercise-induced increase in body temperature and subsequent growth hormone (GH) secretion. In such cases, the regulator for the activation of the hypothalamic-pituitary axis might recognize the increase in core temperature as an indicator of an increase in peripheral tissue energy requirements during exercise. Conversely, the rise in body temperature, and subsequent hormonal responses, may be independent of any exercise stressor or increased energy requirements (Buckler, 1973), but instead, may be the result of changes in the glandular membrane characteristics that enhance hormonal exocytotic release.

Although the influence of changes in core temperature on circulating hormone levels in extreme climates have been reported previously, a recent discussion by Mougios & Deligiannis (1993) attests to the lack of understanding and sound scientific research on the interactions of thermoregulation, neurology, endocrinology and exercise physiology. It is, therefore, important to examine fully the possible relationships that exist between core temperature and exercise, and the associated secretion of various hormones.

6-3. Body Temperature Indices and Thermoregulation:

Normal function in the human body operates within a narrow range of core temperature and a wider range of skin temperature. While it may be technically easier to measure skin temperature, the importance and complexity of core temperature have made it necessary to utilize a number of different mechanisms to measure any thermal changes accurately and practically. Thus,

measurements of core temperature by only one method cannot fully reflect or explain the actual internal temperature. Although the quantification of muscle temperatures, assessed through thermistor probes which were surgically implanted, provided interesting data (Holmer & Bergh, 1974) during thermal studies. However, this invasive technique may not be reasonable in most investigations involving exercise physiology. The actual temperature established for a specific body area is the result of a combination of different factors, including the tissues' particular metabolic rate, blood flow within, to and from the specific area, insulative properties of the tissue, and the temperature gradients surrounding that specific area (Sawka & Wenger, 1992). These temperature inputs can be detected by neural mechanisms, both locally and centrally.

The preoptic area of the anterior hypothalamus appears to be the major thermosensitive site in humans (Adair, 1974). The hypothalamic thermoregulatory system receives information concerning temperature from many areas of the body and acts to control the responses that are necessary to regulate heat production, heat loss and heat storage (Gisolfi & Wenger, 1984). In effect, the hypothalamus synchronizes the outflow of the autonomic nervous system with the responses of various endocrine organs (Gale, 1973).

The human system can actively produce heat and also aid in the dissipation of heat accumulation. There are three mechanisms which the body uses to regulate temperature around the normal set point of 37.0° C, namely: heat flow via blood shunting, heat production through metabolic processes, and evaporation through the water loss of diaphoresis. Thus, convection, radiation and evaporation participate in the temperature control of the body. The stability of the thermoregulatory set-point is influenced by disease, fever, heat acclimation, time of day, and the menstrual cycle (Sawka & Wenger, 1992).

There are two classes of thermal receptors located in the body: skin and central core receptors. These neural receptors consist of free nerve endings which transmit information about

the temperature in the target areas to a central integrator that oversees the control of vasodilation, vasoconstriction and sweating. While it appears that there are a number of thermal receptors within the core, such as the spinal cord, heart and the arterial and venous surfaces, the preoptic anterior hypothalamus appears to have the highest concentration of core thermal receptors (Gale, 1973). The hypothalamus has the unique capability of measuring its own temperature, generating a constant reference signal (37.0° C set point), integrating thermal and non-thermal inputs to adjust the reference point and mobilizing the required physical, chemical, and behavioral thermoregulatory responses (Hammel, 1968).

A number of different skin temperature receptors exist in the human body. Basically, two groups of receptors respond to either heating or cooling with an initial burst of neural activity and an increase in static activity. Another type of skin temperature receptor is activated by both stimuli, but responds with a more continuous discharge of neural activity (Hardy, 1961). Skin temperature varies over a greater range than core temperature. Slight changes in the firing activity of each class of receptor will have different effects on the other receptor type. Acute exposure to a warm environment quickly increases skin in both temperature and vasodilation, and leads to diaphoresis without any concomitant increase in core temperature. Evaporation of this fluid then leads to cooling of the skin, and therefore, the regional blood flow. It is the cooling of the circulating blood that results in heat dissipation. In contrast, acute exposure to cold environments leads to vasoconstriction in an attempt to diminish heat loss and to maintain a constant internal temperature. A longer exposure to cold will activate shivering and non-shivering thermogenesis involving a number of hormonal and neural systems (Gale, 1973). A rise in core temperature, as a result of exercise, activates the heat dissipating mechanisms, but has little effect on skin temperature (Sawka & Wenger, 1992).

During rest, approximately 70 % of the 37.0° C core temperature set point is maintained by the normal metabolic processes of internal organs (Guyton, 1986). In this non-exercising case,

core temperature is higher than that of skin and muscle. With exercise, the thermal by-product of muscular activity accounts for the majority of the heat production as the contribution from internal organ metabolism is diminished.

The degree to which heat-loss and heat-gain mechanisms are utilized within the body to regulate temperature is dependent upon ambient temperatures (Nielsen & Nielsen, 1962). At low environmental temperatures, approximately 70 % of heat loss from the body is due to convection and radiation. As the ambient temperature approaches that of the skin temperature, evaporation becomes increasingly more important until the point where skin and ambient temperatures are equal. In this instance, evaporation accounts for all of the heat loss. When ambient temperature is higher than skin temperature, there can be heat gain to the body from the environment.

6-4. The Effects of Core Temperature Changes on Resting Hormonal and Metabolic Levels:

In clinical medicine, especially pediatric endocrinology, it has been useful to employ different stimuli to test the sufficiency of the hypothalamic-pituitary-adrenal axis. Growth hormone challenge tests have included exercise, protein ingestion and insulin infusion. A certain amount of interest has centered around the use of heat exposure as a test for the stimulation of GH release. Geisen & Meder (1969) have established the hot water bath challenge for assessing GH secretion in short-stature children. Growth hormone secretion was observed to increase as body temperature increased during exposure to hot air (Aldercreutz et al., 1976; Okada et al., 1972a; Okada et al., 1972b), sauna baths (Weeke & Gundersen, 1983), hot water immersion (Buckler et al., 1973), and during both a pyrogen-challenge and viral inoculation (Frohman et al., 1967; Kimball et al., 1968). By realizing the interaction of core temperatures and certain hormone systems, it can be speculated that the heat production during exercise may also play a role in the subsequent GH response during some types of exercise.

In order to understand fully the complexity of the hormone responses interrelated with thermoregulation and exercise, it is necessary to review the responses of the various hormones during cold and hot exposure and a combination of the two. While the hormonal responses to cold stress have been studied extensively with particular emphasis on the sympathetic nervous system and pituitary-thyroid axis, the responses of GH to thermal stress have not received the same attention.

a) Growth Hormone Responses to Cold Exposure (Table 6-1):

In early studies, the GH response to hypothermia was examined by Berg et al. (1966). These authors assumed that a generalized stress and body cooling response could be initiated after the ingestion of 600 g of crushed ice. Hormonal profiles reflecting the activation of the pituitary-thyroid axis were examined. Despite a drop of 0.5° C in the tympanic membrane temperature, the thermal stress failed to demonstrate any significant changes in the circulating levels of GH and thyroid stimulating hormone (TSH) when compared to the euthermic conditions. Berg et al. (1966) did not provide any explanation for their results, except that the cold stress was ineffective at causing an increase in GH secretion.

Table 6-1:

Growth hormone responses during cold stress.

Investigator:	Activity/Challenge:	Temperature and Hormone Response:
Berg 1966	500 g ice ingestion	↓ Tympanic 0.5° C, → GH → TSH
Golstein 1970	2 h 4° C air	↓ Core ° C, ↑TSH, → GH→ Cortisol
Weihl 1981	a) 45 min 25.5° C bath	↓Core 0.75° C, ↑NE ↓EPI →GH →Cortisol
	b) 45 min 33° C bath	↓Core 0.1° C, →NE ↓EPI →GH →Cortisol

While the major emphasis of Berg's investigation centered on the responses of TSH, Golstein-Golaire et al. (1970) studied the effect of exposure to 4° C air for 2 h. In this case, GH and cortisol failed to respond to the low ambient temperature. However, unlike Berg et al. (1966), serum TSH began to increase during the latter stages of the experiment and remained elevated throughout 4 h of recovery. The apparent differences between these two experiments may have been the length of the cold exposure and the degree to which the body temperature decreased. Unfortunately, Golstein-Golaire et al. (1970) failed to measure the core temperature responses, treated male and female subjects as the same group and lacked consistency in the pre-experimental time period by allowing between 5 and 30 min after inserting the I.V. catheter before beginning the experiment. These methodological limitations contribute substantial criticism to any conclusion that Golstein-Golaire et al. may provide.

Weihl et al. (1981) studied the effects of hypothermia in Navy divers during total body immersion for 45 min in water temperatures of 25.5° C and 33° C. Growth hormone and cortisol remained unchanged during both trials despite mean drops in core temperature of 0.75° C and 0.1° C when exposed to the 25.5° C and 33° C water, respectively. Plasma norepinephrine (NE) increased significantly as a function of time spent in the cold water (25.5° C) immersion, but failed to respond above basal levels in the warm water condition. Epinephrine (EPI) levels decreased from initial resting levels in both the warm and cold water immersion studies. Weihl et al. (1981) speculated that the cold stress may not have been severe or long enough to influence the release of GH from the pituitary.

b) Growth Hormone Responses to Heat Exposure (Table 6-2):

In 1972, Okada et al. (1972b) evaluated the GH responses in resting male subjects who were exposed to hot air in a chamber (48° C) for 1 h. Significant elevations in core temperature and plasma GH concentrations above basal levels were observed in all subjects during the hour of

heat exposure, whereas cortisol failed to change beyond the initial resting level during the experiment. Typically, in the situation of a generalized stressor, the cortisol level will increase and respond in conjunction with an increase in GH concentration (Galbo, 1983). Therefore, it was significant that Okada et al. (1972b) demonstrated the separation of GH and cortisol responses to thermal stimuli. During the recovery period from heat exposure, the reduction in GH level mirrored the decline in core temperature. In this study, Okada et al. (1972b) suggested that the GH response to heat exposure was mediated by a diminished substrate availability as the subjects, given a glucose load immediately prior to the experiment, failed to demonstrate any increase in GH secretion with heat exposure. Unfortunately, due to infrequent blood sampling (every 30 min), the time lag or acuteness of the GH response could not be identified. In addition, inadequate methods of measuring body temperature, via sublingual thermometer every 30 min, failed to provide precise measurements of changes in core temperature for each subject. As to the significance of substrate availability and GH responses, only two subjects were evaluated in this portion of the experiment, and therefore, any conclusions regarding heat exposure, GH secretion and substrate availability must be questioned.

Table 6-2:

Growth hormone responses during heat stress.

Investigator:	Activity/Challenge:	Temperature and Hormone Response:
Okada 1972b	1 h 48° C sauna	↑Sublingual 1.2° C, ↑GH →Cortisol
Leppaluoto 1975	15 min 100° C sauna	↑Sublingual 3.0° C, ↑GH
Jurcovicova 1980	a) 30 min 40° C bath	↑Core 2.1° C, ↑GH
	b) 30 min 30° C bath	→Core, →GH
Leppaluoto 1986	2 h 80° C sauna	↑Core 0.8° C, ↑GHRH before ↑GH
Leppaluoto 1987	15 min 72° C sauna	↑Core 0.2° C, ↑GHRH ↑GH

In disagreement with Okada et al. (1972b), Leppaluoto et al. (1975) studied the hormonal responses of subjects exposed to a 100° C sauna for 15 min. As sublingual temperatures rose 3.0° C over baseline levels, plasma GH levels diminished. However, increases in GH were observed as the body temperature declined following the heat exposure. Growth hormone levels peaked 20 min after the hot exposure, and at the identical time when sublingual temperatures had returned to pre-exposure levels. The results suggested to Leppaluoto et al. (1975), that acute heat exposure activated a neurotransmitter stimulus that eventually led to GH secretion. Leppaluoto et al. (1975) also postulated that the GH level may have increased as a consequence of the body's return to normal core temperature, but failed to comment on this concept further.

Jurcovicova et al. (1980) utilized a hot water bath (40° C) to raise core temperature beyond 38° C while investigating the effect of GH on glucose tolerance and insulin secretion. These investigators documented a significant increase in GH secretion that was correlated with the increases in sublingual temperatures. Growth hormone levels gradually diminished following the heat exposure and returned to baseline levels within 60 min of recovery. This same group of subjects failed to produce any increase in GH levels or core temperature when exposed to an isothermic water bath at 30° C for the same time period. Jurcovicova et al. (1980) suggested that a form of core temperature threshold may exist in this system whereby GH responds only after this point has been exceeded.

Later, Leppaluoto et al. (1986) investigated the endocrine responses to repeated 1 h bouts of a 80° C sauna. Subjects were exposed to the sauna twice each day for a seven day period. As was the case in previous experiments, plasma GH and prolactin levels increased significantly during the heat exposure. Although the repeated sauna exposure continued to result in core temperature increases of 0.8-1.1° C, GH levels diminished, and by the final day of testing, had fallen to levels slightly above the pre-experimental values. The strong heat exposure failed to stimulate any increases in TSH, thyroid hormones (T3 and T4), testosterone, leutinizing hormone

(LH) and follicle stimulating hormone (FSH), but did result in significant decreases in cortisol and adrenal corticotrophin (ACTH) levels at the end of the experimental period (Leppaluoto et al., 1986). The authors speculated that the heat-exposure-induced dehydration was associated with the stimulation of GH.

Leppaluoto et al. (1987) described both the Growth Hormone Releasing Hormone (GHRH) and GH response in young and older men during exposure to a 72° C sauna for 15 min. In this case, and unlike their previous investigation (Leppaluoto et al., 1975), the GHRH levels in young men were elevated soon after the heat exposure began. The GHRH increased only slightly, but preceded the increase in circulating GH levels. These hormone responses were accompanied by an increase in body temperature of only 0.2° C. In contrast to the results demonstrated by young men, the strong heat exposure failed to produce any responses of GHRH or GH in the older subjects (Leppaluoto et al., 1987). Although the investigators concluded that the release mechanisms of GHRH and GH become less effective with age, they did not provide any explanation for the increased release of GH due to heat exposure.

c) Hormone Responses to Both Cold and Hot Exposure (Table 6-3):

Okada et al. (1970) investigated the hormonal responses to whole body cooling in subjects exposed to a 4° C environmental chamber for up to 2 h. In addition, the effects of rewarming were observed following the cold exposure as each subject recovered in a 23° C chamber for 2 h. During the cold exposure, Okada et al. (1970) documented slight increases in cortisol and free fatty acid levels from resting values. However, the circulating GH levels decreased as the body temperature dropped below 34° C. The authors believed that the cold exposure was not stressful enough to stimulate an increase in GH secretion. However, when the subjects were rewarmed, and body temperature subsequently restored, GH concentrations steadily rose from the cooling and pre-cooling levels of the experiment. Thus, it seemed plausible to Okada et al. (1970) that the

increase in core temperature from below normal levels was responsible for the increase in GH levels.

Table 6-3:

Growth hormone responses during heat and cold stress.

Investigator:	Activity/Challenge:	Temperature and Hormone Response:
Okada 1970	2 h 4° C air then	↓Core 3.4° C, ↓GH ↑Cortisol ↑FFA
	2 h 23° C air	↑Core 2.1° C, ↑GH
Weeke 1983	a) 90 min eating ice	↓Core 0.8° C, ↓GH ↑NE →TSH →T3 →T4
	b) 90 min 36.3° C bath	→Core, ↓GH ↓NE →TSH →T3 →T4
	c) 60 min 39° C bath	↑Core 1.8° C, ↑GH ↑NE →TSH →T3 →T4
Leppaluoto 1988	30 min 28° C then	↑Core 0.2° C, ↑NE ↓GH ↓Cortisol ↓Prolactin
	2 h 10° C	↓Core 0.4° C, ↓EPI ↓T3 ↓T4 ↓TSH↓LH

Weeke & Gundersen (1983) studied the responses of GH, TSH, T3 and T4 of non-fasting subjects when exposed to 90 min of central cooling during a thermoneutral bath (31° C) and a control hot water bath (37° C). The design of this study allowed for the control of peripheral thermal inputs, and therefore, ensured isolation of the core temperature influences on subsequent hormone responses. During the central cooling, accomplished with ice ingestion, activation of peripheral skin thermoreceptors was prevented by immersing the subjects in the thermoneutral bath. Likewise, the use of central cooling and slight external heating was also evaluated. Following each condition, subjects were exposed to a 39° C bath for 60 min. During the ice ingestion with the 31° C bath, the core temperature decreased by nearly 1.0° C until the recovery re-warming period was started. In contrast, during the ice ingestion with the 37° C bath, the core temperature remained stable for the 90 min and then increased during the recovery period. Thyroid stimulating hormone, T3 and T4, did not respond to either the central cooling or heating.

However, central cooling suppressed GH secretion, while core heating resulted in a profound increase in GH levels. The investigators reported that there was no correlation found between the increases in GH during the heating and the increases in core temperature. Plasma NE increased 2.5 fold over basal levels due to central cooling, and then proceeded to decrease by 45 % during the 90 min heating phase. Plasma epinephrine failed to respond during either of the cooling or heating phases of these experiments. Weeke & Gundersen (1983) reported that the increases in GH observed during heating were not related to the rate of temperature increase in these subjects. From the findings of this study, Weeke & Gundersen (1983) suggested that the GH response during heat exposure may be related to the same mechanisms that were behind the GH and body temperature responses observed during physical work.

Leppaluoto et al. (1988) studied male volunteers first exposed to a 28° C environmental chamber for 30 min and then subjected to 10° C for a further 2 h. While general sympathetic stimulation was evident (the NE concentrations increased significantly), other hormonal profiles did not respond. Serum GH, prolactin and cortisol levels decreased throughout the duration of the experiment. Despite the decrease in core temperature by 0.5° C after 60 min of cold exposure, serum EPI, testosterone, LH, TSH, T3 and T4 failed to change over the course of the cold exposure. Leppaluoto et al. (1988) suggested that the suppression of GH secretion was mediated by the hypothalamic inhibitory mechanisms, but failed to proceed on possible reasons.

Clearly, it appears that core temperature plays an integral role in the regulation of various hormone responses within the human system (Tables 6-1, 6-2, 6-3). While the study of passive body heating and cooling demonstrates a number of interesting hormonal alterations, the investigation of the core temperature, and the subsequent hormonal responses that are seen with physical activity, are areas of exercise physiology not well studied.

6-5. Normal Body Temperature Responses To Exercise:

The elevation of core temperature during exercise represents the storage of metabolic heat which is produced as a by-product of skeletal muscle contractile activity. Physical exercise, in most environments, will increase metabolism and heat production many fold. Although exercise in high temperature and high humidity environments has the potential to cause fatal thermoregulatory difficulties, heat production from physical activity in low temperatures can be just as detrimental to the human system (Guyton, 1989). Costill (1972) has documented clinical hyperthermia in long distance runners under environmental conditions that were quite cool (9° C).

Core temperature, as measured by the use of rectal thermal probes, increases quite rapidly during the beginning phase of running exercise. This initial rapid increase in core temperature is followed by a reduced rate of temperature increase until heat loss equals heat production (Sawka & Wenger, 1992). It is evident from the significant increases in core temperature occurring after the beginning of exercise that the heat loss mechanisms are slow to respond (Wyndham, 1973). If the exercise intensity is maintained at a lower rate and thermal regulatory processes are functioning efficiently, a steady state of heat production-heat loss can exist.

Muscle heat production accounts for up to 90 % of the core temperature during exercise (Stolwijk & Hardy, 1966), and during strenuous activity, muscle temperatures easily surpass core temperature readings. While the temperature of active muscle tissue may increase dramatically, the temperature of non-active muscle remains steady as the core temperature increases (Aikas et al., 1962).

Exercise type plays an important role in the body temperature response to physical activity. When an identical metabolic intensity is utilized, exercise on a cycle ergometer results in a significantly higher core temperature response than arm-crank activity (Nielsen, 1968). Sawka et al. (1984) concluded that during absolute intensity exercises, leg cycling and arm-cranking activity

produced similar core temperature responses. However, when relative intensity measurements were used, the arm-crank resulted in a lower body temperature response.

Our knowledge of the influence of ambient conditions' on the increase in core temperature during exercise is far from complete. The work of Nielsen & Nielsen (1962) suggested that the magnitude of the core temperature increase during exercise was independent of the environmental temperature. While this seemed contrary to most experience, Nielsen & Nielsen (1962) observed comparable core temperature increases during identical exercise activities that were independent of a range of ambient temperatures from 5° C to 36° C. In some agreement with this, Lind (1963) concluded that the body temperature responses to physical exercise are independent of environmental conditions as long as the activity was conducted within a well defined envelope of specific environmental factors which included ambient temperature, humidity and air motion. However, during exercise with a substantial combined metabolic and environmental heat stress, or during periods of high humidity, overall core temperature responses are not independently governed, and may rise uncontrollably when heat loss mechanisms are impaired (Sawka & Wenger, 1992).

During exercise in a cool environment, the increase in core temperature provides for an improvement in skin blood flow in order to transfer heat at a greater rate to the skin surface (Wyndham, 1973). While skin blood flow will be lower during exposure to a cool environment due to local vasoconstriction, heat dissipation per unit of blood flow may be greater from the cooler skin when compared to conditions within a warm environment. This allows for a sufficient core-to-skin heat transfer despite a reduced skin blood flow to the area (Wyndham, 1973) and provides for heat delivery from the core to the outer layers of the body.

6-6. The Effect of Core Temperature on the Hormonal and Metabolic Responses During Exercise:

It has been well established that the increase or decrease in core temperature have an influence on certain hormone systems in the body. The alteration in core temperature can also occur as a result of the heat produced as a consequence of physical exercise. Thus, there appears to be a significant relationship between the exercise, the ambient conditions where the activity takes place, the subsequent body temperature responses to that exercise, and the responses of circulating hormones. By manipulating the core temperature through alterations in the ambient conditions surrounding the subject, the influence of core temperature on these hormone systems can be closely studied. In order to explain fully the control mechanisms of these physiologic responses, it is necessary to investigate the mutual relationships that exist between the exercise, the elevation in core temperature, and the metabolic and hormonal responses that are observed concurrently with these changes. Classically, there have been three exercise types studied in this area: running, cycling and swimming.

a) Running Exercise (Table 6-4):

Frewin et al. (1976) examined the GH responses in a number of subjects that completed treadmill running exercise at 3.5 mph with a 8.6 % grade for 20 min. The exercise tests were conducted under hot (40° C) and cold (10° C) ambient conditions. Oral temperature increased by 1.2° C during the run at 40° C, but increased only slightly during the identical exercise at 10° C. Significant increases in circulating GH concentration were found in the subjects running in the 40° C environment. No statistical changes in GH levels were found in the same subjects completing the identical exercise stimulus in the 10° C environment. By utilizing sublingual methods to monitor temperature changes, these authors demonstrated a 1.1° C and 0.55° C increase in temperature in the 40° C and 10° C exposures, respectively. Unfortunately, by collecting data on only two subjects and employing these methods to provide the data on the body temperature changes, the findings must be questioned. Furthermore, the authors sampled blood too

infrequently to define fully the course of the observed responses. Thus, given the methodological limitations of this investigation, it would seem that ambient temperature was involved in the overall thermoregulatory and hormonal responses observed. Frewin et al. (1976) speculated that the response of GH was the result of a dual adrenergic mechanism that operated when the exercise and thermal stresses were applied simultaneously.

Table 6-4:

Growth hormone responses during running with thermal stress.

Investigator:	Activity/Challenge:	Temperature and Hormone Response:
Frewin 1976	i. 20 min run in 10° C	↓Sublingual 0.5° C, →GH
	ii. 20 min run in 40° C	↑Sublingual 1.15° C, ↑GH
Radomski 1994	2 h aerobic run in 26° C	↑Core 3.3° C, ↑GH related to temp↑

Francesconi et al. (1978) studied a number of hormone systems in exercising and sedentary subjects that were exposed daily to 2 h of heat acclimatization in a 49° C thermal chamber for 8 consecutive days. The exercise group completed a moderate intensity, treadmill walking task, and demonstrated a significant GH response both on a control day completed at 21° C and during all exercises conducted during the heat exposure (49° C). Over the 8 day acclimatization period, there was a downward shift in the responses of GH. In contrast, the sedentary group failed to produce any significant GH responses during the control or acclimatization exposures. Thus, Francesconi et al. (1978) demonstrated a greater sensitivity of GH secretion to light exercise than to extreme heat stress and concluded that GH played an insignificant role during heat acclimation.

Recently, Radomski et al. (1994) examined the relationship between changes in core temperature and certain hormonal levels in subjects running on a treadmill at 70 % of their $\dot{V}O_2$ max for 90 to 120 min at an ambient temperature of 26° C. Core temperature increased at 0.051°

C·min^{-1} for the first 30 min and at 0.014° C·min^{-1} for the remainder of the exercise period. The GH levels increased markedly during the initial 30 min phase of the exercise, but plateaued for the remainder of the testing period. A significant exponential relationship was observed between the rise in core temperature and the subsequent GH responses ($r=0.89$, $p<0.001$). Given their findings, Radomski et al. (1994) postulated that a thermosensitive mechanism(s) within the hypothalamus, independently or in combination with mechanisms described by the oxygen demand/availability ratio, may have played a role in the regulation of GH during the exercise period.

b) Cycle Ergometry Exercise (Table 6-5):

Buckler (1973) demonstrated that the rise in body temperature due to exercise was related in timing and magnitude to the responses of circulating GH levels. In this study, subjects completed a number of different experiments designed to study the effect of body heating on GH secretion. Subjects exercised on a cycle ergometer at 900 kpm for approximately 30 min in 4° C and 21° C environments, with and without donning a rubberized sauna suit, or were exposed to a hot sauna bath to passively promote a rise in core temperature. In each case, when an increase in core temperature was found, plasma GH levels also responded positively. Interestingly, one subject experienced a greater increase in GH levels during the cold exposure than during the heat exposure, but in this case, the subject's core temperature increased more rapidly than in the warmer conditions. From these observations, Buckler (1973) suggested that the rate of temperature change was associated with the response of GH and not the absolute level of body temperature attained with either external heating or exercise. This assumption remained true for GH responses in both cold and hot environments, with and without exercise, and agreed with the previous work completed by Okada et al. (1970). Buckler (1973) proposed that if the rate of temperature change was slow, there would be little or no GH response, but as the rate of temperature change increased, an increase in GH levels would be observed. This concept would support the lack of

any GH response observed in an experiment where the exercise was too light, too heavy and therefore short acting, or when body temperature returned to normal from subnormal levels. It was postulated that the rate of rise of body temperature occurring as the result of exercise was a factor which influenced the GH response and may actually be responsible for the response (Buckler, 1973).

Table 6-5:

Growth hormone responses during cycling with thermal stress.

Investigator:	Activity/Challenge:	Temperature and Hormone Response:
Buckler 1973	20 min @ 900 kpm in 4° C	↑Core 0.054° C•min^{-1}, ↑GH
	20 min @ 900 kpm in 21° C	↑Core 0.057° C•min^{-1}, ↑GH
Claremont 1975	a) 1 h @ 56 % in 0° C	↑Core 1.1° C, ↑lactate
	b) 1 h @ 50 % in 34° C sauna	↑Core 1.8° C, ↑lactate 2X > a)
Raynaud 1983	a) 1 h @ 47 % in 24° C	↑Core 0.45° C, →GH
	b) 1 h @ 59 % in 24° C	↑Core 0.6° C, ↑GH 10X a)
	c) 1 h @ 70 % in 24° C	↑Core 0.93° C, ↑GH 20X a)
	d) 1 h @ 41 % in 33° C	↑Core 0.55° C, ↑GH 40X a)
	e) 1 h @ 54 % in 33° C	↑Core 0.6° C, ↑GH 60X a)
	f) 1 h @ 71 % in 33° C	↑Core 1.05° C, ↑GH 80X a)
Christensen 1984	40 min @ 400 kpm in 4° C	↓Tympanic 0.27° C, ↓GH
	40 min @ 400 kpm in 22° C	↑Tympanic 0.8° C, ↑GH
Dore 1991	a) 45 min @ 70 % in 23° C air	↑Core 0.9° C, ↑GH_T ↑GH_{20K}
	b) 45 min swim @ 70 % in 26° C	↑Core 0.9° C, ↑GH_T ↑GH_{20K}
	c) 20 min 94° C sauna	↑Core 0.8° C, ↑GH_T ↑GH_{20K}

Raynaud et al. (1983) investigated the variability of GH responses in subjects cycling for 1 h at three different intensities (40, 60 & 80 %) of their $\dot{V}O_2$ max. The experiments were completed in either a 24° C or a 33° C thermal chamber. The percentage of $\dot{V}O_2$ and core temperature responses between the two temperature conditions remained constant during the exercise tests. The light, moderate and heavy exercise tests produced increases in core temperature of 0.45° C, 0.60° C and 0.93° C in the 24° C environment and 0.55° C, 0.59° C and 1.05° C in the 33° C test, respectively. However, the GH response to each intensity was the greatest during the warmest chamber conditions. Although Raynaud et al. (1983) measured lactate levels during the exercise periods, they were unable to demonstrate a causal relationship with the GH responses. The authors failed to speculate on the potential physiologic mechanisms underlying their observations.

Christensen et al. (1984) studied the GH responses to passive heating in subjects covered with a hot liquid infused blanket and reflective body insulation in fasting and non-fasting states. While the slope and magnitude of the tympanic temperature was identical during the two experiments, higher levels of GH were obtained in the fasting state when compared to the non-fasting state. These results were then compared to GH responses following 40 min of cycle exercise at 400 kpm in a 22° C environmental chamber. Accordingly, the rate of tympanic temperature increase was greater during the exercise period, but higher peak levels of GH were observed during the passive heat test. Later, Christensen et al. (1984) found that the increase in GH levels demonstrated in response to the cycle exercise completed at 22° C were completely attenuated during the same exercise completed at 4° C. When the tympanic temperature did not increase, GH responses were suppressed. While GH responses were completely suppressed with exercise during the cold exposure, GH levels did increase when the subjects were permitted to rewarm in the 22° C chamber. Christensen et al. (1984) speculated that exercise may not stimulate the GH release, but may actually inhibit the GH secretion evoked by the rise in body temperature during exercise. In this case, the final core temperatures were similar between the heat test and the

exercise study, although the rate at which exercise led to the increase in body temperature was twice as fast as that of the heat exposure study. Thus, the GH response to the heat exposure was actually attenuated due to the rapid rise in body temperature as a result of the metabolic heat produced by the cycle exercise. This raises many questions as to the role of exercise and the rate of temperature change plays as a stimulator of GH release.

Kaciuba-Uscilko et al. (1992) investigated the plasma GH responses to four consecutive exercise bouts completed in a 23° C environmental chamber. Each exercise period, fixed at 50 % of the subjects' $\dot{V}O_2$ max, was followed by 30 min of rest before the next exercise session commenced. From the onset of cycling, core temperature increased above the pre-experimental values (36.5° C). This was followed by a slight decrease during the rest interval. Thereafter, with each successive exercise and rest interval, core temperature levels increased to a further degree and peaked at 37.5° C during the last exercise period. Despite the fairly low exercise intensity, plasma GH levels increased significantly and continued to increase for the entire 2 h of exercise. The pattern of GH secretion was quite similar to that of the core temperature responses by increasing during each exercise period while decreasing somewhat during each rest interval. Cortisol levels responded by first decreasing from initial basal levels and finally increasing during the final two exercise and rest periods. Epinephrine levels increased from the onset of the exercise period while NE responded more slowly and increased only after the second exercise session. After this, the catecholamine levels continued to increase for the duration of the experiment. While glucose concentrations decreased during the experiment, lactate remained constant throughout the 4 exercise:rest intervals. Kaciuba-Uscilko et al. (1992) speculated that the changes in core temperature were the result of the release of thermogenic hormones such as GH, cortisol and catecholamines, which were due, in part, to the decrease in circulating glucose levels in the exercising subjects.

c) Swimming Exercise (Table 6-6):

Swimming represents a uniquely different condition from exercise in the air as water presents a highly conductive and convective medium for heat loss. Galbo et al. (1979) investigated the effects of prolonged swimming at 68 % of $\dot{V}O_2$ max for 60 min in three water temperatures, 21° C, 27° C and 33° C. Core temperature increased during the swim in the 33° C and 27° C water, but decreased during the exercise in the 21° C water. Growth hormone, cortisol and glucagon concentrations increased in the 27° C and 33° C conditions, but failed to increase during the exercise at 21° C. Insulin and glucose levels decreased throughout all three of the different water conditions while lactate levels were highest during the 21° C swim. Norepinephrine levels were higher in the 33° C swim than in the thermoneutral water at 27° C, while both NE and EPI increased during the 21° C exercise. Without fully explaining the GH response, Galbo et al. (1979) concluded that cold exposure led to greater lactate responses, and that muscular glycogenolysis was enhanced in the cold study due to increased NE concentrations.

Table 6-6:

Growth hormone responses during swimming with thermal stress.

Investigator:	Activity/Challenge:	Temperature and Hormone Response:
Galbo 1979	a) 1h @ 68 % in 21° C	↓Core 0.8° C, →GH ↑NE ↑EPI
	b) 1 h @68 % in 27° C	↑Core 0.7° C, ↑GH ↓Insulin
	c) 1 h @ 68 % in 32° C	↑Core 1.3° C, ↑GH ↑NE ↑EPI
Dulac 1987	8 h 32 km in 18.5° C	↓Core 1.8° C, ↓GH ↓Insulin

Dulac et al. (1987) studied the hormone responses in long distance swimmers completing a 32 km race in 18.5° C water. The post-exercise GH remained unchanged or decreased from pre-exercise values during the 8 h competition, while plasma NE, EPI, cortisol and lactate levels

increased significantly. Cortisol was highest in the subjects that experienced the greatest drop in core temperature (mean = 35.1° C). Unfortunately, variable work expenditure, exercise duration, body composition, diet and infrequent blood sampling (only pre and post samples) combined to cast doubt as to the exact interpretation of these data.

6-7. Literature Conclusions:

Given that the hypothalamus is the central regulator of both core temperature and pituitary GH secretion, it is of interest that the body temperature responses to exercise may also play some role in the GH responses to exercise. Generally, the influence of physical activity during heat exposure has been shown to induce an increase in GH. Conversely, the reverse has also been shown to be true, the effect of exercise during body cooling suppresses GH responses. Raynaud et al. (1983) demonstrated that exercise that promoted a greater increase core temperature also resulted in greater GH levels. Okada et al. (1970) demonstrated that subjects exposed to 4° C for up to 2 h failed to show any change in GH levels. While cooling resulted in both a drop in body temperature and GH levels, rewarming of these subjects in a 23° C environment produced a profound increase in GH secretion. With this in mind, Buckler (1973) proposed that if the rate of temperature change was slow, there would be little or no GH response, but as the rate of temperature change increased, an increase in GH levels would be observed. Thus, a dispute has developed as to the significance of the rate of the temperature change versus the peak core temperature experienced in response to exercise, and the subsequent GH response.

The role of body temperature in the hormonal and metabolic responses to physical exercise has not been fully explained. It is, therefore, necessary to conduct an investigation involving the manipulation of the core temperature changes experienced during exercise. In this case, conditions should be chosen that would either accentuate or suppress the core temperature responses to the exercise stimulus. Unlike previous investigations, the study must encompass a high degree of

reliability and include a comprehensive blood sampling schedule, analysis of GH, lactate and oxygen consumption levels, and at the same time, control for other factors that may have an influence on the secretion of GH such as age, fitness, percentage body fat and level of fasting.

6-8. Pilot Study: Manipulation of Core Temperature During Exercise:

A number of investigators have attempted to regulate the increase in core temperature experienced during exercise. Davies et al. (1976) were able to diminish the rise in core temperature in runners that exercised for 60 min at 75 % of their $\dot{V}O_2$ max. In this investigation, the subjects had their skin sponged with water at regular intervals throughout the exercise. The effect of skin wetting was to enhance the evaporative sweat loss by approximately 10 %, thereby producing a fall in core temperature of 0.02° C below that observed when evaporation was not enhanced.

While investigating the effects of exercise on slow wave sleep, Horne and Moore (1985) studied the core temperature responses in subjects running at 75 % of their $\dot{V}O_2$ max for two consecutive bouts of 40 min. The subjects ran on a treadmill in an environmental chamber with the temperature controlled at 21° C and 40 % humidity. Body temperature increased in the control experiment by an average of 2.3° C over the length of the exercise period. During the experimental condition, Horne and Moore (1985) were able to significantly diminish and delay the rise in core temperature through the use of assisted evaporation. In this case, the subjects were cooled with air from electric fans while wearing light cotton garments that were moistened with the spray from water bottles. This assisted evaporative technique significantly slowed the rate of core temperature rise when compared to the same exercise bout without any cooling intervention.

Recently, Melin et al. (1994) examined the effect of wind speed on the core temperature responses in male runners that were exercising at 70 % of their $\dot{V}O_2$ max. The subjects ran for 40 min in an ambient temperature of 25° C to 26° C and relative humidity of between 35 % and 45 %.

Melin et al. (1994) demonstrated that wind speed had a major effect on the time course of the core temperature changes observed during the exercise period when compared to control trials.

In an attempt to attenuate and potentiate the rise in body temperature during exercise, our laboratory has investigated the responses of subjects during treadmill running under a number of different ambient conditions, including varying temperatures (5° C, 7° C, 8° C, 10° C and 26° C), humidity readings (4 %, 25 % and 85 % relative humidity) and wind speeds (variable or constant).

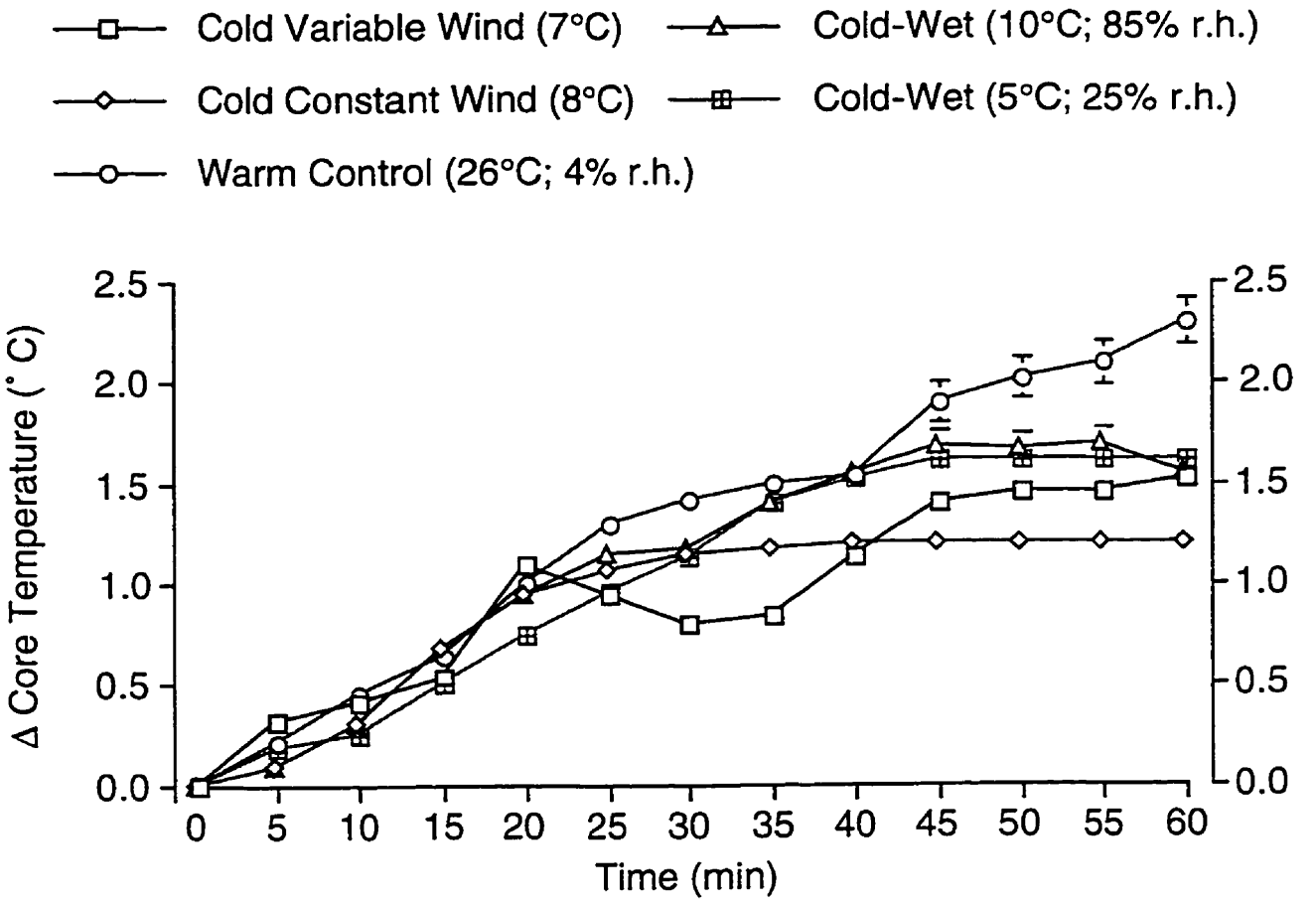

Figure 6-1: *Core temperature responses to moderate aerobic exercise.*

In this case, the subjects ran at a constant intensity (70 % of $\dot{V}O_2$ max) for a 60 min period during which changes in core temperatures and heart rates were recorded. It is evident from these

data that cold exposure, or assisted evaporation ("Wet" experiments) through the constant spraying of water on the subjects' over-garments and the influence of electric fans, did not significantly diminish the rise in core temperature. These subjects exhibited nearly identical heart rate responses to the exercise tests, but had very inconsistent and non-significant effects on the rise in core temperature (Figures 6-1). The warmest environmental chamber temperature always produced the highest end-exercise core temperature. A low environmental temperature (7° C), with variable wind, produced the lowest overall end-exercise core temperature, while the assisted evaporation during cold exposure (5° C) lead to the next lowest overall core temperature. Thus, we were unable to clamp core temperature responses during exercise by Horne's technique (Horne & Moore, 1985).

There may be a number of differences between our methods and those of Horne & Moore (1985). In one set of experiments, our subjects exercised in a much colder (5° C - 10° C) environmental chamber which may have caused an exaggerated vasoconstrictive response (Figure 6-1). Indeed, our subjects did verbally complain of the cold and the use of spray water bottles. In addition, these subjects wore long sleeve tops and bottoms made of 100% polyester which may have helped to insulate the body instead of assisting in the cooling effect of evaporation. In a second set of experiments conducted in a warmer chamber, the subjects dressed in light 100% cotton short sleeve shirts. Again, in disagreement with Horne & Moore (1985), core temperature increased at the same rate and to the same maximum as during the control experiments. It was obvious from these data that more research on the effects of environmental temperature, humidity and wind on core temperature must be completed before the overall question of hormonal regulation and core temperature can be challenged.

Further investigation by our laboratory attempted to control the rise in core temperature during exercise by wearing a number of different Canadian Forces (CF) cooling garments (Figure 6-2). These garments were designed to facilitate core temperature cooling in our Air Force

personnel during operational activities in extreme environments.

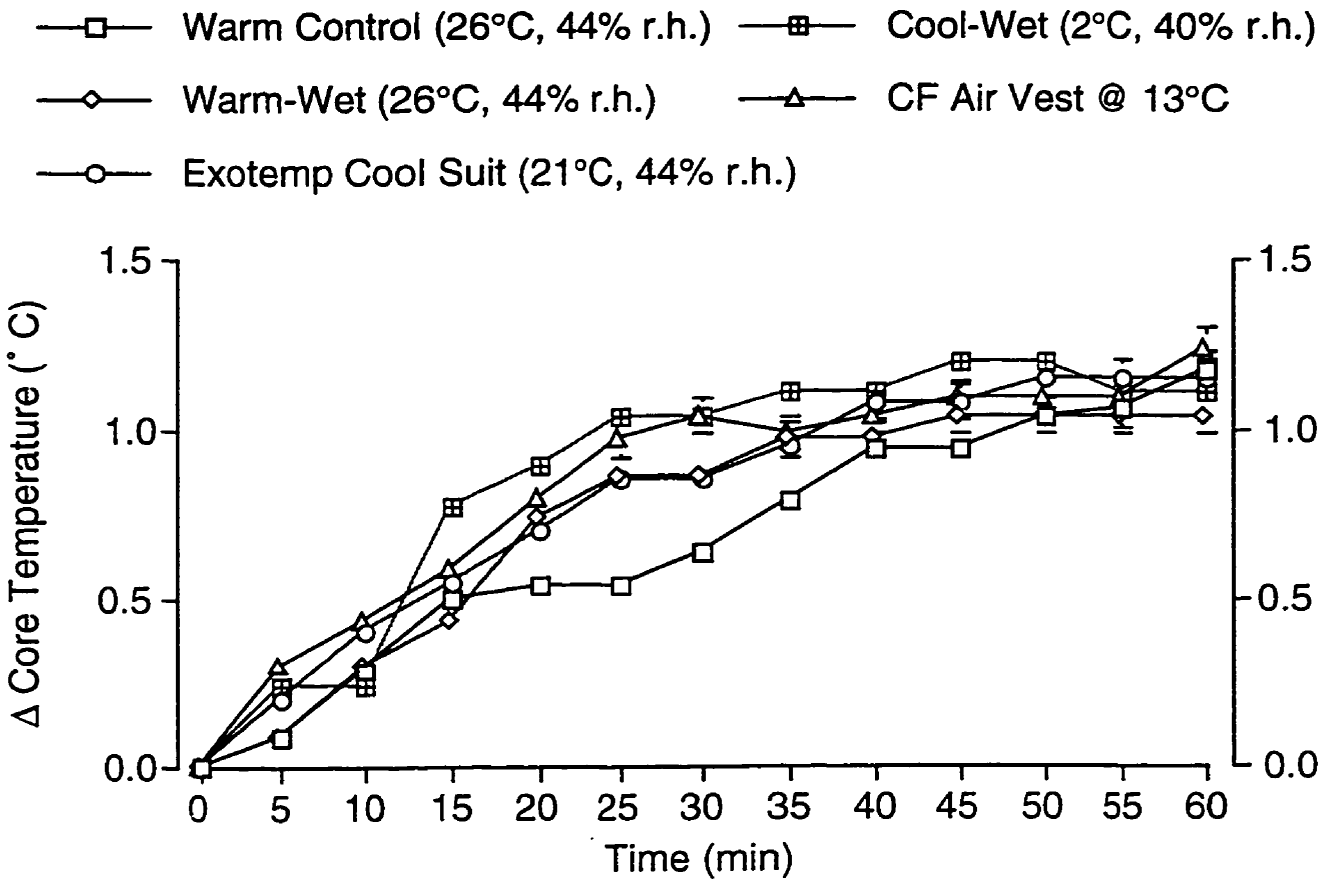

Figure 6-2: Core temperature responses during moderate aerobic exercise.

Our study attempted to suppress the increase in core temperature during running at 70 % of the subjects' $\dot{V}O_2$ max for 1 h. Figure 6-2 demonstrates that the use of assisted evaporative cooling (Wet) at 2° C and 26° C, the Exotemp Cool suit infused with 5° C water, the C.F. Air Vest infused with 13° C air, failed to influence the core temperature responses when they were compared to the control exercise conducted at 26° C.

Recently, Young et al. (1993) investigated the effect of body temperature on the aerobic responses observed during endurance training. In this case, the subjects were exercised on a

underwater cycle ergometer during submersion in 20° C and 35° C water. While 8 weeks of endurance training failed to demonstrate any difference in aerobic improvements between the two conditions. However, cycling exercise in the 35° C resulted in a sharp increase in body temperature whereas the identical exercise and intensity of effort in 20° C failed to lead to an increase in core temperature (Young et al., 1993).

In our continued attempts to control the core temperature response to exercise, our group investigated the metabolic and thermal responses experienced while performing exercise on a cycle ergometer which was placed at the bottom of a water immersion tank (Figure 6-3).

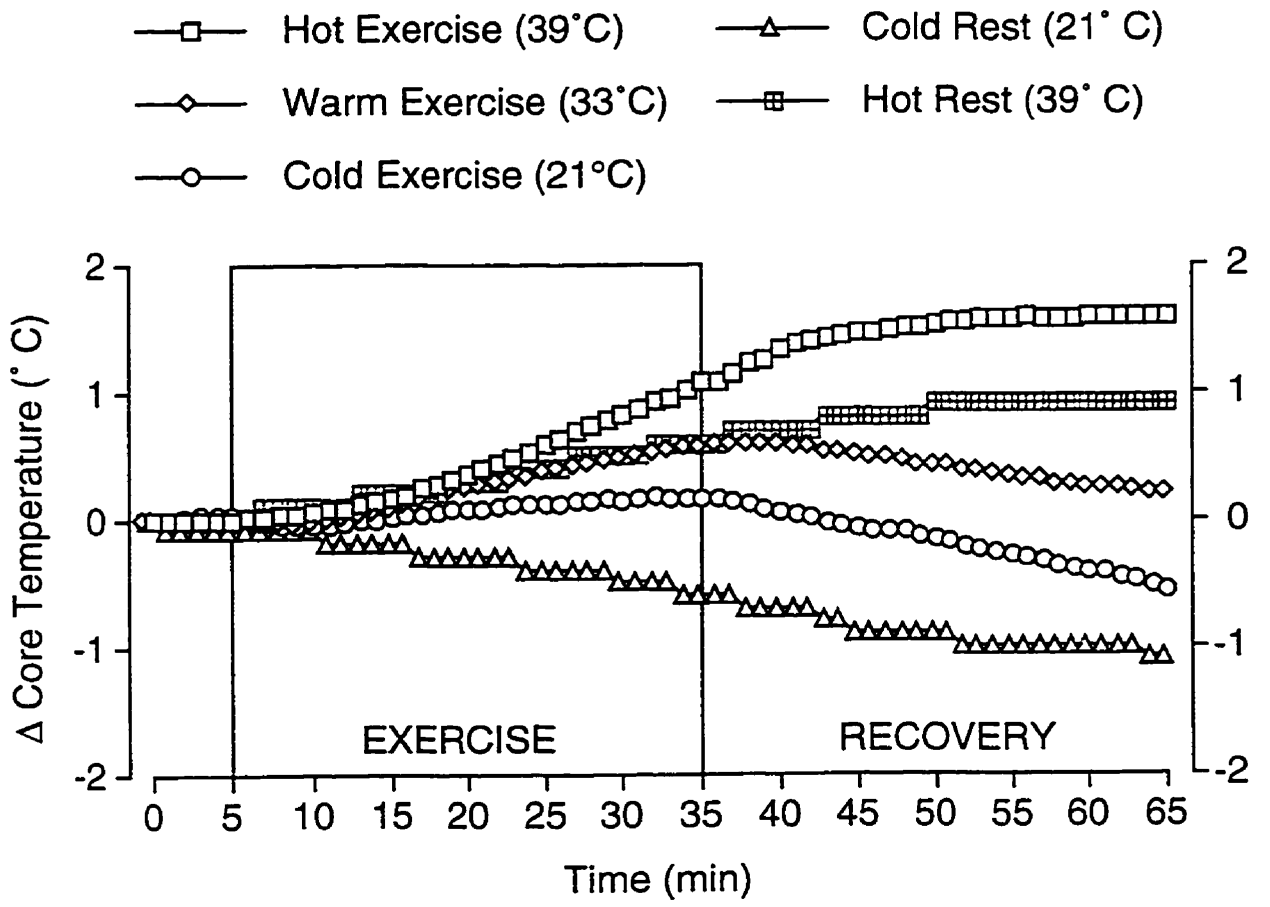

Figure 6-3: *Core temperature responses during moderate intensity submersion cycling.*

In this case, the subject performed moderate aerobic leg cycle exercise while being completely immersed to mid-chest water depth. The exercise intensity was fixed at 70 % of the subjects' $\dot{V}O_2$ max. By varying the temperature of the water, our laboratory has been able to attenuate and potentiate the rise in core temperature commonly experienced during physical exercise. Clearly, as Figure 6-3 demonstrates, we were able to separate the core temperature responses found with the identical exercise stress by using different water temperature and length of exposure combinations.

To document further the suppression of core temperature during exercise during under water cycling, and to test the duration of this effect, we studied several subjects completing 45 min of exercise at 70 % of their $\dot{V}O_2$ max (Figure 6-4).

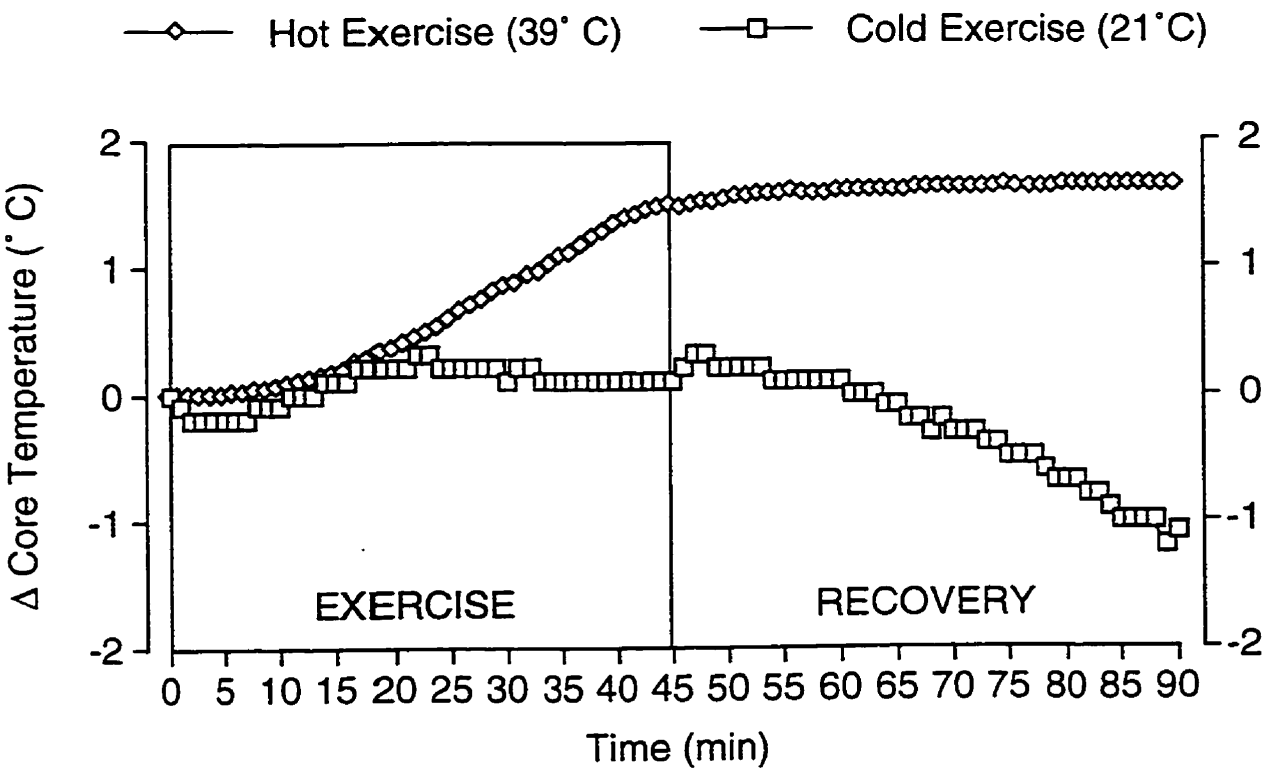

Figure 6-4: *Core temperature responses during moderate intensity submersion cycling.*

6-9. Objectives:

The specific objectives of this investigation were to:

i. Develop a technique to clamp core temperature increases during physical exercise in order to separate the components of energy expenditure and temperature during exercise.

ii. Determine using this technique, whether the increase in core temperature observed during exercise, rather than energy expenditure, is primarily responsible for the increase in hormone response during exercise.

6-10. Hypothesis:

The hypothesis tested in this investigation was that:

i. The temperature increase that occurs during physical exercise is not a factor in the increases in GH secretion during the exercise.

6-11. Materials and Methods:

This study was completed at the Physiology and Immunology Laboratories of the Defence and Civil Institute of Environmental Medicine at the Canadian Forces Base Toronto. Additional testing and analysis were also conducted at various departments within the University of Toronto.

i. Subjects: Eight healthy males from the university and military populations, that were not involved in any exercise training, volunteered as subjects for this experiment. On their initial visit to the laboratory, each subject was educated to the methods and time commitments involved in the investigation. Afterwards, each subject was informed of all risks that were associated with the experiment and signed an informed consent document that was previously approved by the Ethics Committees of the University of Toronto and the Defence and Civil Institute of Environmental Medicine. All subjects were then examined by a military physician. Medical screening excluded any subject from the experiment who was not free from any acute or chronic medical condition. Furthermore, all eight subjects had remained free of infection for a minimum of 6 weeks prior to the study, and none of the group were currently taking any medications. Subjects abstained from eating (albeit a standardized morning meal), smoking, caffeine and sexual activity for 12 h before any of the testing procedures. Each subject visited the laboratory on six separate occasions, two visits for physical tests and familiarization and four visits for protocol testing purposes.

ii. Physical Tests, Familiarization and Protocol Design: On the first visit to the lab, at least two weeks before the protocol testing was to commence, each subject had their physical characteristics determined (Table 6-7). The percentage of body fat for each subject was determined using under water weighing (Behnke et al., 1942) and skinfold thickness (Durin & Womersley, 1974).

Each subject was then completely familiarized with the experimental protocol and the immersion laboratory. Figure 6-5 accurately details the laboratory setup used in this investigation.

The immersion tank measured 1.3 m (w) * 2.1 m (l) * 1.4 m (d) and had a capacity of approximately 4000 L. Also, during this visit to the laboratory, each subject completed a short exercise cycle test in 33° C water in order to familiarized the subject with cycling under water on the ergometer. An electronically-braked cycle ergometer (Collins Pedalmate, Warren E. Collins, Braintree MA, USA) was modified and sealed to prevent water leakage into the mechanism. Furthermore, the sealed crankcase unit was kept under constant positive pressure by air infusion with the use of a Ametek Flow Control R-1 air pump (Applied Electrochemistry, Pittsburgh, USA) to prevent water contamination of the working mechanisms. The ergometer pedals were outfitted with foot brackets while the entire apparatus was stabilized to the bottom of the tank with 50 kg of lead weights. In addition, each subject wore a diving weight belt (4 kg) to assist in remaining seated on the ergometer during each test. In order to standardize the effects of clothing, all subjects were instructed to wear swimming trunks, and were provided with NIKE Aqua socks, during each testing period.

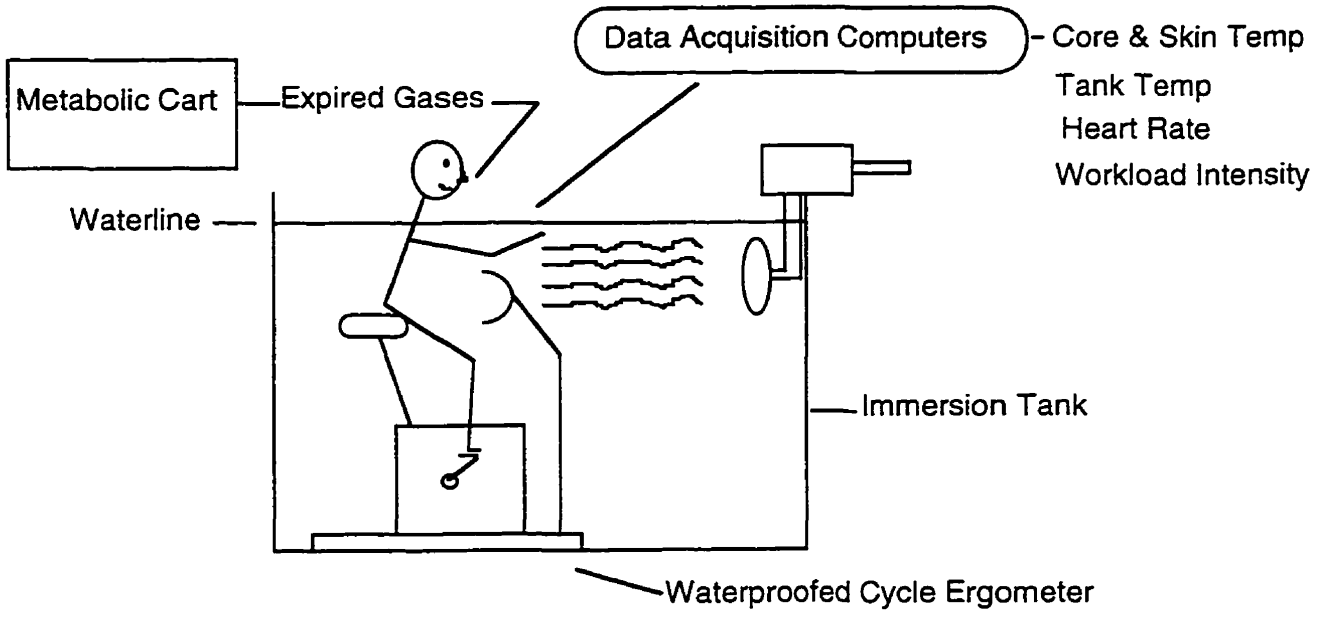

Figure 6-5: *Laboratory set-up for experiments.*

On the second laboratory visit, one week prior to beginning the experiment, the predicted $\dot{V}O_2$ max of each subject was determined using a graded cycle ergometer test while immersed to mid-chest level in 33° C water. Expired gas analysis was conducted for the length of the test using continuous gas sampling and analysis (DCIEM Metabolic Cart, Downsview ON, Canada). The apparatus was calibrated before and after use, by means of a 3 L syringe and precision-analyzed cylinder gases. The heart rate during the test was recorded with an Accurex heart rate monitor (Polar Electro, Port Washington, USA). The test consisted of a 2 min rest period while sitting in the water followed by a 75 W warm-up period of an additional 2 min. The pedaling pace was 60 rpm. Thereafter, the workload was increased by 25 W every min until the subject could no longer continue the test. The $\dot{V}O_2$ max was established as the greatest level of oxygen consumed during the exercise test. The data are presented in Table 6-8.

The respiratory and work output data were used to calculate the workload intensities for each subject. In this case, 65 % of the subject's $\dot{V}O_2$ max during the immersion test was used for the workload in all experiments. The two exercises protocols used in the experiment equaled 65 % of the subjects' $\dot{V}O_2$ max, and were of equal total external work output and total exercise duration. with an identical work-rest interval, but were conducted at two different temperatures.

To account for the additive effect of the water resistance on the work intensity, each subject's test results were modified using procedures established at DCIEM (Lecourt, 1986; Gilmour et al., 1988). In this case, previous experimentation by the Diving Medical Facility at DCIEM had determined that the additional effect of water resistance accounted for approximately 75 W above the resistance encountered while normally cycling in air. This effect was accounted for in the final presentation of the actual workloads utilized and was standardized between the two water temperature conditions (Table 6-8).

In addition to the familiarization and $\dot{V}O_2$ max tests, the subjects completed a total of four trials in the immersion tank. Each immersion trial, two exercise and two resting control sessions, lasted for 85 min in total, with the first 5 min simply acclimation in the immersion tank. In the Exercise (E) trials, each subject exercised on the cycle ergometer for 40 min and then remained sitting on the cycle ergometer in the immersion tank for an additional 40 min. In the Control (C) trials, each subject remained at rest for the entire 85 min period.

The experimental protocol consisted of an exercise and a control trial at two different water temperatures. The Cold (C) water temperature was 23° C, and was used to blunt core temperature increases during exercise. The Hot (H), 39° C, water trial was used to potentiate the rise in core temperature seen during the physical exercise. The sequence of the four tests was fashioned in a randomized style and each test was separated by at least 1 week. All four tests were performed by each subject, which allowed each subject to serve as his own control. The tests were labeled as:

	Hot	Cold
Control	H-C	C-C
Exercise	H-E	C-E

Each subject was immersed to a mid-chest level water line (1.3 m maximum) while seated on the cycle ergometer. Water in the immersion tank was circulated with a modified trolling outboard motor (Minn Kota Turbo 35) on medium speed and powered by a standard marine battery (Motormaster Nautilus Gold 12V60 AMP) in order to guarantee a consistent water temperature throughout the tank. Tank temperature was continuously monitored and recorded during each of the tests via a data acquisition system (described below) and was adjusted when needed with the

addition or removal of water. Water quality was maintained using standard techniques and was replaced on a daily basis.

The subjects refrained from eating (albeit a standardized morning meal), smoking, and sexual and physical activity a minimum of 12 h prior to the start of each day of testing. On the day of the test, all subjects ingested a standardized 235 mL lactose free (355 cal) Ensure Plus liquid meal (Ross Laboratories, Montreal, Canada) at 0800 h. The subjects were not permitted to ingest any other nutritional sources until after the completion of that particular day's testing. All experiments were performed at the same time of day to minimize the influence of circadian rhythms on the findings.

The time sequence of events for the remainder of each trial is described in Figure 6-6.

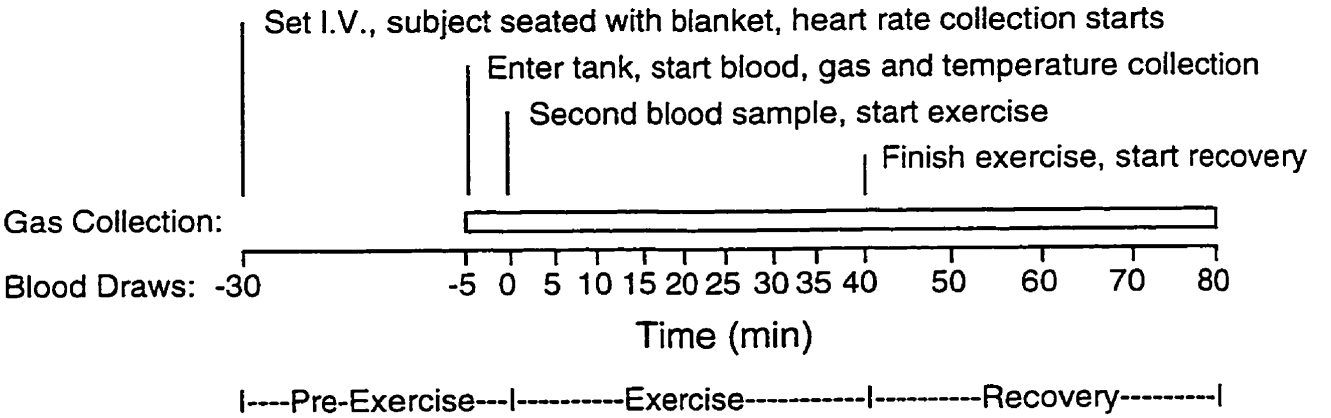

Figure 6-6: *Timing sequence for exercise and blood collections in H-C, H-E, C-C and C-E trials.*

After the pre-test (-5 min) blood sample, each subject was moved into position on the cycle ergometer in the immersion tank. Each subject positioned himself on the cycle ergometer with his arms out of the water and resting on a support board which was utilized for the entire test session.

Collection of temperature responses and analysis of the expired gases were also initiated at the -5 min point and continued until the end of the experiment (85 min) (Figure 6-6).

At time 0 min, the subject would either remain resting for 80 min (control condition) or cycle at 65 % of their individual $\dot{V}O_2$ max for 40 min and then rest for an additional 40 min in the immersion tank (exercise condition). Two resting control (C) trials, one at each temperature, were conducted with each subject merely remaining seated on the ergometer in the immersion tank for the entire 85 min. Two exercise (E) trials, one at each temperature (H and C). were conducted for the remaining two tests. The two exercise tests consisted of the identical work intensity (65 % of $\dot{V}O_2$ max) on the cycle ergometer for 40 min followed by an additional 40 min of recovery while remaining in the immersion tank.

Each individual experimental trial was terminated if:

(1) the subject wished to withdraw from the test;
(2) the rectal temperature decreased below 35.0° C or increased above 39.3° C;
(3) the heart rate exceeded 95 % of maximum for longer than 3 min;
(4) after 85 min of immersion exposure.

After the cold water tests, each subject was placed in a hot water tub (39° C) in order to bring the core temperature back to its pre-experimental levels. Each subject was not allowed to leave the testing facility until their core temperature and heart rate had returned to the pre-testing level.

iii. Measures: At 15 min prior to beginning each experiment (1300 h), the subject inserted a rectal temperature probe (Baxter Pharmaseal, Valencia, CA, USA) approximately 12 cm into his rectum. The probe was secured in place by tape and a harness to prevent accidental displacement during the

trial. Each specific probe was handled exclusively by the particular subject and was disinfected with a standard chemical sterilizing solution (Glutacide, Pharmax Ltd, Mississauga, Canada) after each trial. Skin temperature data were collected from the upper right chest, upper middle back and on the mid-cranium (Figure 6-7). In this case, the subjects were fitted with three skin temperature thermistors (Yellow Springs Inc., OH, USA) on areas of the skin that were previously prepared by razor shaving, vigorous rubbing with alcohol swab and the positioning of a final covering of Skin-Prep protective dressing wipe (Smith & Nephew, Largo FL, USA). In addition, each subject was instrumented with a NPT25 core temperature thermometer (DEEP Body Thermometers, Cambridge, England) directly over the heart. Data from this monitor were continuously collected by the data acquisition computer system (described below).

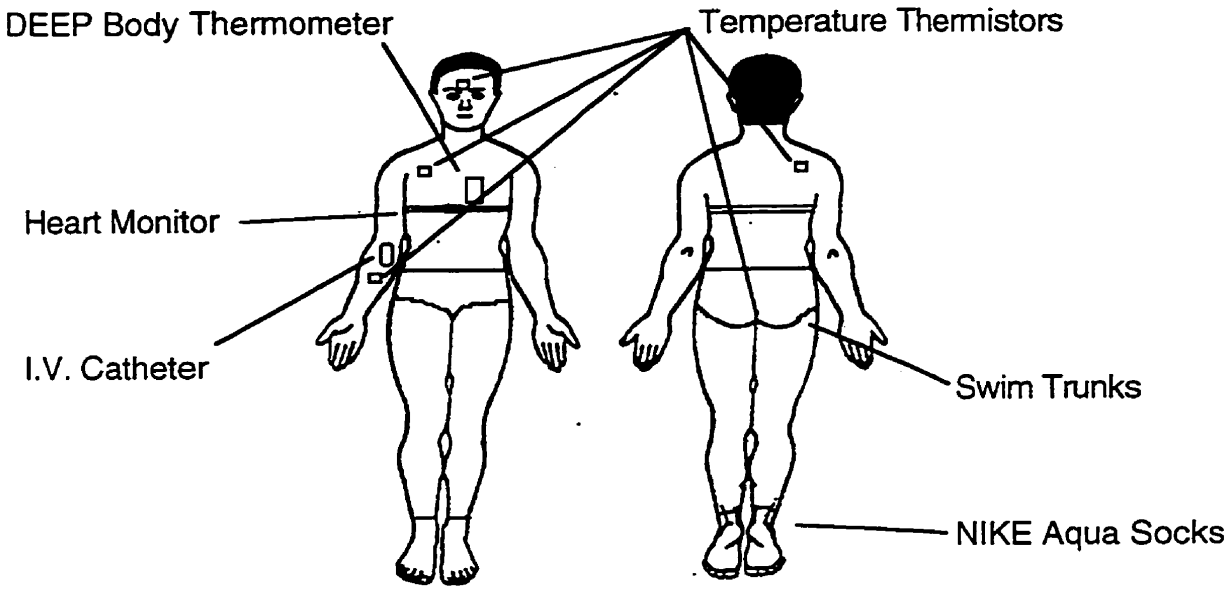

Figure 6-7: Probe and thermistor placement.

At approximately 1300 h, a sterile I.V. catheter (Insyte 20 GA, 2.5 cm) affixed with an injection adapter (Medex 1 7/16") was inserted in the anticubital area prior to the beginning of the exercise. The catheter was kept patent through the use of a heparin-saline flush (Hepalean

Organon Teknika, Toronto ON, Canada). The site of entry for the catheter was protected from the water tank environment by a Tegaderm transparent I.V. dressing (3M Medical, St. Paul, MN, USA).

To record heart rate response to the control and exercise sessions, a heart rate monitor (Polar Electro, Port Washington MI, USA) was attached to the mid-chest at the level of the diaphragm. Data from the five temperature probes (core, chest, back, head and heart) were collected for the length of the entire experiment (85 min) with a data acquisition system (Hewitt-Packard HP75000 Series B, Mississauga ON, Canada) and accompanying software package (Red Barron Data Logger, Mississauga ON, Canada). After each subject completed the experimental preparation, they remained sitting in the subject preparation room (22° C) for 30 min while covered completely with a blanket and a small heating pad (NECO, Mexico) over the IV catheter.

At -5 min, the first blood sample and heart rate (pre-test) measurements were obtained with the subject in the seated position. Samples for hormone analysis were collected in sterile vacutainer tubes, allowed to clot and then centrifuged at 3500 rpm for 15 min at room temperature (21° C). After separation, the serum was aspirated from the tube and stored in microcentrifuge tubes which were then immediately frozen at -80° C until analysis. The samples for haematologic analysis were collected in vacutainer tubes which contained exactly 0.4 mL of liquid EDTA (15%) solution, and gently rocked at room temperature until analysis. Haematologic analysis was conducted within 90 min from the time of sampling. Samples for metabolite analysis were collected in tubes containing sodium floride (NaF) powder (30 mg). Immediately after filling each tube, the contents were mixed by gentle repeated inversion. Immediately after adequate mixing, a 25 μL sample for lactate analysis was taken from the tube containing NaF powder. This sample was vigorously shaken (not stirred) with 200 μL of really, really ice-cold perchloric acid ($HClO_4$) and then frozen at -80° C until analysis. The remainder of the tube containing the NaF was centrifuged at 4° C and the separated plasma immediately frozen and stored at -80° C until analysis.

During the first 40 min period, blood and heart rate samples were obtained every 5 min, while during the last 40 min (rest) of the immersion, blood and heart rate samples were taken every 10 min (Figure 6-6).

iv. Analyses: Serum GH levels were analyzed using a commercially available radioimmunoassay kit (Allegro Double Antibody, Nichols Institute Diagnostics, San Juan Capistrano CA, USA). Analysis for cortisol was conducted by using a solid phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles CA, USA). Radioactive counts were determined on a Cobra Auto-Gamma Counter (Packard Model 5002, Meriden CT. USA). The haematologic analysis was conducted on a Coulter JT Model DA26-6 interfaced with a Coulter DTH2-AS Data Acquisition terminal (Coulter, Hialeah FL, USA). Lactate concentrations were determined with a lactate enzyme kit (Boehringer Mannheim, Mannheim, Germany) as described by Maughan (18). In order to correct for the plasma volume shifts experienced during this investigation, all hormonal values were corrected by the method described by Dill & Costill (1974).

The oxygen demand/availability ratio was calculated according to the method described by VanHelder et al. (1987). The oxygen demand was calculated by measuring the cumulative oxygen consumption over the time (t) of the exercise period and was expressed by the following equation:

$$\text{Oxygen Demand} = \int_0^x \dot{V}O_2 \cdot dt \text{ (where x = exercise time)}$$

Blood lactate concentrations were measured for the corresponding time period and oxygen availability was expressed as being inversely proportion to the blood lactate concentration at that time:

Oxygen Availability = 1/f (where f = [lactate at time x]/[lactate at time 0]

The oxygen demand/Availability ratio (O_2 D/A) was then expressed as:

$$O_2 D/A = [\int_0^X \dot{V}O_2 \cdot dt] \cdot f$$

and compared to the corresponding changes in GH concentrations

v. Statistical Analysis:

As no significant differences were found in any of the measured blood factors between the pre-test (-5 min) and baseline (0 min) samples, all exercise and recovery levels were normalized with respect to the average of the resting levels and compared using a repeated measures ANOVA (Superanova, Abacus Concepts Inc., Berkeley CA, U.S.A.). Post-hoc analysis was completed using a means comparisons of the data. The integrated area under the response curve was calculated using the trapezoidal rule for unequally space X values. In addition, relevant correlations for each hormone were also calculated and compared between the H-C, H-E and C-E protocols (Statview, Abacus Concepts Inc., Berkeley CA, U.S.A.). The level of statistical significance accepted was $p<0.05$. Mean data are presented with SE in figures and SD in table presentations. Solid symbols indicate statistical significance ($p<0.05$) from time 0 min or from compared trial in specific figure. Where error bars do not seem to be presented, the particular error for that graph was too small to be included in that specific figure.

6-12. Results:

i. Subject Characteristics:

The physical characteristics for each of the subjects are presented in Table 6-7.

TABLE 6-7:

Physical characteristics of subjects (n=8).

Characteristic (unit):	Mean ± SD	Range
Age (y)	27.3 ± 6.0	19-31
Body Mass (kg)	80.9 ± 11.9	69.5-104.5
Height (m)	1.8 ± 0.06	1.7-1.8
Body Mass Index (kg/m^2)	25.0 ± 2.1	22.4-28.1
Body Fat (skinfold) (%)	15.0 ± 3.3	9.2-18.8
Body Fat (UWW) (%)	12.9 ± 3.5	8.4-17.4

Data collection for this experiment began in mid-May and was completed by the beginning of September of that same year. There were no significant changes in the subject's fitness level or medical condition during this time (Table 6-8).

TABLE 6-8:

Aerobic testing and workload determinations.

Characteristic (unit):	Mean ± SD	Range
Maximal $\dot{V}O_2$ (Absolute) ($L \cdot min^{-1}$)	3.4 ± 0.5	2.6-4.1
Maximal $\dot{V}O_2$ (Relative) ($mL \cdot kg^{-1} \cdot min^{-1}$)	41.5 ± 5.1	31.5-47.9

Ergometer Workload During Testing (W)	180.6 ± 20.8	150-200
Workload With Water Resistance (W)	255.6 ± 20.8	225-300

ii. Effect of Core Temperature Clamping:

Figures 6-8, 6-9 and 6-10 describe the mean core temperature changes observed during the two control and two exercise conditions. The use of two different water temperatures in this experimental protocol was sufficiently effective for both decreasing and potentiating the core temperature responses to the given aerobic exercise stressor. These figures clearly demonstrate the separation of the core temperature responses during the two identical exercise activities.

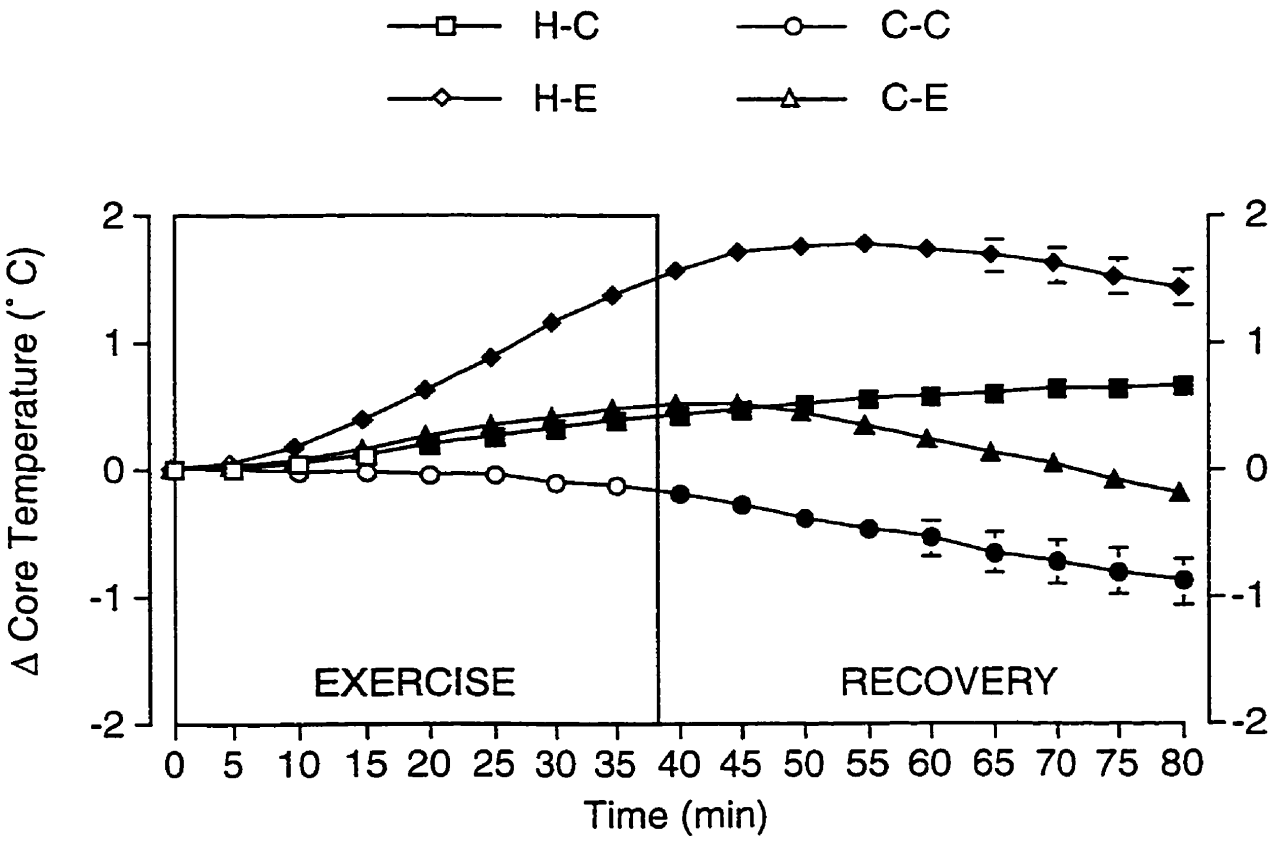

Figure 6-8: *Core temperature responses during immersion trials. Solid symbols indicate significance from time 0 ($p<0.05$).*

The H-E trial became significantly different ($p<0.05$) from the C-E trial and its own baseline values within 10 min after starting the immersion cycling (Figure 6-9). During the H-E trial, the core temperature reached 38.9 ± 0.2° C by the end of the 40 min of exercise, peaking at 39.1 ± 0.4° C at 15 min post-exercise. When cycling in the C-E trial, the increase in core temperature from the start of the exercise period became significant after 15 min. Furthermore, the temperature at the end of exercise period had increased by only 0.52 ± 0.3° C to 37.8 ± 0.3° C, and then proceeded to decline to 37.5 ± 0.3° C during the recovery period. The mean difference between the two exercise trials was almost 1° C during the exercise period and this increased to a difference of almost 1.75° C above the C-E trial during the recovery phase.

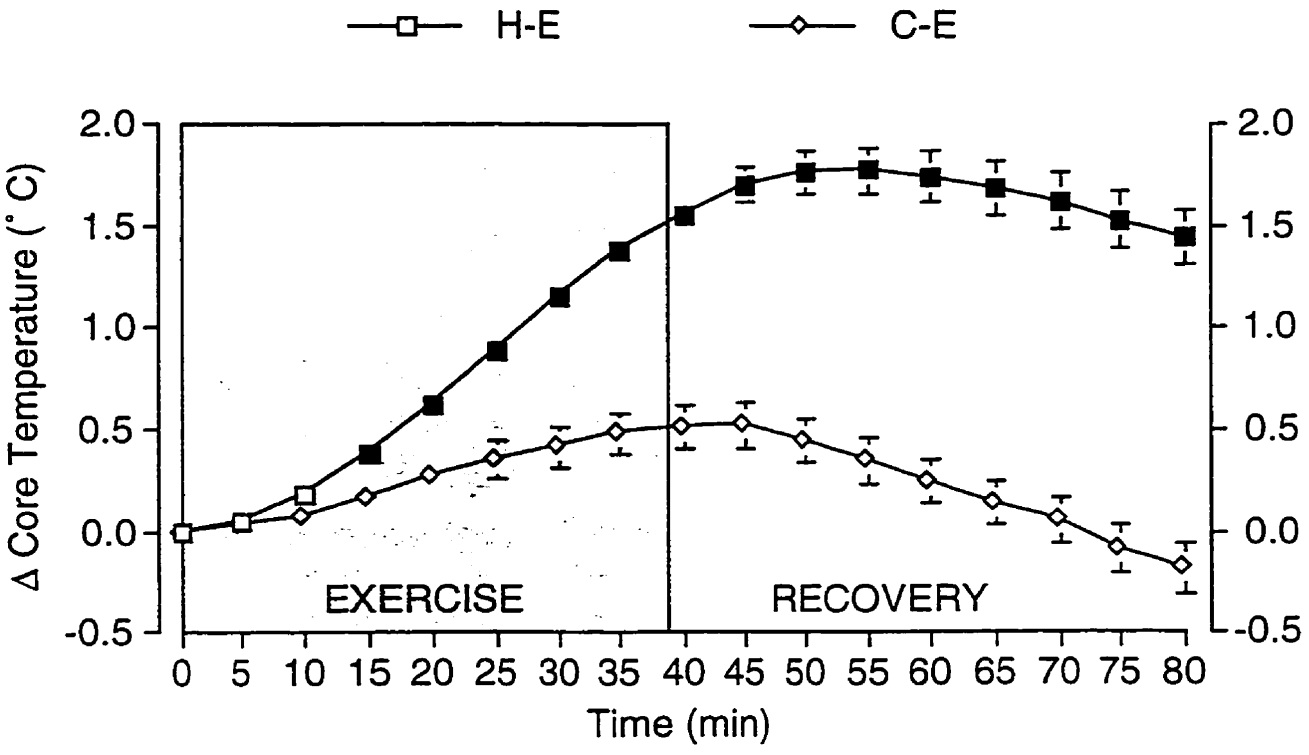

Figure 6-9: *Core temperature responses between H-E and C-E. Solid symbols indicate significance from C-E ($p<0.05$).*

The temperature responses in the H-C condition closely resembled those in the C-E condition. The H-C trial became statistically significant from its baseline values after 15 min of exposure. In comparison to the C-E trial, a similarly equivalent temperature of 37.7 ± 0.3° C was observed after sitting in the H-C condition for 40 min, after which the core temperature increased to 37.9 ± 0.3° C after a total exposure period of 80 min (Figure 6-10). In the C-C condition, the core temperature dropped progressively to 37.0 ± 0.3° C after 40 min and continued to drop during the recovery period to 36.4 ± 0.6° C after a further 40 min of measurements during the recovery period. The H-C trial was different from the C-C values after 20 min of exposure ($p<0.001$).

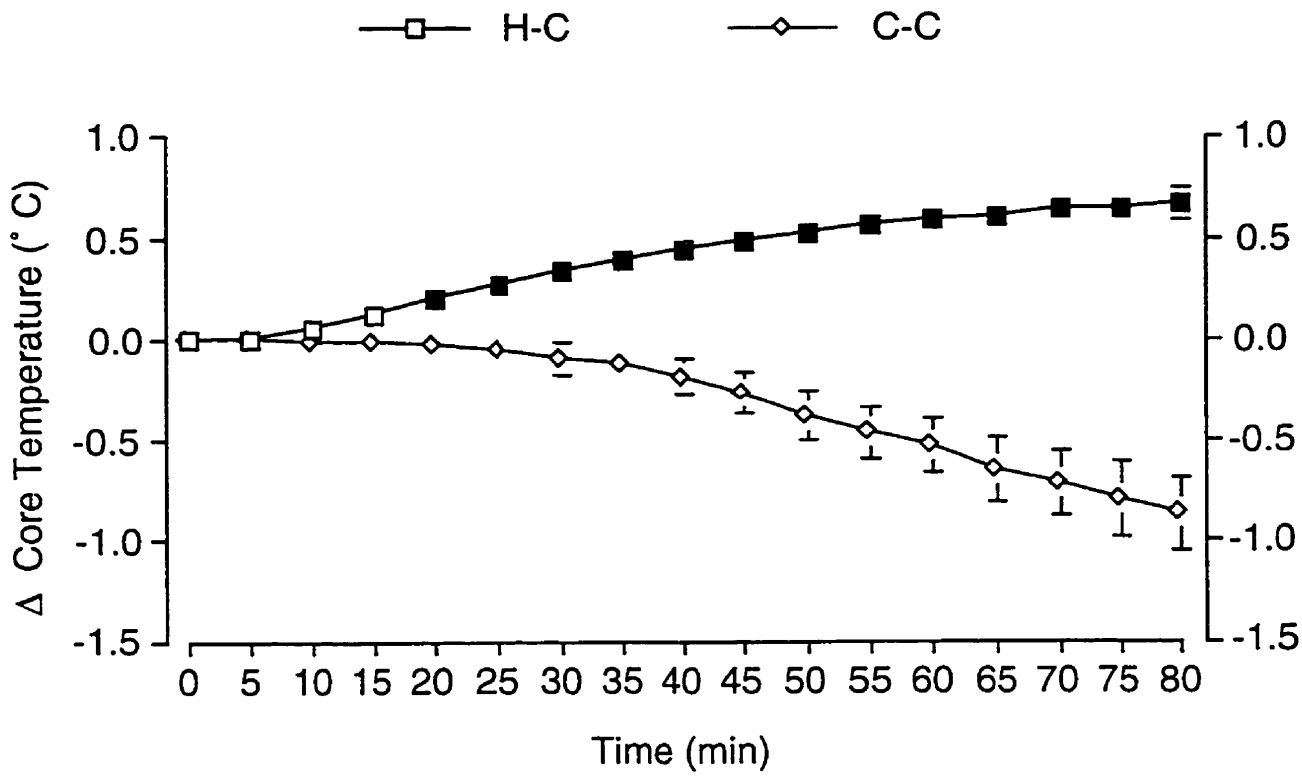

Figure 6-10: *Core temperature responses during H-C and C-C. Solid symbols indicate significance from C-C ($p<0.05$).*

iii. Oxygen Consumption:

The mean oxygen uptake observed during the two control and two exercise conditions is shown in Figure 6-11. During both the exercise and recovery periods, the oxygen consumption did not differ significantly between the warm and the cold conditions. Towards the end of exercise in the H-E period, the workloads of two subjects had to be adjusted downwards in order to allow them to finish the entire 40 min of exercise, resulting in a statistically significant lower value for oxygen consumption at 35 to 40 min during the H-E trial compared to the C-E trial.

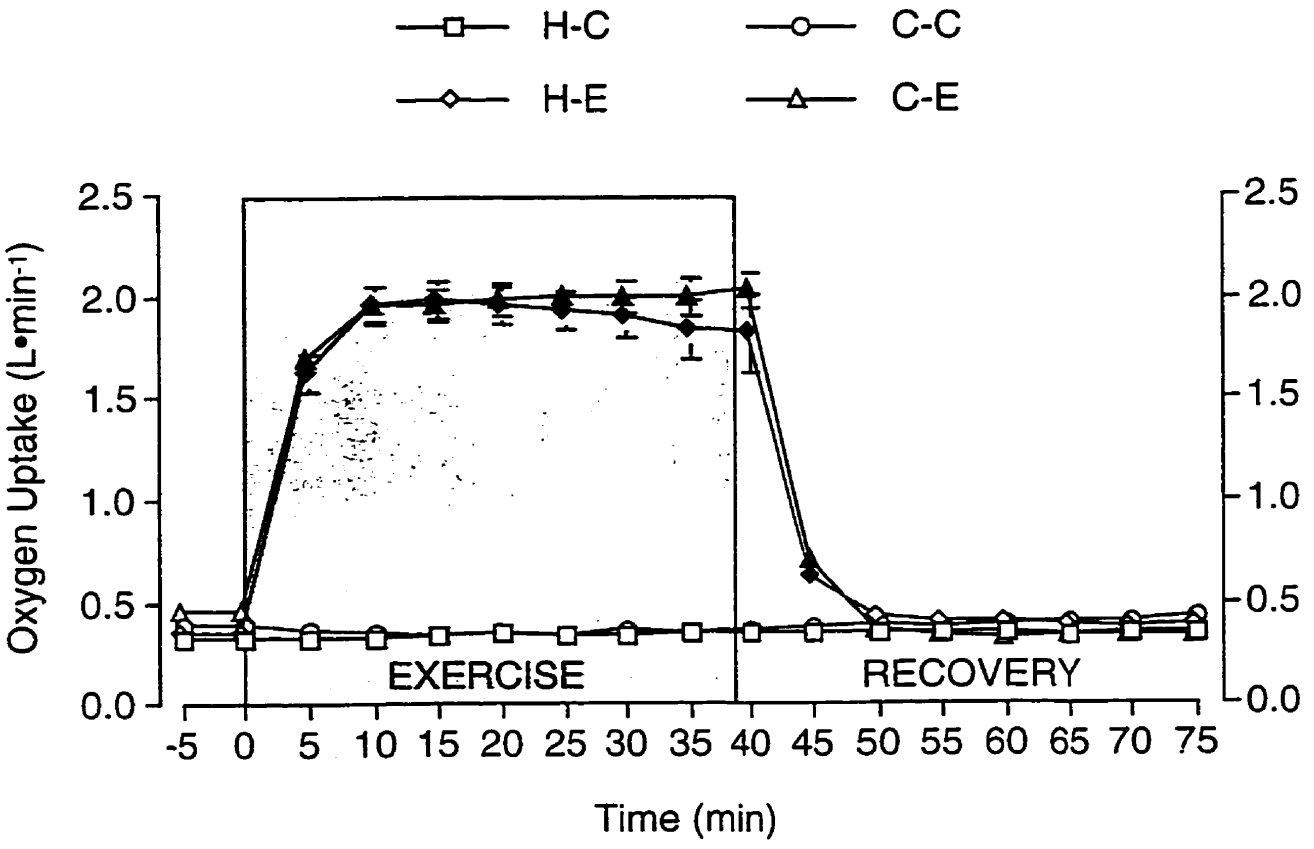

Figure 6-11: *Oxygen consumption during immersion trials. Solid symbols indicate significance from time 0 ($p<0.05$).*

No significant changes in oxygen consumption over the baseline values, or between the two control trials, were observed in either the H-C or C-C trials.

iv. Heart Rate

Figure 6-12 describes the mean heart rate responses observed during the two control and two exercise conditions. While heart rates increased substantially during both of the exercise trials, the H-E condition consistently demonstrated higher heart rates than those levels found during the C-E exposure.

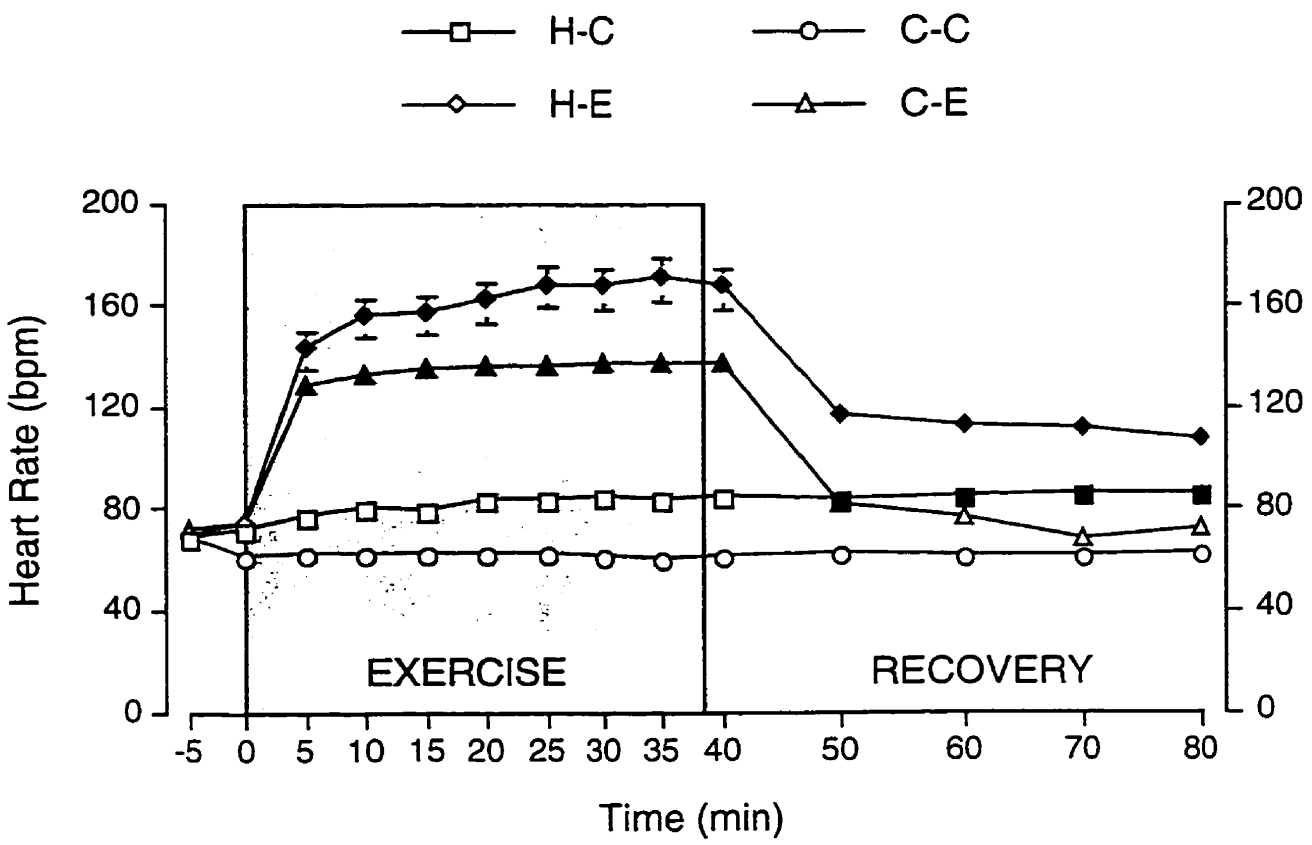

Figure 6-12: *Heart rate responses during immersion trials. Solid symbols indicate significance from time 0 ($p<0.05$).*

There were significant changes in the heart rate responses over the baseline values between the two control trials ($p<0.05$). In this case, the significantly higher heart rate was observed in the H-C condition for subjects remaining at rest, when this was compared to the C-C trials.

v. Growth Hormone (GH):

Figures 6-13, 6-14 and 6-15 describe the mean GH responses observed during the two control and two exercise conditions.

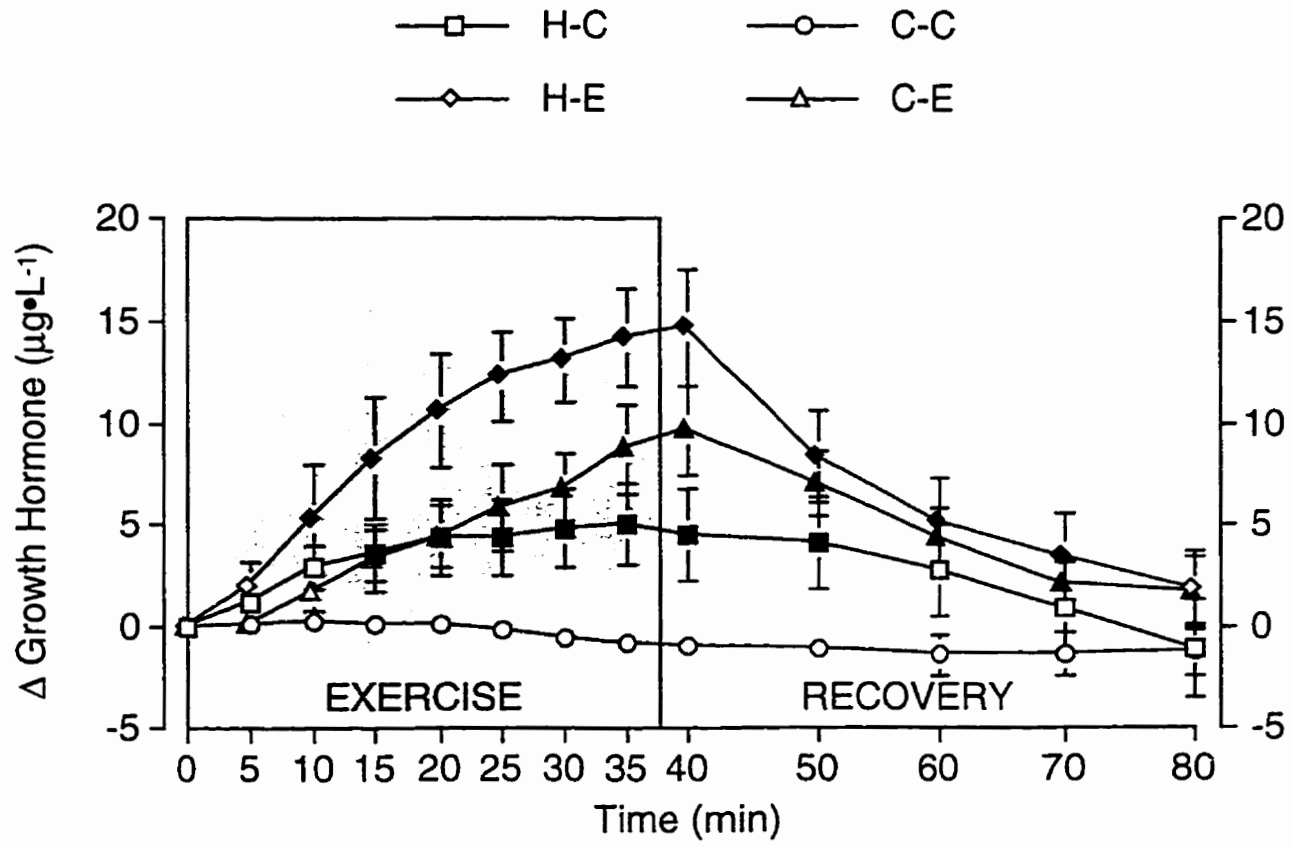

Figure 6-13: *Growth hormone responses during immersion trials. Solid symbols indicate significance from time 0 ($p<0.05$).*

The GH responses to the two exercise trials mirrored each other in pattern, but not in degree (Figure 6-14), being almost twice as great in the H-E as in the C-E trial. When exercising in the hot water, the GH concentration increased by some 15 $\mu g \cdot L^{-1}$ to reach a peak of 19.9 $\pm$ 4.4 $\mu g \cdot L^{-1}$ at the immediate end of exercise. The changes from baseline values were significant from 10 through 80 min of exposure. There was an exponential decline in GH over the next 40 min of recovery to a level of 7.0 $\pm$ 1.7 $\mu g \cdot L^{-1}$. Exercise in the C-E trial produced a much smaller incremental increase in GH levels, to 11.7 $\pm$ 3.0 $\mu g \cdot L^{-1}$, which, while statistically significant from

15 through 60 min, was marginally less than the peak level of 12.1 ± 3.7 $\mu g \cdot L^{-1}$ reached when sitting at a similar core temperature. As Figure 6-14 clearly demonstrates, the H-E trial resulted in GH levels that were statistically different from the values found with the C-E trial from 10 to 40 min of exercise only. There were no differences between the two exercise trials during the 40 min of recovery.

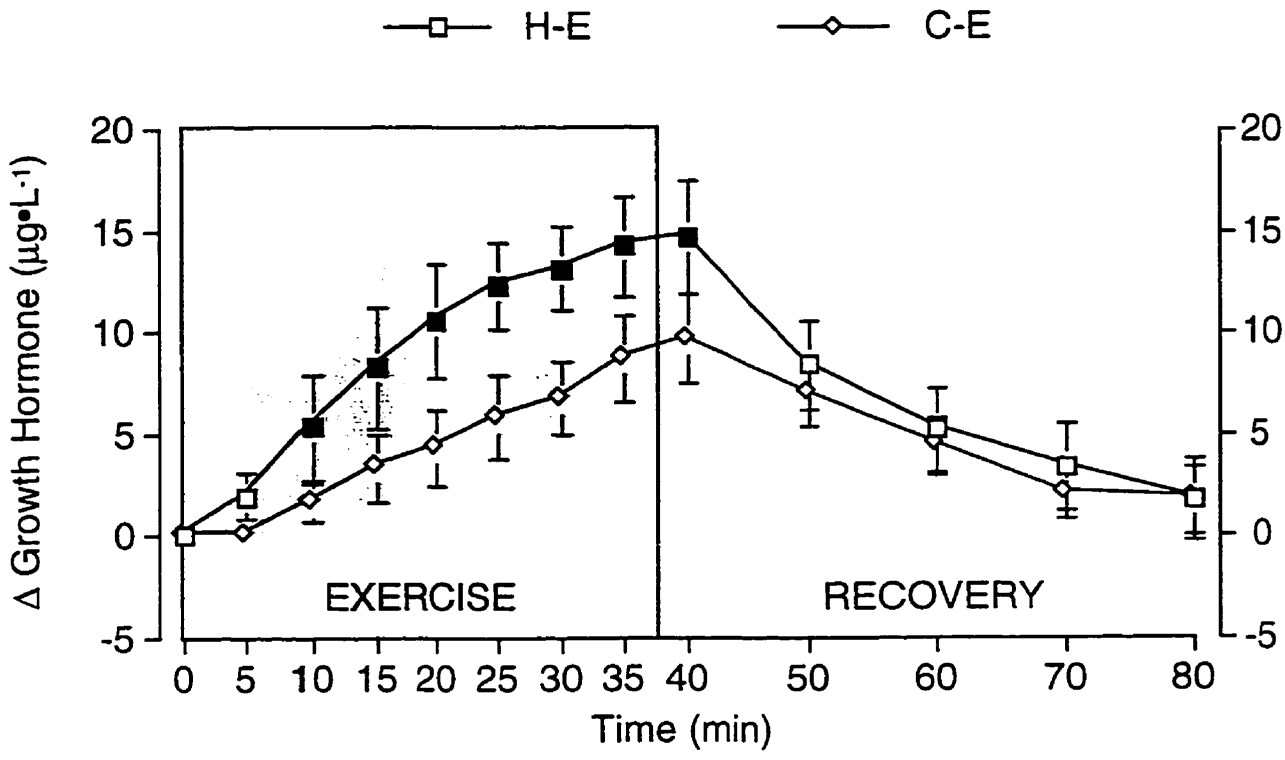

Figure 6-14: *Growth hormone responses during H-E and C-E. Solid symbols indicate significance from C-E ($p<0.05$).*

Figure 6-15 accurately demonstrates the responses of GH while remaining at rest (seated) in the C-C trial. In this case, there was a negative change from baseline in GH concentration from 0.8 ± 0.5 $\mu g \cdot L^{-1}$ at the beginning of the immersion exposure to 0.5 ± 0.3 $\mu g \cdot L^{-1}$ after 80 min of sitting. The H-C trial resulted in a response of circulating GH levels that were significant from the 15 min to the 60 min period of exposure when compared to those levels found in the C-C experimental condition.

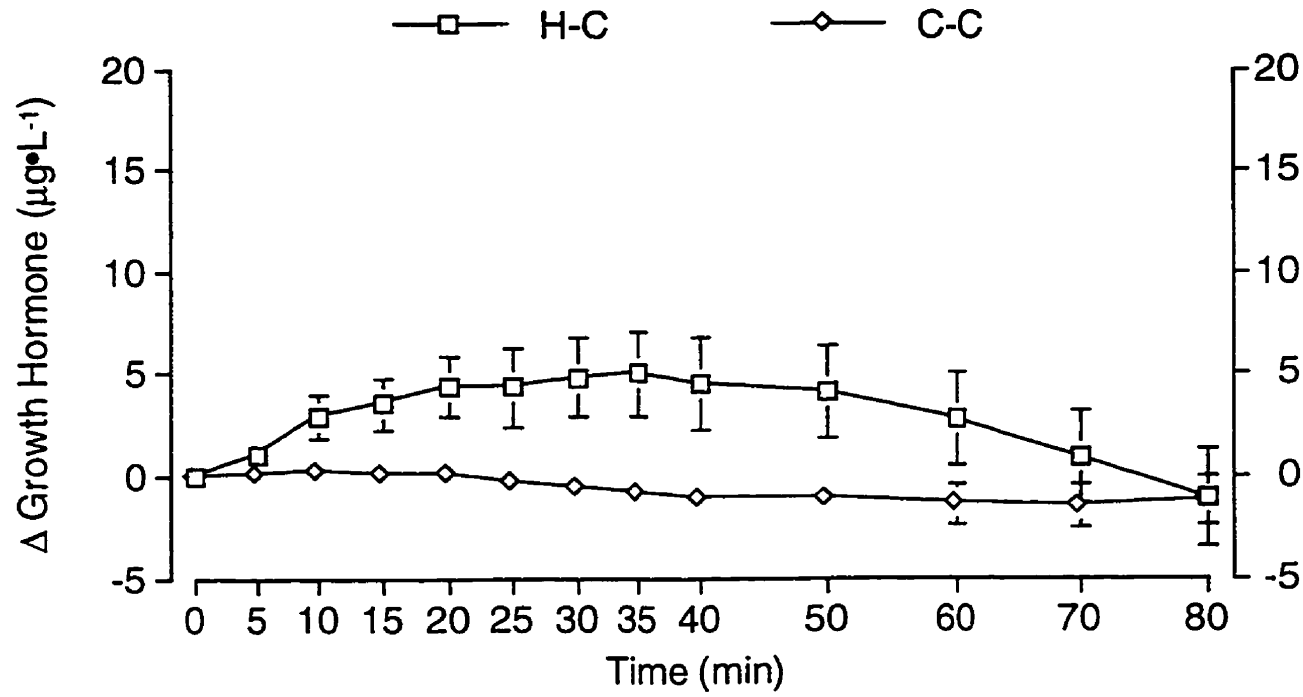

Figure 6-15: *Growth hormone responses during H-C and C-C. Solid symbols indicate significance from C-C ($p<0.05$).*

The total GH area response (80 min) was 487.6 ± 209.5 µg•L^{-1} in H-C, 1062.0 ± 184.8 µg•L^{-1} in H-E, 11.4 ± 6.3 µg•L^{-1} in C-C and 579.2 ± 143.9 µg•L^{-1} in the C-E trial (Figure 6-16).

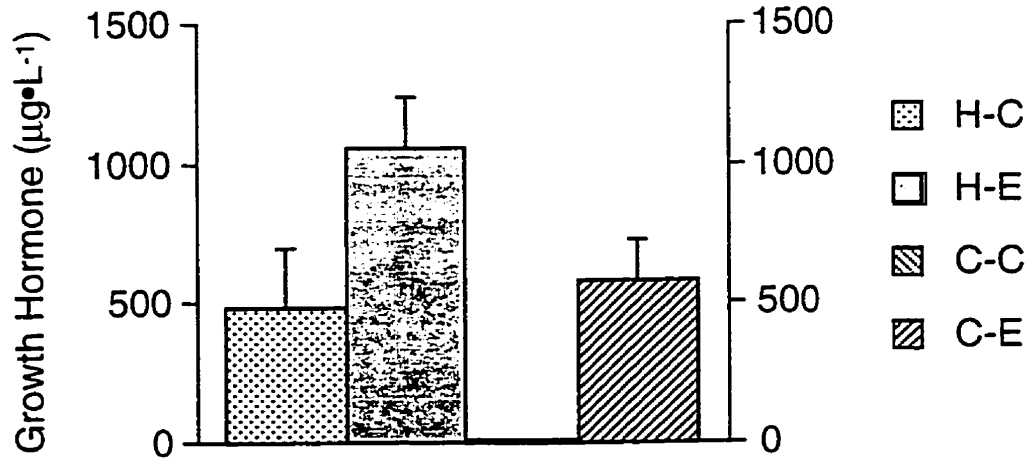

Figure 6-16: *Incremental area of GH in H-C, H-E, C-C and C-E.*

vi. Cortisol:

Figures 6-17, 6-18 and 6-19 demonstrate the mean cortisol responses observed during the two control, H-C and C-C, and the two exercise conditions, H-E and C-E.

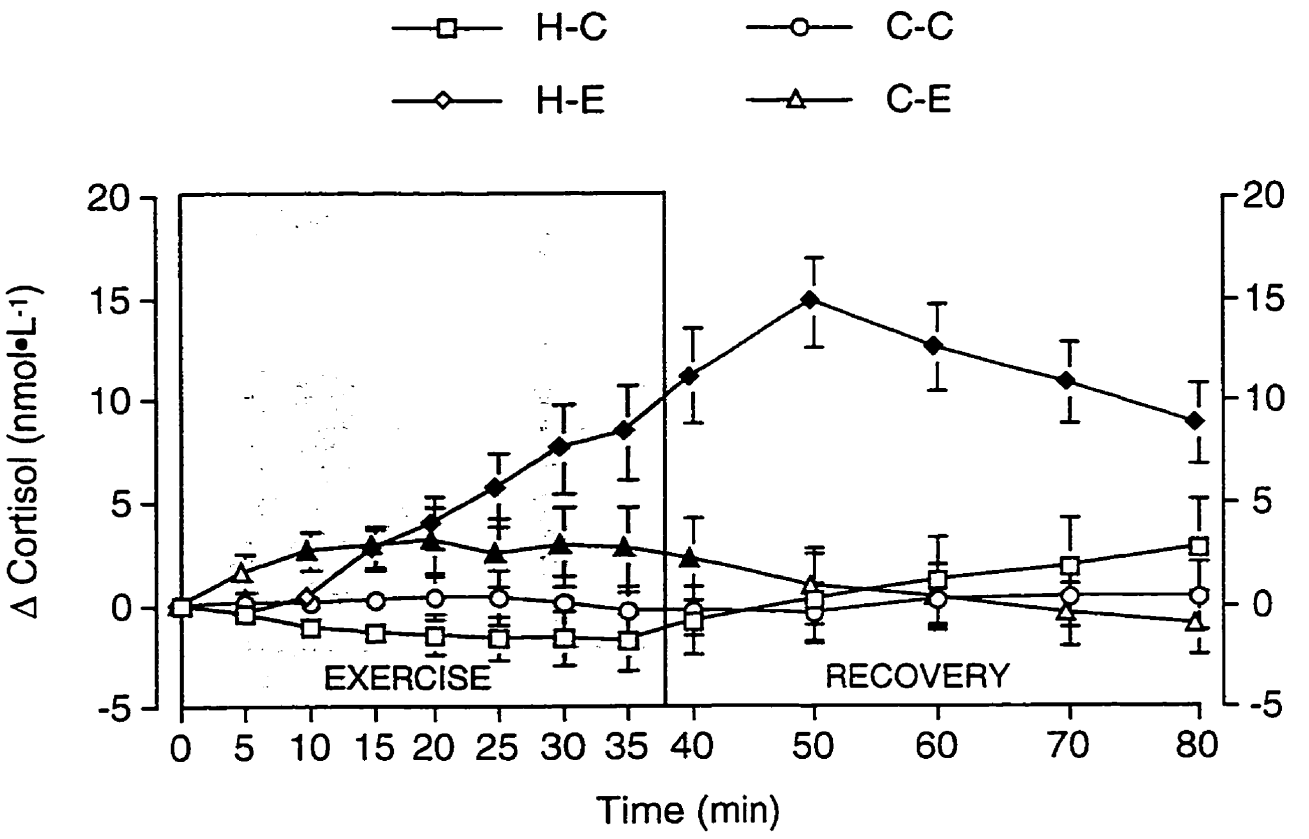

Figure 6-17: Cortisol responses during immersion trials. Solid symbols indicate significance from time 0 ($p<0.05$).

In the H-E condition, cortisol concentrations closely mirrored the changes in core temperature. Exercise in the H-E condition produced a substantial increase of 12 nmol•L^{-1} in cortisol levels by the end of the 40 min exercise period. The cortisol increases reached a peak of 18.6 $\pm$ 2.4 nmol•L^{-1} at the end of the exercise test, with a further increment (total 15 nmol•L^{-1}) to

achieve a peak of 22.2 ± 2.1 nmol•L^{-1} at 10 min following the exercise period. These increases in cortisol levels from baseline were first significant after 15 min of the exercise period. This was followed by a subsequent decline in parallel with the decrease in core temperature. Exercise in the C-E trial produced a small (3.5 ± 1.6 nmol•L^{-1}), but statistically significant, change over baseline in cortisol concentrations during 10 to 40 min of exercise. The cortisol curve for the C-E condition differed from that for the H-E conditions at all times except during the first 15 and 20 min of exercise (Figure 6-18).

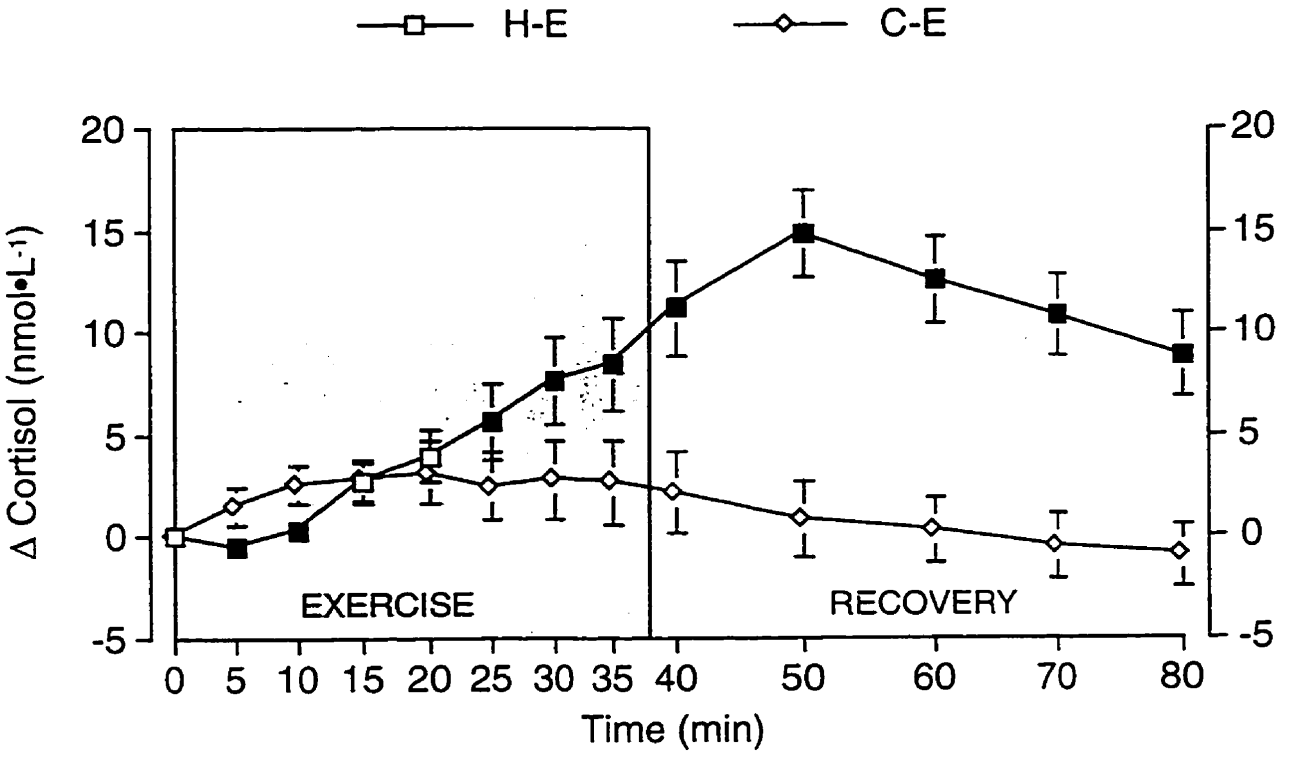

Figure 6-18: Cortisol responses during H-E and C-E. Solid symbols indicate significance from C-E ($p<0.05$).

No significant changes over baseline values were observed in subjects sitting in either of the H-C or C-C environmental conditions. However, a significant difference was observed between the two control trials between the 20 to 30 min periods and the final sample at 80 min (Figure 6-19). In this case, the C-C trial resulted in higher cortisol concentrations.

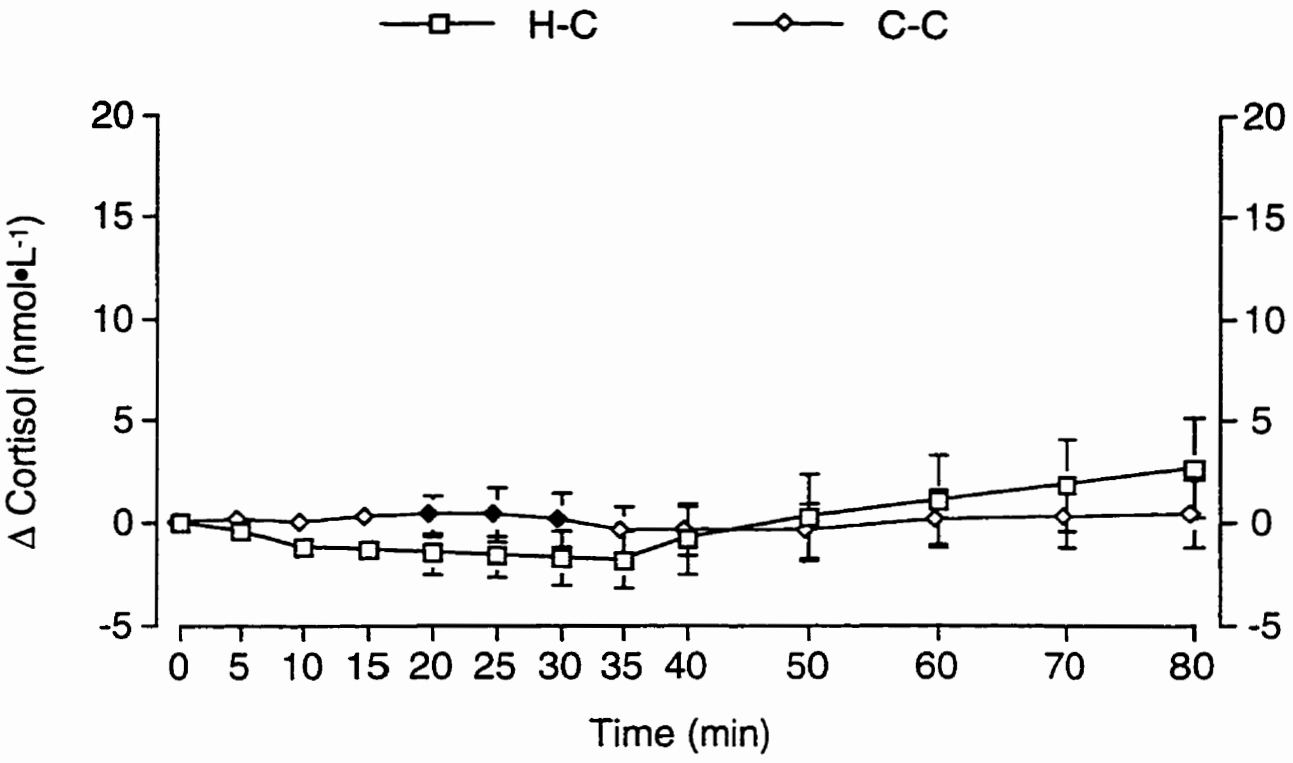

Figure 6-19: *Cortisol responses during H-C and C-C. Solid symbols indicate significance from C-C ($p<0.05$).*

Figure 6-20 shows the total cortisol responses (80 min) for the H-C, H-E, C-C and C-E.

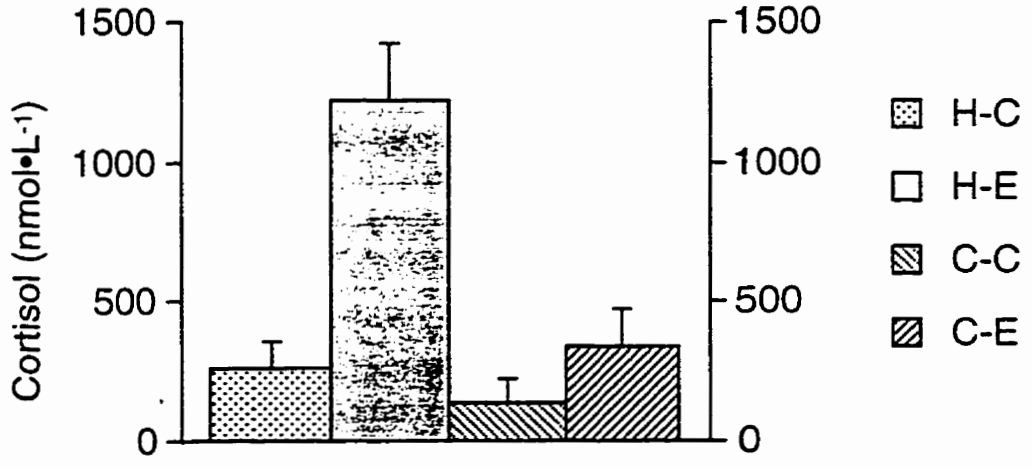

Figure 6-20: *Incremental area of cortisol in H-C, H-E, C-C and C-E.*

The total cortisol response was 258.3 ± 103.9 nmol•L^{-1} in H-C, 1226.1 ± 201.1 nmol•L^{-1} in H-E, 139.1 ± 83.3 nmol•L^{-1} in C-C and 337.4 ± 133.3 nmol•L^{-1} in the C-E trial.

vii. Lactate:

Figure 6-21 describes the mean lactate responses observed during the two control and two exercise conditions.

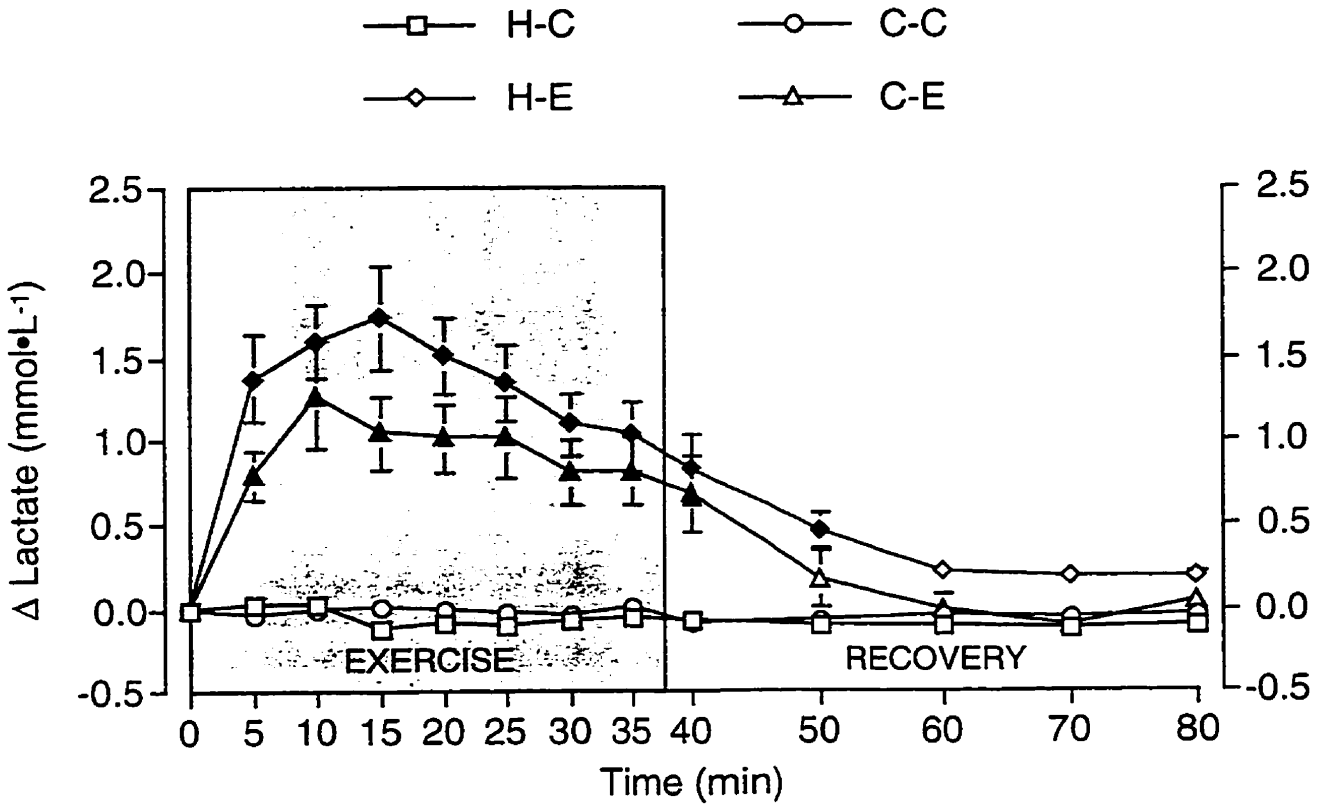

Figure 6-21: *Lactate responses during immersion trials. Solid symbols indicate significance from time 0 ($p<0.05$).*

An immediate statistical increase in lactate levels over baseline values occurred during both of the exercise conditions. During the H-E trial, the lactate levels peaked at 1.96 ± 0.3 mmol•L^{-1} at

15 min into the exercise period and then proceeded to fall during both the exercise period and throughout recovery to a level of 0.4 ± 0.1 mmol•L^{-1}. The H-E lactate responses were significantly more elevated than those of the C-E trial from the 5 min point to the 30 min mark and again at both the 50 and 70 min sampling times. Lactate levels in the C-E trial peaked at the 10 min mark of the exercise challenge and then proceeded to decline from the 1.73 ± 0.3 mmol•L^{-1} to reach a low point of 0.4 ± 0.2 mmol•L^{-1} at the end of the experiment. There were no significant changes in lactate levels over the baseline values during the two control conditions, the H-C or C-C trials (Figure 6-21).

Figure 6-22 demonstrates the total lactate responses (80 min) for the H--C, H-E, C-C and C-E trials.

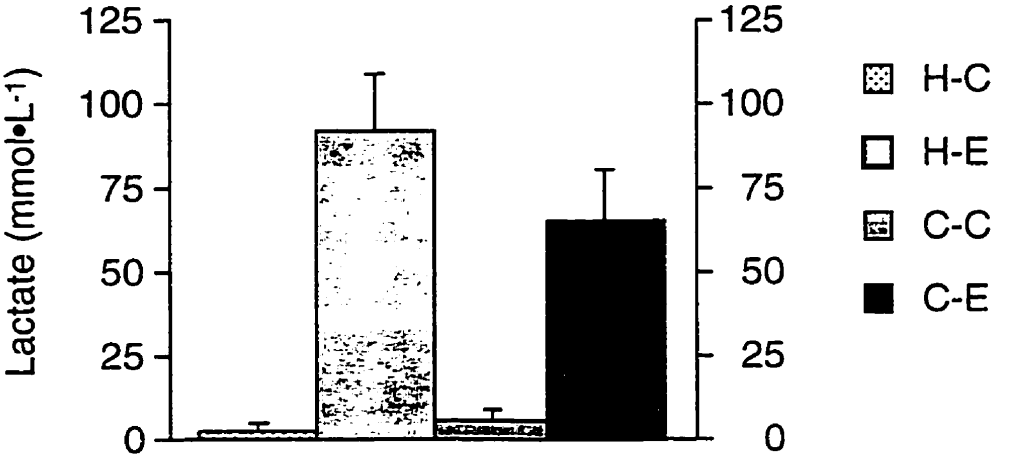

Figure 6-22: *Incremental area of lactate in H-C, H-E, C-C and C-E.*

viii. Correlations:

A correlational analysis for GH and core temperature is presented in Figure 6-23.

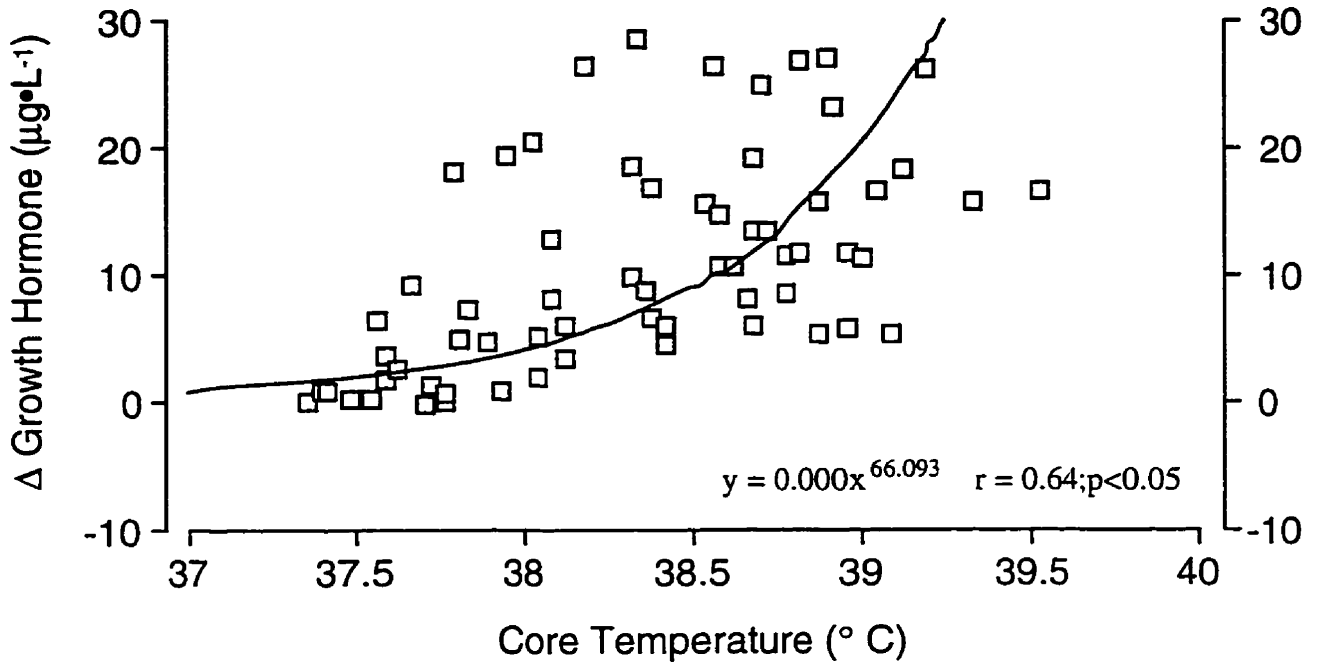

Figure 6-23: *Correlation between growth hormone and core temperature responses in H-E*

A significant power relationship was found between the normalized GH responses and the corresponding core temperature measurements during the 40 min H-E trial ($r=0.64$, $p<0.05$).

A correlational analysis for the GH versus the oxygen demand/availability ratio of H-E is presented in Figure 6-24.

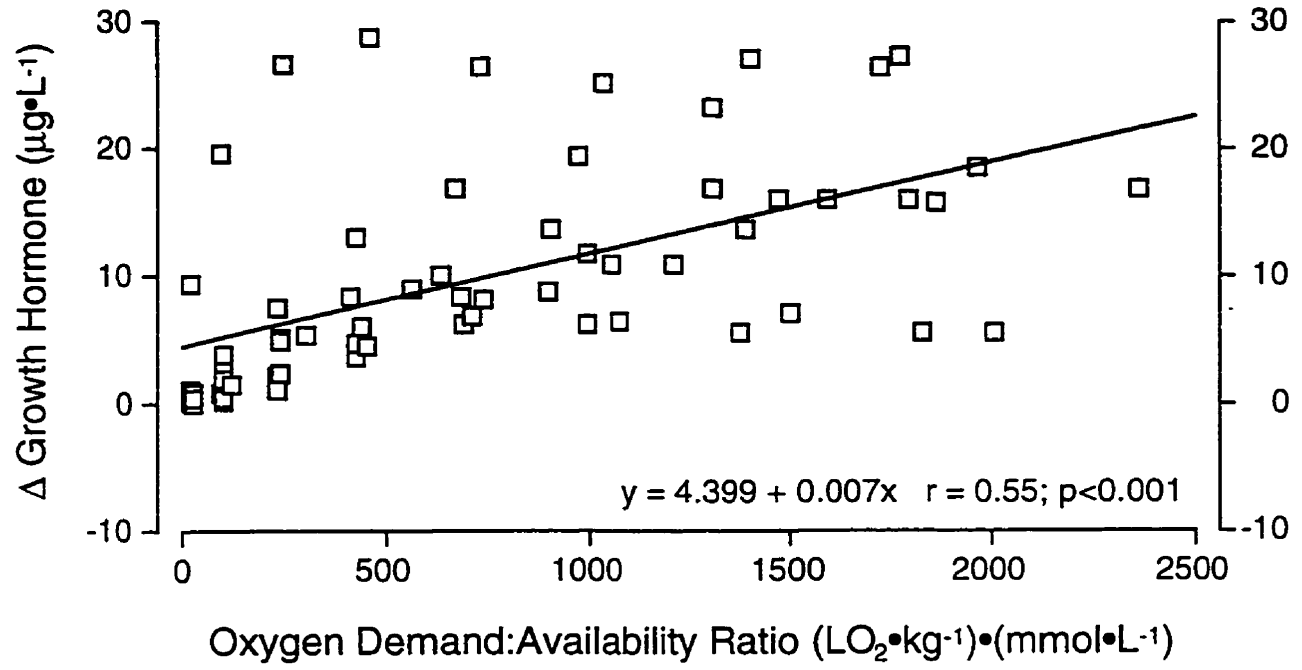

Figure 6-24: *Correlation between growth hormone and oxygen demand/availability ratio in H-E.*

A significant linear relationship between the GH responses and the calculated oxygen demand/availability ratio ($r=0.55$; $p<0.001$) was found in the H-E trial (Figure 6-24).

Figure 6-25 demonstrates that the cortisol responses found in the H-E trial were correlated to the corresponding core temperature changes during the exercise period ($r=0.58$; $p<0.05$).

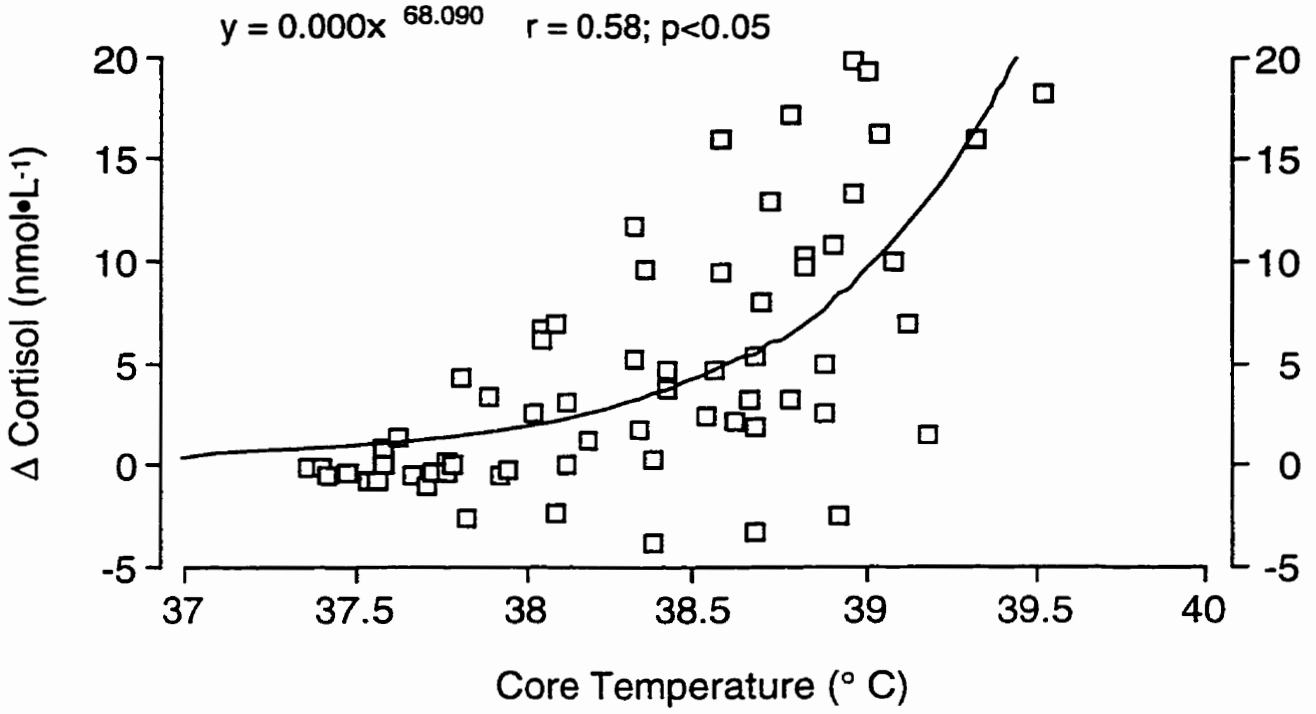

Figure 6-25: *Correlation between cortisol and core temperature responses in H-E.*

6-13. Discussion:

The experimental conditions chosen for the present study seem well-suited to determining the respective contributions of core temperature increases and exercise to the hormonal changes observed during physical activity. When exercising in the H condition, a substantial increase in core temperature occurred, but when exercising, at exactly the same intensity in the C-E trial, only a minor thermal change was detected. Furthermore, by clamping the increase in core temperature during the moderate intensity exercise, we were able to significantly suppress the GH responses which were previously shown to occur during the exercise completed when the core temperature was allowed to increase (H-E).

It is well known that the hypothalamus is the central regulator of both core temperature and pituitary GH secretion. It has also been established that passive heating of the body increases the secretion of GH (Okada et al., 1972b). Thus, the increases in GH secretion commonly observed with exercise could be related to an exercise-induced change in core temperature. We have demonstrated a statistically significant GH suppression during exercise by preventing the core temperature from increasing. Thus, the influence of core temperature on GH secretion during exercise may be related to events occurring at the muscle site or may be related to a thermoregulatory response initiated by the hypothalamus.

For a number of years, the role of lactic acid during exercise was not believed to be important to the enhanced secretion of GH. In 1977, Klimes et al. (1977) studied the infusion of lactic acid into subjects to disrupt the blood acid-base equilibrium, but failed to demonstrate any change in the concentrations of GH. As a result of this, and other work (Sutton et al., 1976), the role of lactate as a possible metabolic regulator of GH secretion was severely challenged and discounted by many (Galbo et al., 1983). However, since intracellular and plasma environments are quite different, plasma lactate changes during infusion studies cannot be assumed to indicate the

intracellular milieu, nor can infused lactate be expected to move into muscular tissues to trigger a response from chemical-metabolic receptors.

An investigation by Luger et al. (1992) disputed the claims of Klimes et al. (1977) and Sutton et al. (1976). In concentrations equivalent to lactate production during exercise in the range of 70-90 % of $\dot{V}O_2$ max, Luger et al. (1992) infused sodium lactate into resting subjects, and demonstrated a significant increase in plasma GH levels. The increases in GH closely paralleled the infusion and increase in circulating lactate levels. Thus, Luger et al. (1992) challenged the view of Klimes et al. (1977) that has pervaded the scientific literature since its publication, and as such, the role of lactate production in the regulation of GH during exercise must be reconsidered.

More recently, a report from Kraemer's group also investigated the role of acid-base alterations in the responses of GH during exercise. In this case, Gordon et al. (1994) compared the GH responses to a 90 s maximal-effort cycle ergometer test under two conditions, alkalosis and control. The control experiment resulted in significant GH elevations that were above resting values during the entire post-exercise recovery period (30 min). In contrast, the alkalotic trial demonstrated GH concentrations that were statistically above baseline levels only at the end of the recovery period. Significant differences in blood pH were also observed in the control and alkalotic conditions, with the more acidic condition resulting in the greatest GH responses. Gordon et al. (1994) concluded that an increase in blood hydrogen ion concentration was responsible for the GH response to the acute high-intensity anaerobic exercise test.

The work of Luger et al. (1992) and Gordon et al. (1994) provides evidence that the lactate responses associated with exercise are involved in the regulation of GH secretion. Our findings show a significantly increased lactate response in the H-E trial that was statistically greater than the lactate responses during the same exercise stimulus completed in the C-E (Figure 6-26).

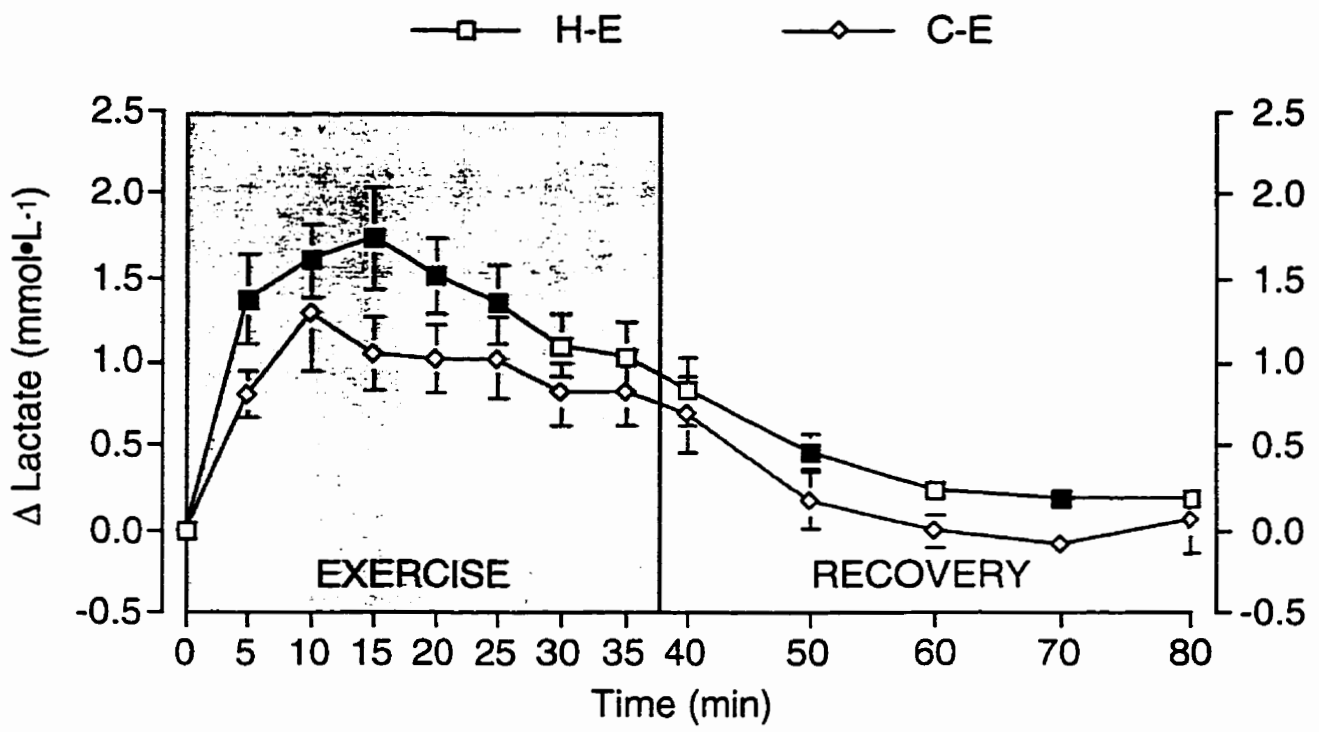

Figure 6-26: *Comparison of lactate responses in H-E and C-E.*

Thus, the varied responses of lactate during the H-E could help explain the differences in the GH responses that were also seen during this investigation. The responses of lactate during exercise in contrasting ambient conditions has been investigated in the past (Claremont et al., 1975; Fink et al., 1975).

Fink et al. (1975) investigated the metabolic responses of intermittent cycling exercise of 138 W, completed during both heat (41° C) and cold (9° C) exposure. Oxygen uptake, lactate and rectal temperature were significantly higher during the exercise conducted under the hot conditions than during the cold conditions. By comparing pre and post-exercise biopsy results, it was demonstrated that muscle glycogen utilization was greater during heat exposure. These results established that exercise in the heat resulted in a greater lactate production and glycogen utilization (Fink et al., 1975), and thus, greater anaerobic metabolism. Fink et al. (1975) speculated that the greater anaerobic activity under the hot conditions was the result of a decreased blood flow to the

active muscle that caused a decrease in oxygen availability to the working muscle. The decrease in blood flow to the exercising muscle would be the result of a decrease in cardiac output and a redistribution of blood flow to assist in heat transfer from the core to the skin. In agreement with Fink et al. (1975) and Claremont et al. (1975), Rowell et al. (1965) and Rowell et al. (1968) demonstrated that greater reductions in liver blood flow occur during exercise at elevated environmental temperatures, resulting in higher circulating levels of lactate.

Claremont et al. (1975) investigated the cardiovascular and metabolic responses to identical exercise at extreme environmental conditions, 0° C and 35° C. In this case, the subjects cycled at a fixed exercise intensity (55 % of their $\dot{V}O_2$ max) for up to 60 min in each of the two conditions. The post-exercise recovery period was completed in a 25° C environmental chamber. During exercise and recovery, oxygen consumption, ventilation, heart rate, lactate levels and muscle, skin and rectal temperatures were recorded. Physical activity in the hot condition produced a significant increase in heart rate, lactate, and in skin, muscle and rectal temperatures over the same exercise in the cold condition. Claremont et al. (1975) suggested that vasodilation and central blood volume shunting would decrease liver blood flow during exercise in the heat, and therefore, account for the increased lactate concentration as a consequence of the decreased hepatic clearance.

Results from this investigation demonstrate a markedly different intracellular milieu in response to the identical physical stress during the two thermal conditions. It has been suggested that the increased rate of glycolysis during exercise in the heat was due to a reduction in oxygen availability as a result of a decrease in muscle blood flow (Claremont et al., 1975; Fink et al., 1975). A reduction in the oxygen delivery to the leg muscles could be the result of a decrease in cardiac output and a redistribution of blood flow to facilitate the heat transfer to the skin. Furthermore, a reduction in hepatic blood flow, and therefore, lactate clearance, would lead to an accumulation of blood lactate. This would explain the subjects' increased feeling of fatigue and the significantly higher lactate responses in the H-E experiments. The studies of Fink et al. (1975)

support the concept that the oxygen supply to the working muscles is reduced during heavy exercise in warm environments, and thereby accelerates the rate of muscle glycogen depletion and lactate accumulation under these conditions. Given that lactate was higher in the H-E, this scenario would lead to the enhancement of the factors relevant to the oxygen demand/availability ratio.

While the role of lactate in the secretion of GH during exercise remains some-what controversial, the dual role of the hypothalamus in core temperature regulation and pituitary GH secretion must also be examined. It is clear from our investigation that the regulation of GH secretion during both physical and heat stress is interrelated to the rise in core temperature during the exercise. The detection of an increase in core temperature by the hypothalamus could be fixed to the absolute level obtained, or to the rate of change experienced, during the thermal stress. Histologically, it appears that the GH-secreting somatotrophs of the anterior pituitary are anatomically and functionally connected with higher, thermoregulatory centres. The preoptic area of the anterior hypothalamus appears to be the major thermosensitive site in homeotherms (Adair, 1974); dopaminergic terminals of the median eminence, while involved in the secretion of prolactin (Tindal, 1978), also arise from the anterior hypothalamus and may be responsible for the differential secretion rates of GH in the case of heat stress.

Buckler (1973) stated that the most important factor in relating the GH response to core temperature was the rate at which the body temperature rises rather than the actual final core temperature achieved. This was indicated by the highly significant correlation between the logarithm of the maximum rate of GH increase and the maximum rate of body temperature increase (Buckler, 1973). Further investigation demonstrated that this correlation held when the rise in body temperature resulted from causes other than exercise. Radomski et al. (1994) demonstrated that the rate of core temperature was biphasic, with an increase during the first 30 min of 0.051° $C \cdot min^{-1}$, followed by a rise of 0.014° $C \cdot min^{-1}$ for the following 60 min. However, in our present investigation, the rate of core temperature increase was 0.04° $C \cdot min^{-1}$ during the initial exercise

phase of the H-E, i.e. the first 40 min. In the C-E trial, where a significantly less GH response was documented, the rate of core temperature increase amounted to only 0.013° C•min^{-1}. In addition, an even smaller GH response was recorded in the H-C trial where the rate of core temperature increase was limited to 0.011° C•min^{-1}. Thus, the differences in rate of temperature change between the H-E, H-C and C-E were concurrent with the differences in GH secretion (Figure 6-27).

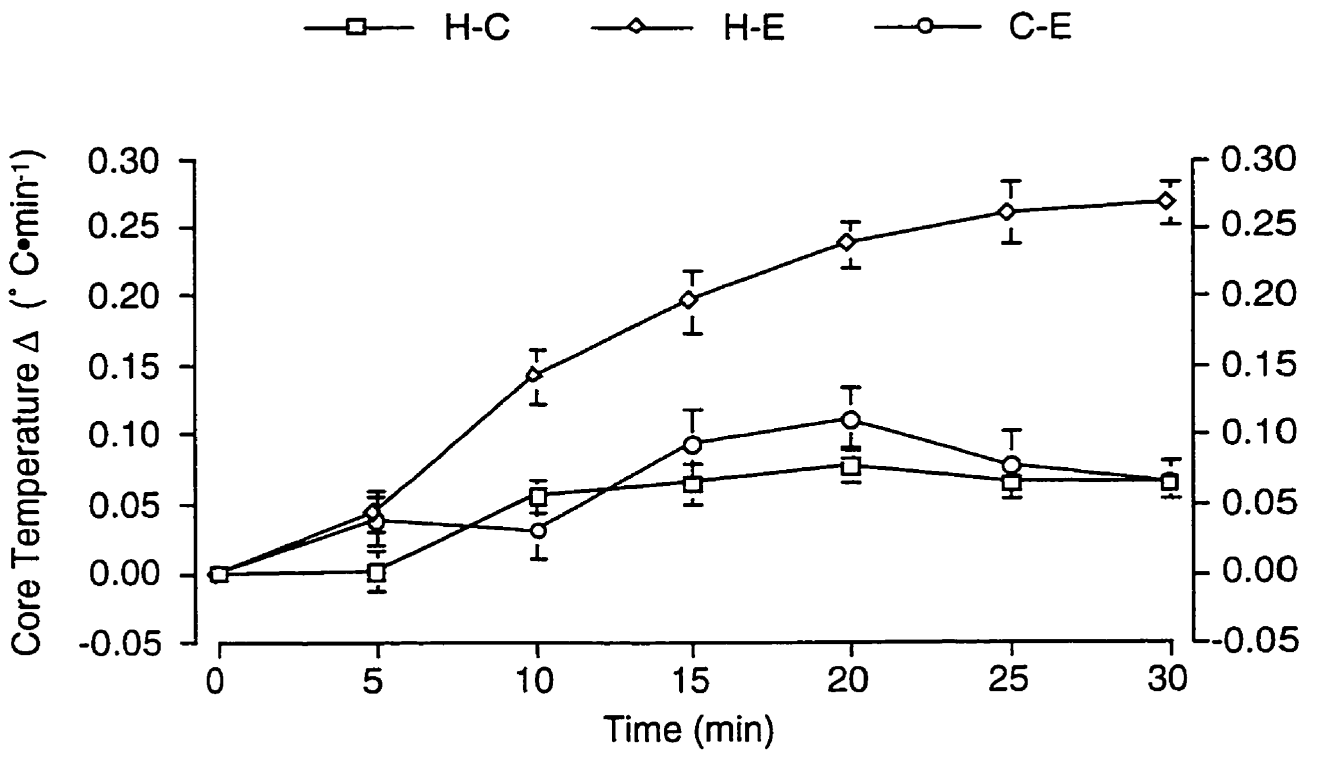

Figure 6-27: Rate of temperature change in H-E, H-C and C-E.

In this investigation, the concentrations of plasma NE and EPI were not determined. It is very likely that exercising in the hot condition, while core temperature increased up to 2 ° C above resting levels, caused considerable discomfort which represented an additional stress to the subject. The analysis of catecholamines would have been useful in this case. Accepting that the possibility of an additional stressor due to the hyperthermia was present, it could be suggested that the enhanced GH secretion was related to an increase in circulating NE secretion, secondary to the heat

stress. This would seem to conform to our understanding of the adrenergic control of GH secretion.

These present findings, that the rate of core temperature increase is important to the subsequent GH response, would agree with the results of both Buckler et al. (1973) and Radomski et al. (1994). It also appears that the initial rate of core temperature increase is very important in determining the magnitude of the overall GH response. There was a three fold rate of temperature change in the H-E trial over that of the C-E, which resulted in a doubling effect on the amount of GH released. In addition, the rate of core temperature change was nearly identical in the C-E and H-C trials, and no statistical differences were found in the GH responses between the two trials.

6-15. Conclusions:

We report here the combined metabolic and hormonal responses to both, exercise and control trials, in young healthy male subjects. Under very closely controlled conditions, the influence of core temperature and exercise on the release of GH, cortisol, lactate and oxygen consumption have been assessed. This investigation has differed from previous studies due to the inclusion of more frequent blood sampling and breath by breath ventilatory gas analysis, use of both a homogenous subject pool, and controlled exercise on an electronic cycle ergometer. Furthermore, the results of these trials have not been contaminated by the "head-start" influence of either the H or C condition as suggested by other investigators. The subjects in our study began to exercise as soon as they were situated in the water tank, and from the results of the blood, metabolic and thermal analysis, were not influenced by the brief exposure prior to commencing the prescribed exercise bout.

The results of this investigation demonstrate the potentiation and suppression of core temperature responses to exercise with the use of immersion cycling at different temperatures, hot

and cold. The rise in serum GH associated with any particular workload has been shown to be reduced by slowing the rate of rise of body temperature by a cold environment, or increased by accelerating this rate artificially (Figure 6-24). In addition, factors that we have previously contributed to the secretion of GH during exercise appear to be intensified during exercise in the heat, and may also be responsible for the difference in GH levels during the H-E and C-E trials. We report a significant correlation between the increase in GH secretion during exercise and the corresponding changes in core temperature values. Furthermore, we also report a significant correlation between the GH levels during the H-E and the parallel oxygen demand/availability ratio.

6-16. Suggestions for future work:

We can only speculate in this investigation, but it is foreseeable that a portion of our results can be explained by an increase in the secretion of GHRH. Leppaluoto et al. (1987) have demonstrated the ability of an acute and short duration of sauna exposure (15 min at 72° C), which allowed for a total increase in core temperature of only 0.2° C, to lead to an increase in GHRH. This was subsequently followed by the enhancement of serum GH levels. Interestingly, the maximum core temperature was achieved at 15 min after the exposure while the maximum levels of GHRH levels were recorded during the first 5 min of the sauna exposure. Growth hormone levels increased to a maximal level during the 15 min period that followed the hot exposure, and then the concentrations preceded to level off following this crest. Later, Leppaluoto et al. (1988) suggested that the decreases in the serum levels of GH and prolactin that occur after the cold exposure reflect a true decrease in their secretion rates which are mediated by hypothalamic inhibitory mechanisms. Given our current findings, and the results of Leppaluoto et al. (1988) and Leppaluoto et al. (1987), it seems quite prudent to reproduce this present study, and to investigate the responses of the releasing hormone, GHRH and the inhibitory hormone, somatostatin.

CHAPTER 7

The Regulation of Growth Hormone Secretion During Different Physiologic States: Influence of Physical Activity, Alpha(α)-Adrenergic Blockade and Core Temperature Clamping.

7-1. Thesis Summary:

The control mechanisms regulating GH secretion by the anterior pituitary have not been completely elucidated. Physiological factors involved in GH secretion include: gonadal steroids, age, body composition, nutritional state, exercise, body temperature, and the sleep-wake cycle. Not all types of exercises result in the increased secretion of GH. At present, the exercise variables that have been investigated in this response include intensity, duration, total work output, physical fitness, continuous versus intermittent, the type of exercise (aerobic versus anaerobic), muscle volume, speed of muscle contraction, acid-base balance, hypoxia, gender, and nutritional state. Understanding the regulation of GH secretion during physical activity is important, since this hormone not only regulates growth, but also protein synthesis, partitioning of nutrients and mobilization and storage of fuel, and body composition. Its importance in post-pubertal homeostasis is only now becoming clear.

Growth hormone secretion is mainly regulated by the interplay of two hypothalamic hormones, GH-releasing hormone (GHRH), and somatostatin (SS) which inhibits GH release from the pituitary. The regulation of these two GH controlling hormones is managed through a number of neurons interacting with the neurosecretory cells of the hypothalamus. While a number of other neurosecretory substances have been implicated in the regulation of the regulatory hormones, such as serotonin, galanin, γ-aminobutyric acid and opioids, the major neurotransmitter systems involved in the secretion of GHRH and SS from the hypothalamus are:

1. Adrenergic - (norepinephrine and/or epinephrine)
2. Cholinergic - (acetylcholine)
3. Dopaminergic - (dopamine)

It is obvious that the neural regulation of the GH releasing factors is accomplished through an interaction of the prevalent neurotransmitters within the hypothalamus (Figure 7-1). Each system involved in this regulation is dependent upon a number of specific stimuli. Furthermore, each system seems to be capable of influencing each other to adjust the overall activation level.

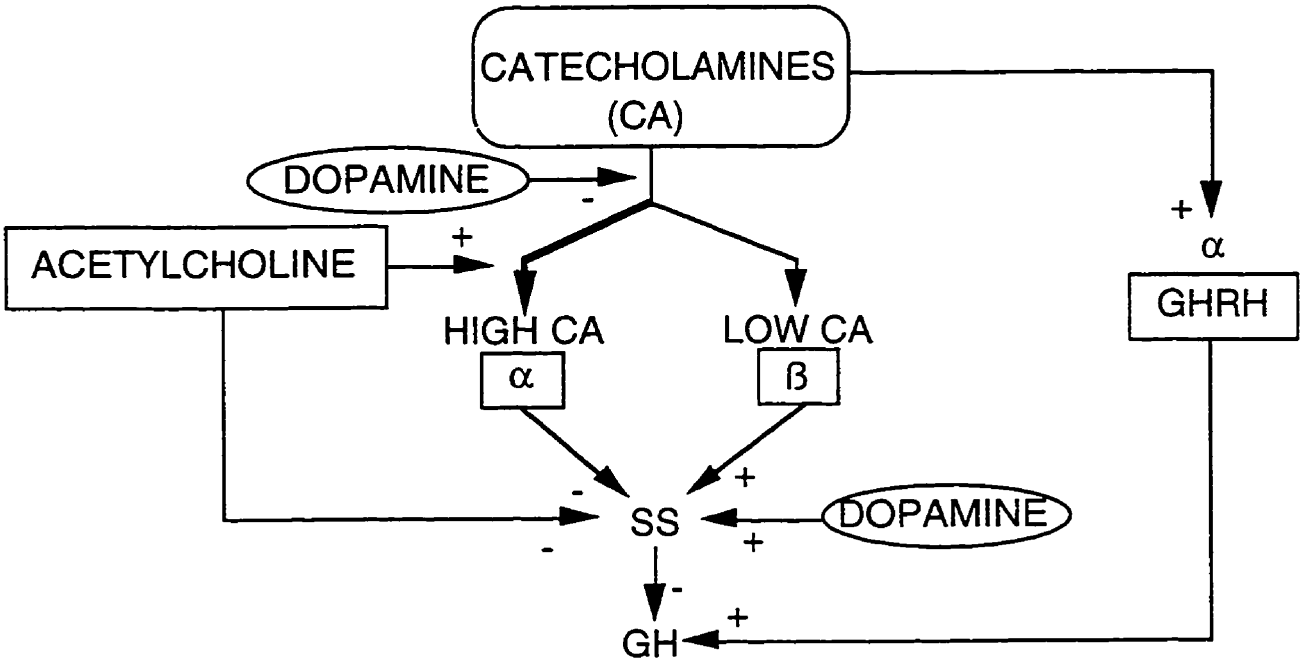

Figure 7-1: Neural regulation of growth hormone secretion.

Thus, the main component of the GH controlling system appears to be norepinephrine (NE). While the neurotransmitter systems are interrelated in the regulation of GH, so too are the releasing factors.

Recent evidence suggests that SS interacts with GHRH neurons to inhibit their activity, as well as antagonizing GHRH binding to its receptors and GH gene transcription and GH release (Figure 7-2). In this case, the systems' components interact with each other to bring about a controlled and rather speedy response to a stimulus.

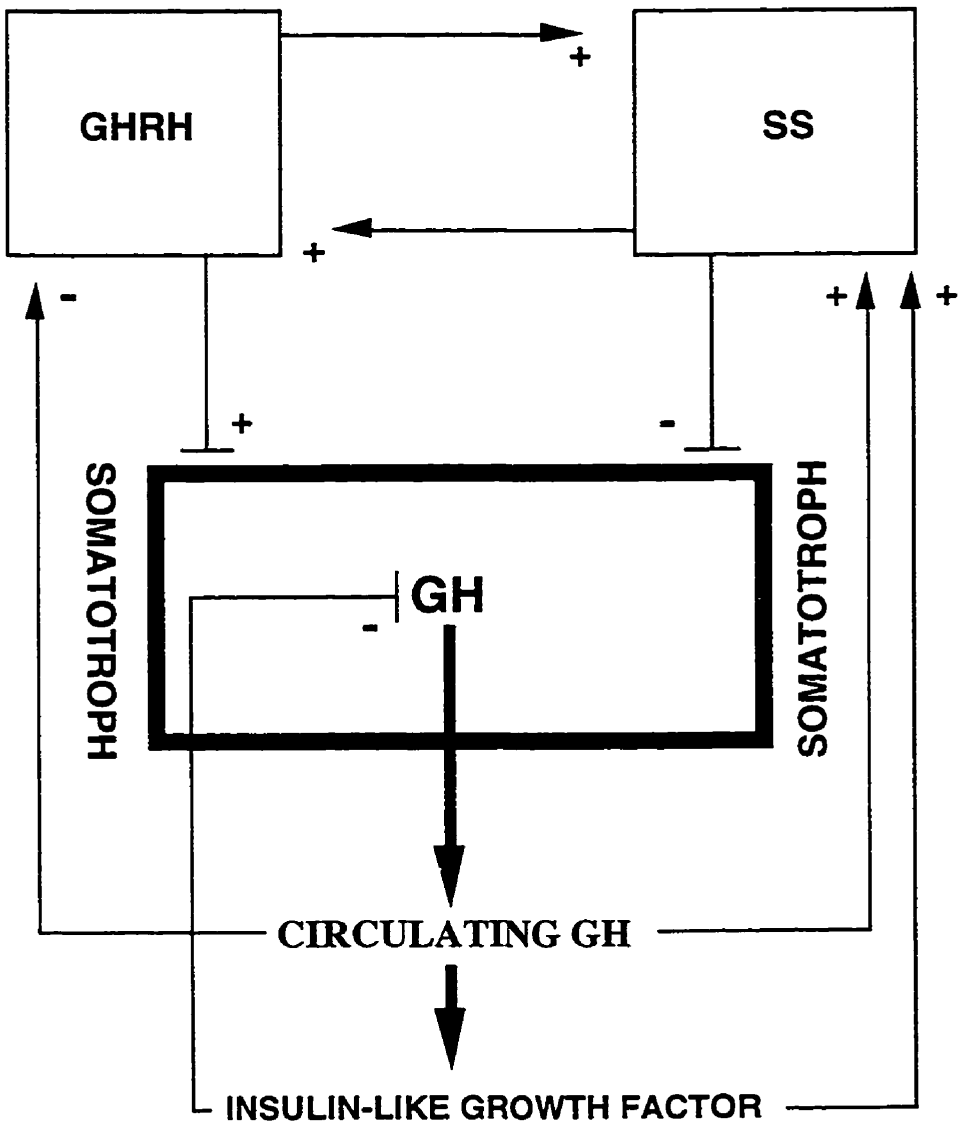

Figure 7-2: Feedback regulation of growth hormone secretion.

(Adapted from Devesa et al., 1992)

In this system, the stimulation of GHRH-producing cells in the hypothalamus brings about an increase in the production and secretion of GHRH which travels via the hypothalamic-pituitary portal system to interact with cells of the anterior pituitary (Devesa et al., 1992). As the GHRH is released from the hypothalamic cells, it interacts with SS-producing cells in order to stimulate the production and increase the secretion of SS. This feedback loop is also observed in the case of SS, with its release leading to the increased release of GHRH. As the two hypothalamic hormones reach the anterior pituitary cells, they interact with their target cells, the somatotrophs. Secondary to GHRH stimulation, the somatotrophs bring about an increase in the production and secretion of GH. The increased levels of GH have dual feedback destinations; they inhibit the secretion of more GHRH and stimulate the secretion of SS. Concurrent to its own down-regulation, the circulating GH levels begin to increase the production of Insulin-like Growth Factor (IGF), which helps carry out many of the actions of GH. This secondary mediator has a direct effect on decreasing the secretion of GH from the pituitary and increasing the hypothalamic release of SS (Devesa et al., 1992). Thus, there is a large amount of physiologic interaction between GHRH, SS, GH and IGF.

While the importance of the sympathetic catecholaminergic nervous system to the secretion of GH cannot be over stated, a number of other hormonal systems are involved in the secretion of GH (Figure 7-3). In this case, the influence of testosterone, and its aromatized product estrogen, glucocorticoids and thyroid hormone, all act to influence the secretion of GH from the pituitary. The role of testosterone and estrogen appears to be to increase the adrenergic activation of catecholamine fibers in the hypothalamic area. This brings about a modification of the adrenergic output, which either inhibits or stimulates SS and GHRH secretion, depending on the concentration levels (Devesa et al., 1992). Both the thyroid hormone and glucocorticoids have facilitatory roles on the GHRH and SS hypothalamic cells, the pituitary receptors of these releasing hormones, or the actual exocytotic release of GH.

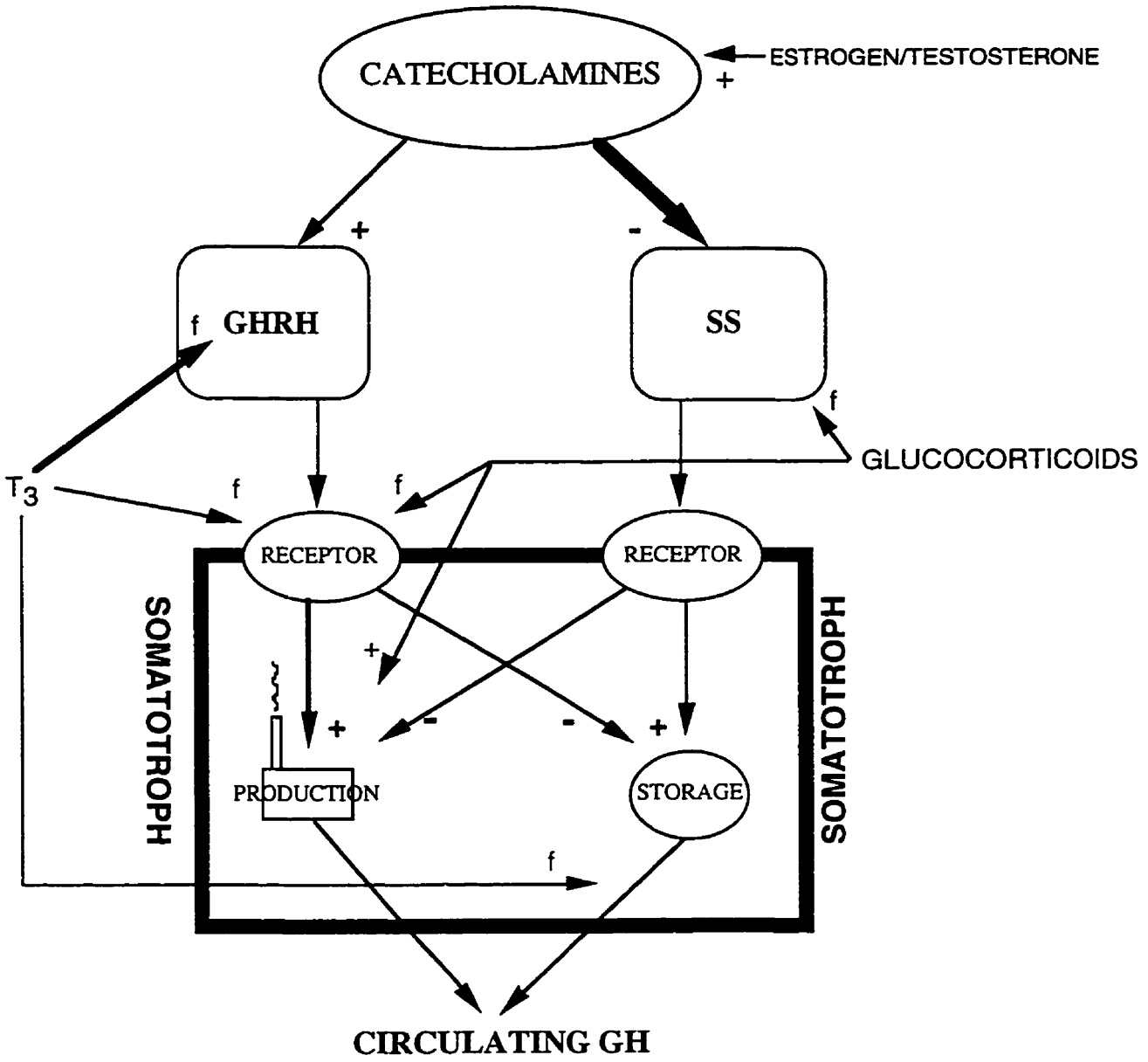

Figure 7-3: Hormonal input into GH regulation.

(Adapted from Devesa et al., 1992)

Thus, the normal control of GH secretion is a complicated interaction of many factors. The control of GH secretion during exercise is further complicated by bringing a host of other factors into the regulatory picture. A number of investigators have forwarded several theories relating to the control of GH secretion during physical activity (Deschenes et al., 1991; Galbo et al., 1983;

VanHelder et al., 1987). These theories have included the oxygen demand/availability ratio, the initial oxygen deficit, the concentration of lactate or lactic acid, the level of sympathetic output and a motor cortex-induced central command hypothesis. It has been our goal to further investigate this physiological mechanism(s) and to offer a hypothesis on its regulation.

The objectives of this thesis were to elucidate further the control of GH secretion during various types of exercise. The objectives of this thesis were as follows:

i. Determine the influence of load and frequency characteristics of resistance exercise protocols on GH secretion.

ii. Determine the influence of different muscle volume characteristics during resistance exercises on the subsequent secretion of GH.

iii. Determine the contribution of the alpha-adrenergic nervous system to the GH secretion responses during resistance exercise protocols.

iv. Determine the contribution of core temperature enhancement and suppression on the subsequent secretion of GH during moderate aerobic exercise.

In Chapter 3, we investigated heavy resistance exercise. Figure 7-4 demonstrates the GH responses to two intermittent resistance exercise protocols of equal total external work output, duration and work-rest intervals. The two protocols of leg extension exercises were designed by varying the load and frequency of movement characteristics. Repetitions in the lighter load and faster exercise protocol (L-F) produced greater GH responses when compared to the heavier load and slower exercise protocol (H-S). Furthermore, the L-F protocol also had the greatest NE, EPI and lactate responses when compared to the H-S trial.

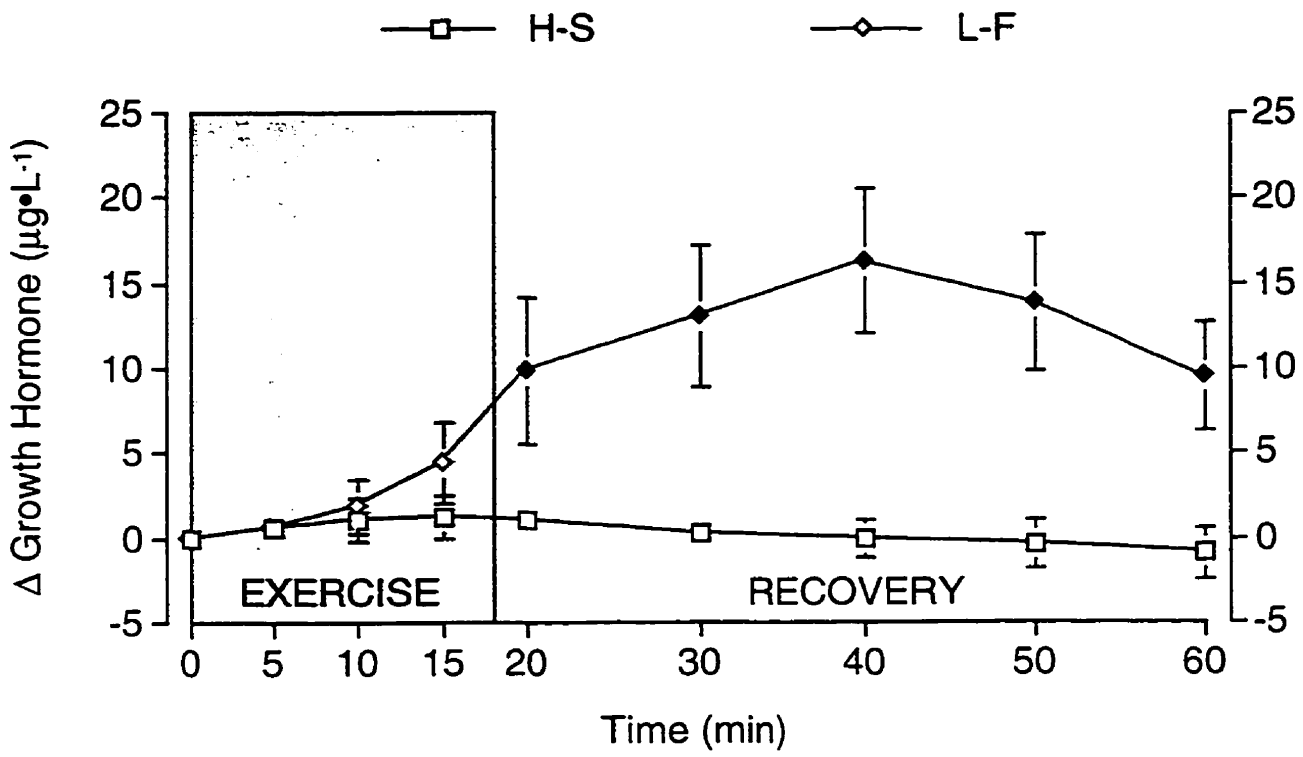

Figure 7-4: Growth hormone responses to H-S and L-F protocols.

The results of this study suggested that the plasma GH response during resistance exercise were related to the speed of movement and the work-rest interval characteristics within each exercise set. The results also suggested that the adrenergic nervous system was involved in the regulation of GH as a significant correlation between the responses of GH and NE was demonstrated in the L-F protocol. In contrast, the H-S protocol failed to respond in either GH, NE, EPI or lactate during both the exercise and recovery periods. The major finding of this study was that velocity of muscle contraction as well as the interval between each repetition were important determinants of GH stimulation. We speculated that the short duration of inactivity between repetitions in the H-S protocol was sufficient to restore blood flow to the exercising muscles. Conversely, the repetitions of the L-F were completed in a consecutive manner, which would have lacked the re-perfusion characteristics of the H-S protocol, and therefore, greater increases in the products of anaerobic glycolysis were detected. We suggested that these products,

be they an increase in lactate, H^+ or a decrease in pH or PO_2, were detected by the exercising tissues. These chemical-metabolic receptors would then respond, through activation of the adrenergic nervous system, with an eventual increase in GH secretion.

In Chapter 4, using the same type of study design, we investigated arm flexion exercise. Figure 7-5 demonstrates the GH responses to two resistance exercise protocols of arm flexion movements. Like the first experiment, the protocols were of equal total external work output, duration and work-rest intervals, but had varying load and frequency of movement characteristics. As was the case in the previous experiments, the lighter load and higher repetition exercise protocol (L-F) produced the greatest GH responses over the responses of the heavier loaded and lower repetition exercise protocol (H-S).

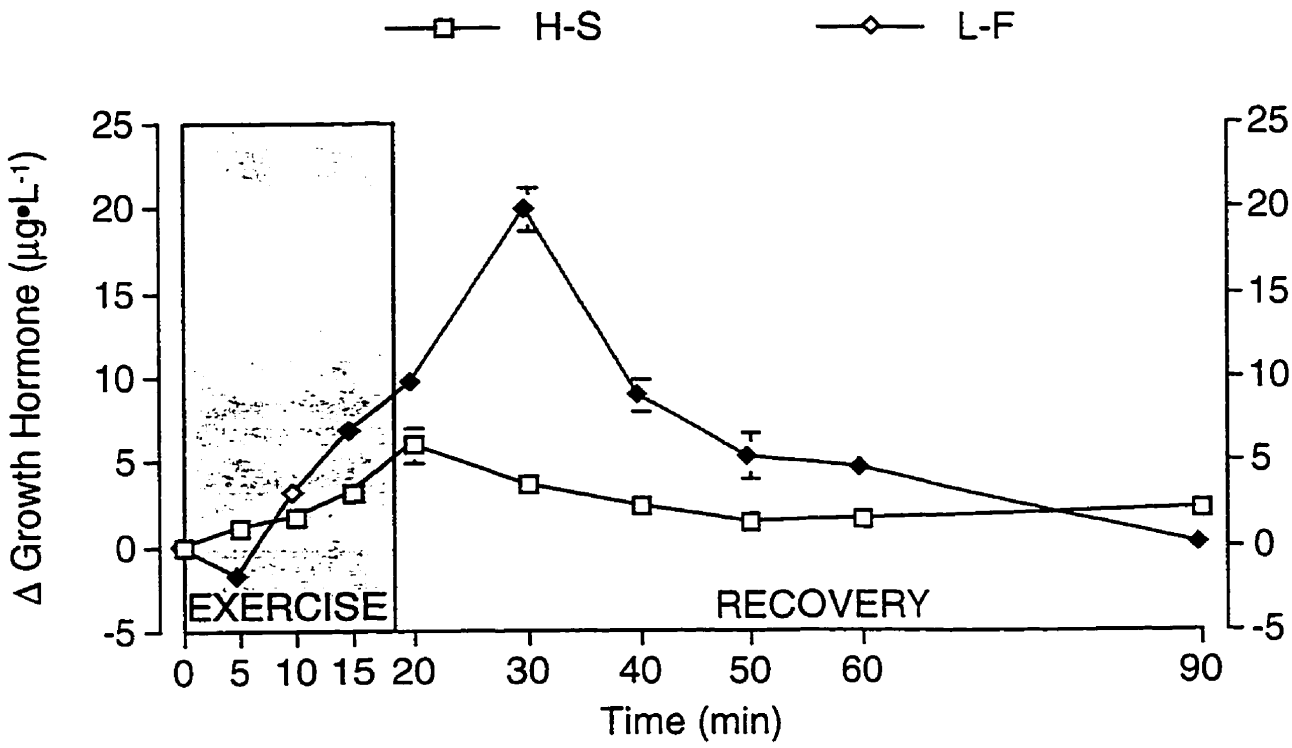

Figure 7-5: Growth hormone responses to H-S and L-F.

The plasma GH responses during the flexion resistance exercises were related to the speed with which the flexion movement was completed. The high correlation between the lactate and GH responses during the exercise period suggested a possible regulatory mechanism for the hormonal responses observed during the resistance exercise. Furthermore, the results of this investigation were compared to the findings in the same group of subjects performing 7 sets of concentric-only leg extension exercises (Chapter 3). In this case, the arm exercise produced a response of significantly more GH and lactate than the observations made from the identical exercise type, duration and work-rest interval (30-150 s), but completed using leg extensions. While we did not analyze the responses of the adrenergic nervous system, we did establish that significantly different metabolic and hormonal responses occurred between the two studies. The issue of blood flow to the exercising limbs was also discussed in this study; in this case, the exercise was conducted at a level at least equal to that of the heart, and possibly more elevated than the level of the heart. These factors would make the possibility of ischemic exercise more likely. Given these characteristics, we reported higher GH and lactate levels in this particular exercise than the same subjects completing the leg extensions of Chapter 3.

In Chapter 5, we investigated the influence of the adrenergic nervous system to the responses of GH during resistance exercise by employing an α-adrenergic block using phentolamine. The results of previous investigations had suggested that NE, acting through the stimulation of α-adrenergic receptors, either centrally or peripherally, was involved in a cascade that regulates the release of pituitary GH during various types of resistance exercise. Like Chapters 3 and 4, the hormonal responses to the two resistance exercise protocols of equal total external work output and work-rest intervals were compared. The two resistance exercise protocols differed in the load and frequency characteristics only. We hypothesized that the use of the α-adrenergic blockade would not change the pattern of GH responses to the two exercise protocols. Figure 7-6 describes the GH responses to the two resistance exercise protocols completed with and without α-adrenergic block.

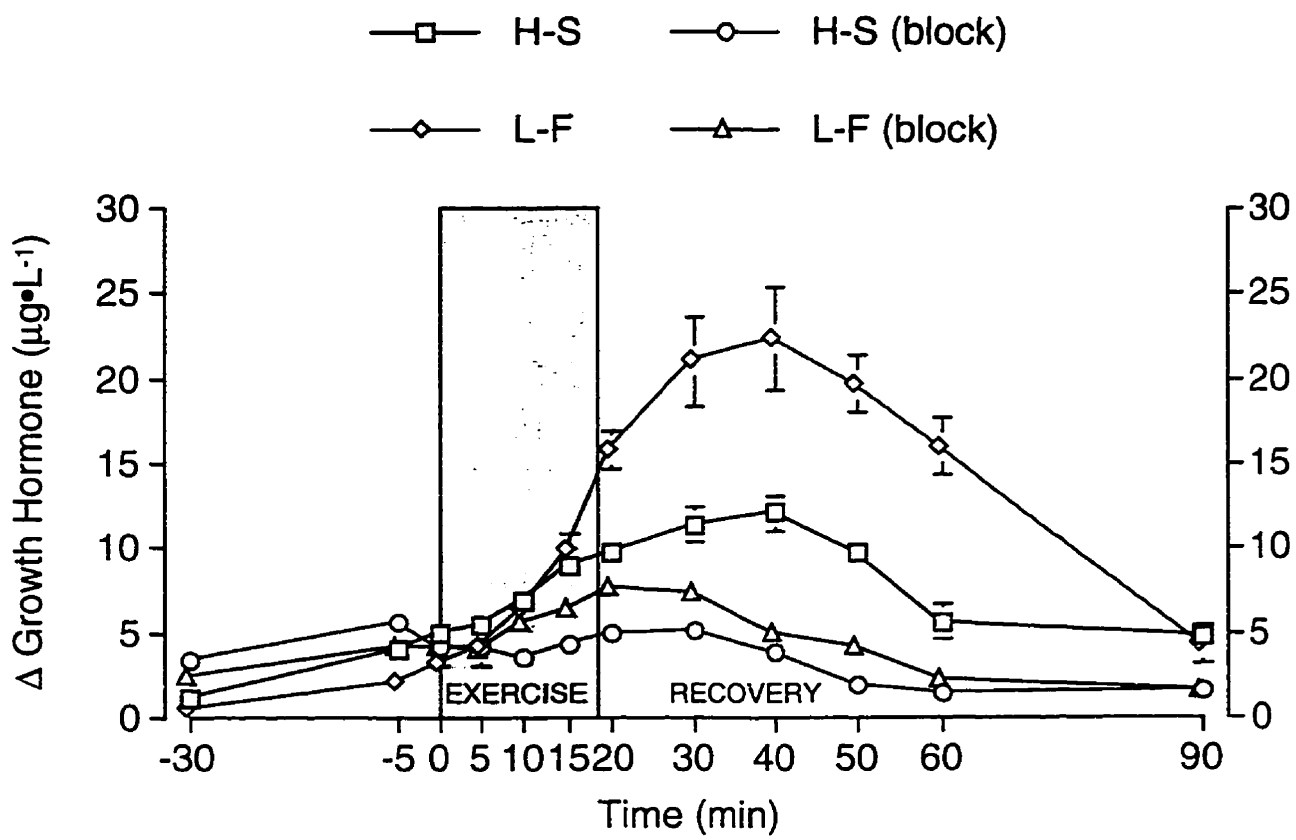

Figure 7-6: *Growth hormone responses in H-S and L-F with and without α-adrenergic block.*

During the infusion of the saline placebo, GH levels increased significantly in both exercise protocols from the beginning of exercise, peaking at 20 min into the recovery period. The total GH response was 2.9-fold higher in the L-F trial than in the H-S trial (833.9 ± 113.4 $\mu g \cdot L^{-1}$ vs. 285.4 ± 58.9 $\mu g \cdot L^{-1}$, respectively). The use of phentolamine blockade of the α-adrenergic receptors during the exercise and recovery periods significantly inhibited the GH responses to the two exercise protocols as well as eliminating any differences in GH-responses between the two protocol designs (H-S and L-F). The mean total incremental GH responses with phentolamine were 58.9 ± 26.9 $\mu g \cdot L^{-1}$ and 95.9 ± 16.2 $\mu g \cdot L^{-1}$ in the H-S (block) and L-F (block) trials (n.s.), respectively. We concluded that, because the differences in plasma GH responses between the H-S and L-F protocols were eliminated by the blockade of α-adrenergic receptors via the infusion of phentolamine, the GH responses to heavy resistance exercise are related to the activation of α-

adrenergic receptors, rather than to circulating levels of NE, EPI or glucose.

A closer inspection of our data, and that of previous published literature of the use of phentolamine during exercise, revealed that the GH levels, while completely blocked at the beginning of the exercise period, steadily increased towards the end of the exercise period. This observation gave support to the idea that another regulatory mechanism was influencing the release of GH from the pituitary, an influence outside of the adrenergic nervous system. It was suggested that core temperature may also be involved in the regulation of GH secretion during exercise.

In Chapter 6, we studied the impact of core temperature to the GH responses during moderate aerobic under-water cycling exercise. The GH responses to two control and two exercise tests during hot and cold mid-chest level water immersion were examined.

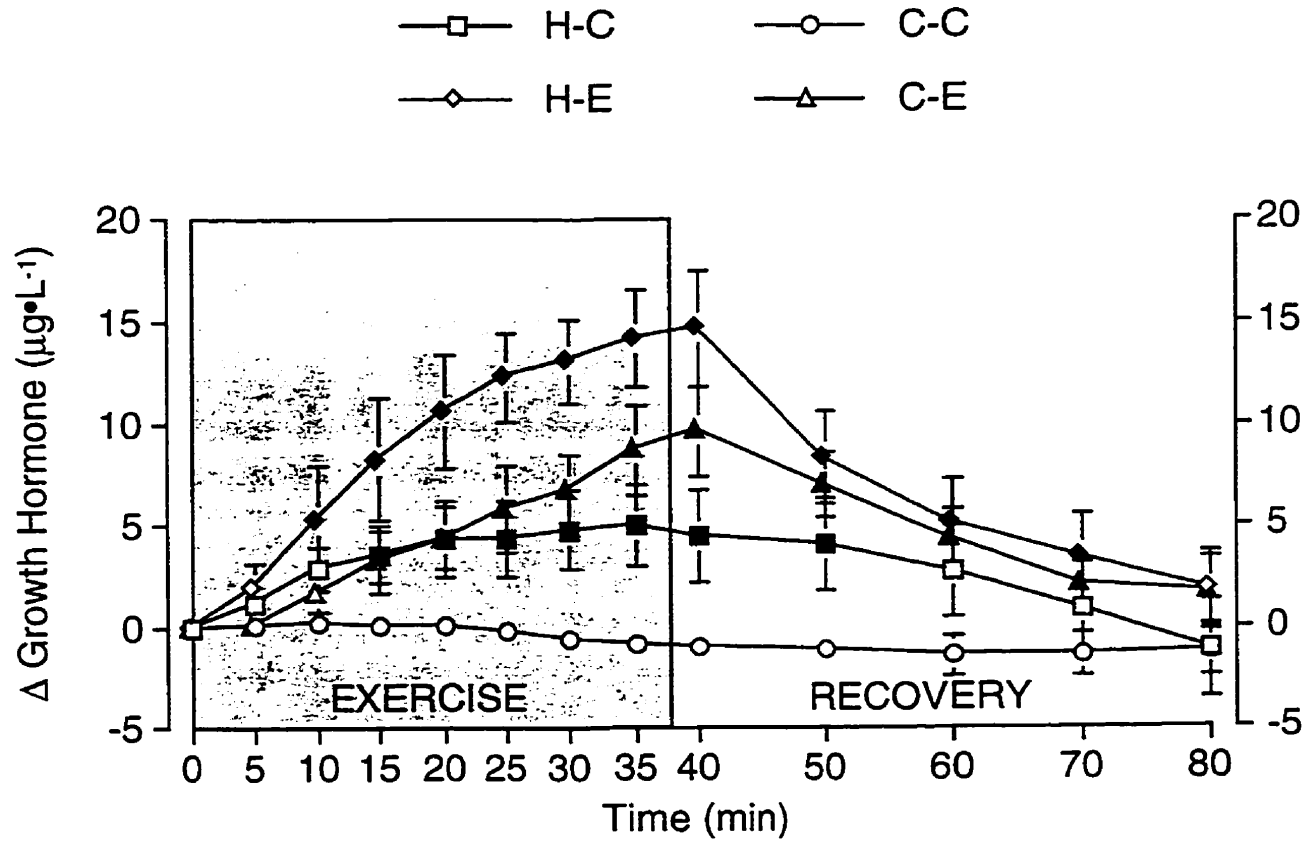

Figure 7-7: Growth hormone responses to immersion cycling.

The control trials consisted of 80 min of resting in the immersion tank. The exercise stimulus was 65 % of the subject's $\dot{V}O_2$ max and lasted for 40 min, at which time the 40 min post-exercise recovery was started. The objective of this study was to examine if the increase in core temperature that accompanies physical activity was a factor responsible for stimulating the hormonal and metabolic responses observed during moderate aerobic exercise.

The results demonstrated that in the hot exercise trial, core temperature increased during the exercise period, peaking at 39.1 $\pm$ 0.4° C at 10 min post-exercise. In the cold exercise condition, the core temperature was clamped at 37.8 $\pm$ 0.3° C by the end of the exercise period and then dropped to 37.5 $\pm$ 0.3° C during recovery. In the hot control trial, the temperature responses during the first 40 min closely matched the cold exercise trial, whereas resting in the cold control condition produced a steady decrease in core temperature to 36.4 $\pm$ 0.6° C. The increases in core temperature during the hot exercise, cold exercise and hot control corresponded with increases in GH levels. The hot exercise trial resulted in greater GH responses than the cold exercise and hot control trials, which were closely matched. Core temperature clamping reduced the exercise-induced increase in GH concentration by essentially half. Furthermore, exercise in the hot trial produced higher lactate levels that were significant from the cold exercise trial from the 5 to 30 min period of exercise, and during the 50 to 70 min period of the recovery phase. Upon closer inspection of the data, it was determined that the rate of temperature change was important to the exercise-induced GH response. The combination of exercise and hot temperature allowed for a higher rate of temperature increase, while the resting in the hot water and the combination of exercise and the cold environment, produced equivalent rates of temperature increase. The responses of GH followed the differences in rate of temperature change, with the highest GH levels in the hot exercise trial and equal GH responses in the cold exercise and hot resting trials. Thus, during moderate aerobic exercise, the rate of core temperature increase was an important factor in the regulation of GH secretion.

Figure 7-8 depicts the regulation of GH secretion during exercise as we now understand it.

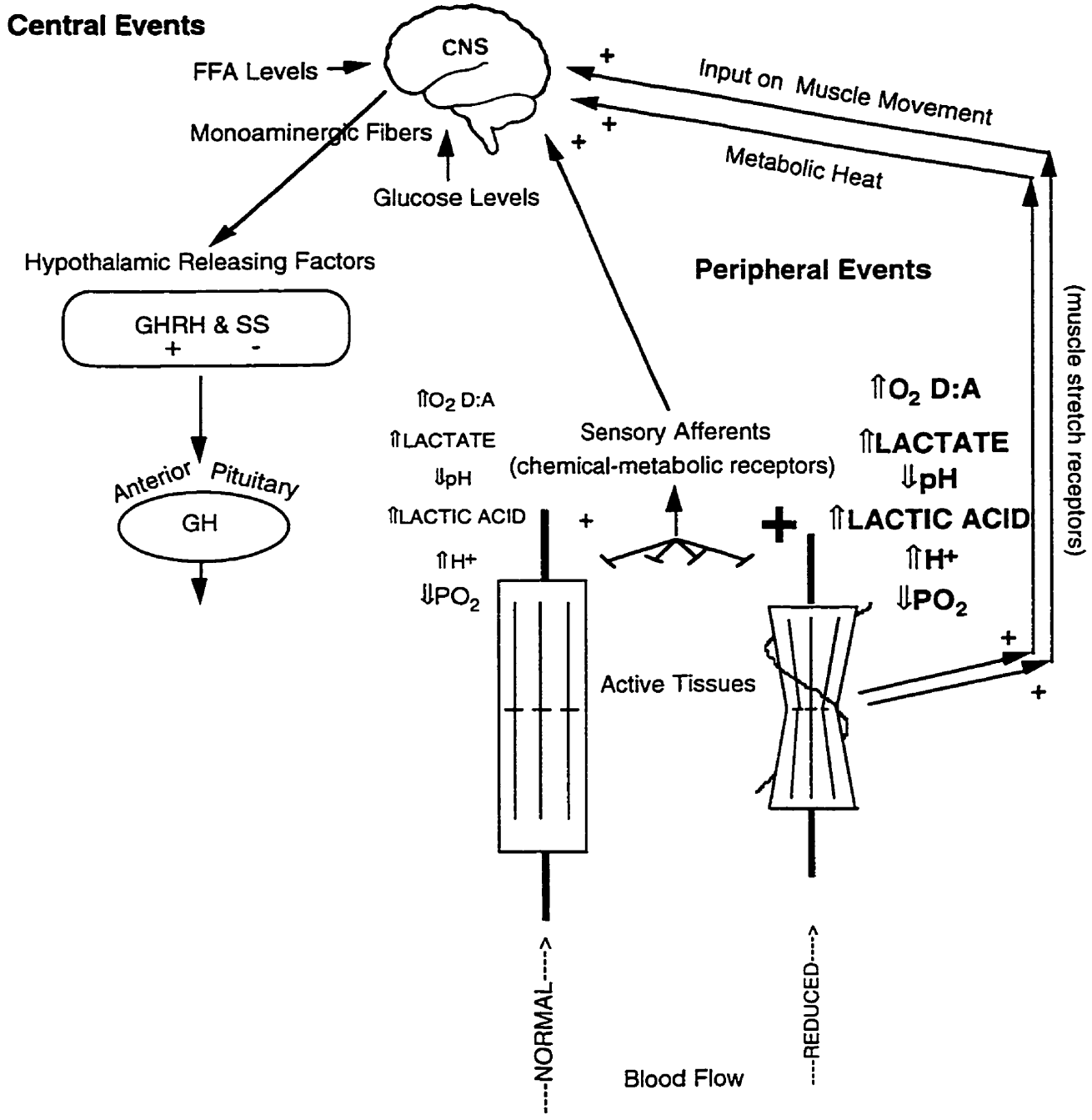

Figure 7-8: Model for the control of GH during exercise.

Given the results of our previous experiments and those of Gordon et al. (1994) and Luger et al. (1992), the role of alterations in acid-base balance must be re-examined. We have previously demonstrated a number of investigations that lend support to the substantiation of the hypothesis surrounding the intracellular detection of chemical-metabolic changes within the exercising tissues and its regulation of the subsequent GH responses. Indeed, the oxygen demand/availability ratio, an accepted regulator of GH secretion during exercise, has been suggestive of changes occurring at the level of the active muscle. It is our belief that, during specific exercise types, the products of rapid anaerobic glycolysis communicate with the central nervous system by way of afferent input from the chemical-metabolic receptors within the active tissues. This communication eventually brings about an increase in GH secretion mediated by adrenergic activation.

The different GH responses in the H-S and L-F exercise protocols were the result of differences in intracellular environments that were influenced by varying blood flows. The non-contractile period of approximately 3 s between each repetition in the slower contracting and heavier loaded exercise (H-S) were likely responsible for the different findings. Thus, a different lag time between the repetitions may have resulted in differences in local blood flow to the exercising muscles. With this in mind, the diminishing supply of blood flow during the 21 repetitions of the L-F protocol would have led to an increased anaerobic glycolysis and subsequent lactic acid production. The H-S protocol would have allowed for a more sufficient restoration of blood flow between repetitions, and thus, the removal of metabolic waste by-products and the re-supply of oxygen and other blood nutrients. Thus, the role of blood flow to the exercising muscles, and the resulting ischemic exercise, is an important component of the GH response to resistance exercise.

We have also suggested that the core temperature responses observed are responsible for the secretion of GH during moderate aerobic activity. It is known that the hypothalamus is involved in the regulation of body temperature and that passive body heating, through the influence

of hot sauna, warm water or heating pad exposure, results in an increase in the secretion of GH (Okada et al, 1970; Okada et al., 1972b). In our present case, the rate of temperature change may be detected by thermal receptors within the hypothalamus, as a condition representing an increased energy expenditure. The hypothalamus would then respond with hormone secretion directed at increasing the supply of glucose to the body's tissues.

Finally, the use of a feed-forward mechanism, similar to the thesis proposed for the control of breathing during exercise, as a mechanism involved in the control of GH secretion during exercise must also been examined. In this case, a central command stimulus would stimulate the release of GH from the anterior pituitary in conjunction with, or at least at the same time as, motor signals from the central nervous system (Kjaer et al., 1987). Previous investigations have demonstrated, as is the case in the control of breathing during exercise, the existence of a two-phase hormonal response, an initial fast-response in activation which is followed by a slower and more sustained activation response. Kjaer et al. (1987) proposed that at the onset of exercise, impulses from motor centres in the brain (central command-fast response), as well as from the working muscles, elicit a work load-dependent increase in sympathoadrenal activation and in the pituitary hormone secretion. Although a number of investigators have suggested the existence of a central command mechanism, the intricacy of the central nervous system has thus far made it impossible to acknowledge or deny (Galbo, 1983; Kjaer et al., 1987).

CHAPTER 8

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APPENDIX 1

The Effect of Core Temperature on the Selective Immune Parameters During Rest and Exercise

1-1. Abstract:

Objectives: The objectives of this study were to examine how leucocyte and hormonal responses to endurance exercise were modified by clamping body temperature through immersion in cool water.

Design of the Study: A controlled, cross-over trial was completed. Eight healthy men aged 27.3 ± 6.0 years were exposed to four 80 min periods of water immersion to mid-chest level, two at a water temperature of 23° C (C), and two at a temperature of 39° C (H). During the first 40 min of two exposures, one at each temperature, the subjects performed a 40 min period of cycle ergometer exercise (E) at an oxygen consumption of approximately 2 $L \cdot min^{-1}$ (65 % of aerobic power). After the exercise period or throughout the control trials (C), the subjects remained sedentary in the immersion tank until the 80 min was over. Each subject performed one control (C) and one exercise (E) trial at each of the two temperature conditions, H and C. Therefore, the four experimental conditions were identified as Hot-Control (H-C), Hot-Exercise (H-E), Cold-Control (C-C), Cold-Exercise (C-E).

Analyses: Core temperatures were measured by thermal probe. Peripheral venous blood was collected every 5 min during the first 40 min of exercise or rest (9 samples) and then every 10 min for the remaining 40 min of recovery (4 samples), during which time the subjects remained immersed in the water. Oxygen consumption was continuously monitored using breath by breath analysis. All blood samples were analyzed for red cell count, hematocrit and total and differential

white cell count by Coulter counter. Growth hormone (GH) and cortisol concentrations were determined by radioimmunoassay. All immune and hormone values were adjusted for changes of plasma volume by the method of Dill and Costill (1974).

Results: In the H-E trial, core temperature increased during the exercise period, peaking at 39.1 ± 0.4° C at 10 min of post-exercise. In the C-E condition, the core temperature was clamped at 37.8 ± 0.3° C by the end of the exercise period, dropping to 37.5 ± 0.3° C during recovery. In the H-C trial, the temperature responses closely matched the C-E trial, whereas resting in the C-C condition produced a steady decrease in core temperature to 36.4 ± 0.6° C. In the C-E, trial increases in white cell (W), monocyte (M), lymphocyte (L) and granulocyte (G) responses were also reduced by approximately 50 % from the levels found in the H-E trial. Core temperature clamping reduced the exercise-induced increase in GH concentration by essentially half, while the increases in cortisol were basically abolished. Backward stepwise multiple regression analysis revealed that the heat clamp abolished the previously observed associations between white cell counts and cortisol in the H-E condition, and weakened the associations with GH concentrations for all cell counts.

Conclusions: The technique of clamping core temperature during exercise used in this study allowed us to examine the independent effects of metabolic heat production, and of energy expenditure during exercise, upon both the immune and endocrine systems. Our data also suggest that approximately 50 % of the leukocytosis observed during prolonged endurance exercise is attributable to a raised core temperature and associated hormonal changes; if the core temperature is prevented from increasing during exercise, cortisol concentrations are insufficient to influence white cell counts.

1-2. Introduction:

There is a considerable gap in the research literature concerning exercise, exercise-induced hyperthermia and immune function. Although physical activity influences marked changes within both the innate and adaptive aspects of the immune system, mechanisms underlying these exercise-induced changes remain unclear. Recently, Brenner et al. (1994a) concluded that the overall immune responses observed during passive heating are essentially very similar to the exercise-induced changes. Because of the lack of literature in this area, it is, therefore, prudent to investigate the relationships that exist between core body temperature, exercise and immune function, and specifically, to determine the immune responses during exercise with and without the use of a core temperature clamp. Furthermore, the influence of the hormonal and biochemical changes that occur during exercise must also be determined in order to provide an explanation of this interaction between body temperature, exercise and immune function.

1-3. Immune Function: Influence of Passive Heating

Changes in white cell counts result from the cardiovascular and hormonal changes that are associated with heat stress (Stephenson et al., 1985). In this case, an increase in cardiac output and blood flow result in the demargination of leukocytes from the endothelia of venules. Hormonal changes, such as elevations in growth hormone (GH), catecholamines and cortisol are frequently observed during hyperthermia and are certainly interrelated in this physiologic response (Kappel et al., 1991a; Kappel et al., 1991b).

Buhring et al. (1977) exposed subjects to a hot water bath of 40° C for 50 min. While the total number of white blood cells (WBC) was enhanced during average core temperature increases of 1.35° C, the lymphocyte number either remained constant or had a tendency to decrease during the length of the investigation. Later, Downing et al. (1988) described more detailed immune

responses in subjects treated to a 40° C water bath for 2 h. With an average increase in core temperature of 2.25° C, total WBC, granulocytes and lymphocytes increased significantly while monocytes remained constant or started to decrease.

That same year, Lackovic et al. (1988) compared the effects of body heating and cooling on total Natural Killer (NK) cell activity. While NK activity was enhanced during 30 min of exposure to both the 39° C water bath and the 4° C environmental chamber, Lackovic et al. (1988) suggested that the condition's particular immuno-modulation was the result of increased GH levels in the hot water immersion and norepinephrine (NE) in the cold condition.

Recently, Kappel et al. (1991a) exposed male subjects to a hot water bath at 39.5° C for 2 h. As core temperature increased by 2.5° C, total WBC, granulocytes, monocytes and lymphocytes also increased significantly. Further investigation by Kappel et al. (1991b) provided a breakdown of lymphocyte subset changes during the induced-hyperthermia. In this case, there was a decrease in the number and percentage of T-cells (CD3) and T-helper cells (CD4) and an increase in the counts for T-suppressor (CD8) and NK cells (CD16, CD56, CD57). As a result of these changes, the T-helper:suppressor ratio was also reduced.

1-4. Immune Function: Influence of Exercise-Induced Hyperthermia:

Schechtman (1987) investigated the effect of changes in body temperature on immune function in subjects cycling at 60 % of their $\dot{V}O_2$ max for 60 min and compared this condition to the immune responses seen when the identical core temperature was induced through passive heating. In both instances, core temperatures reached an average of 38.5° C. In both conditions, the IFNγ and IL_2 lymphokine production by mitogen-stimulated mononuclear cells was increased significantly. However, the lymphokine production was significantly greater during the passive heating than during the exercise condition.

In 1994, Brenner et al. (1994b) considered the effect of heat stress and exercise on the NK cell subpopulation of lymphocytes. Subjects would either remain at rest for 180 min or exercise at 50 % of their $\dot{V}O_2$ max for two bouts of 30 min. The exercise bouts were separated by 45 min and conducted in two ambient conditions, either 23° C or 40° C with 30 % R.H. Average core temperature in the resting condition increased by 0.7° C after 3 h of exposure, while during the exercise sessions in the 23° C and 40° C conditions, core temperatures increased by 1.1° C and 1.5° C, respectively (Brenner et al., 1994b). The NK number and activity increased in both exercise conditions, while there was no detectable quantifiable or functional change in NK activity during the resting conditions.

Severs et al. (1996), using the identical experimental conditions as Brenner et al. (1994b), found no changes in any of the cell count percentages when subjects rested for 3 h in either environmental condition, but did report a consistently enhanced granulocytosis, lymphocytosis and monocytosis during each of the exercise bouts. The $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ lymphocyte differential counts were all enhanced with exercise and continued to be highly expressed over the 60 min of recovery. Severs et al. (1996) have also reported lymphocyte proliferation rates that were attenuated during the repeated exercise bouts of this investigation.

Shek et al. (1994) demonstrated a significant relationship between core temperature increases and the immuno-modulation experienced by subjects running at 70 % of their $\dot{V}O_2$ max for 90 to 120 min. During the exercise period, $CD3^+$, $CD4^+$, and $CD8^+$ T-cells were all significantly elevated. There was a smaller increase in the $CD4^+$ count response which resulted in the T-helper:suppressor ratio being depressed with exercise. The T-cell number decreased throughout recovery to a point that was 50 % of the level found in the pre-exercise analysis. Thus, the T-helper:suppressor ratio was also improved during the recovery period. Interestingly, the B-cell number was not significantly affected by the exercise period, although IgM production by

pokeweed mitogen-stimulated lymphocytes was significantly depressed after 90 min of activity. Both $CD16^+$ number and cytotoxicity were increased during exercise and depressed throughout recovery. This NK cell number was depressed by at least 50 % for over 7 days following the exercise bout. Shek et al. (1994) concluded that the exhaustive physical activity alters the lymphocyte distribution pattern and effector function that may lead to an exercise-induced immune compromise.

1-5. Literature Conclusions:

The leucocytosis of exercise has been well described by Garrey and Bryan (1985), and has since been reviewed in detail by McCarthy and Dale (1988). However, it is less clear whether the observed changes are related to the exercise-induced changes in cardiac output, to the associated micro-traumata (Kayashima et al., 1995), to hormone release (Crary et al., 1983; Tonnesen et al., 1987; Tvede et al., 1994), to the increase in core temperature (Brenner et al., 1995a; Brenner et al., 1995b), or to some more general stress response as suggested by Hoffman-Goetz & Pedersen (1994).

Clearly, there are limits to the enhancement of immune function through increases in body temperature and optimization of physical activity. On the outside of this therapeutic range of temperatures, the immune system will begin to dysfunction. Associated with the increases in core temperature seen during physical exercise, are fluctuating responses, both positive and negative, of certain hormone systems and metabolic responses that may also be responsible for this immune enhancement/compromise scenario. The regulation of GH, cortisol and catecholamines during exercise and their combine and/or individual influence with the immune system needs to be clarified. Thus, further research in the areas of exercise endocrinology, thermoregulation and immuno-modulation will help elucidate the exact interrelationships that exist in these

circumstances. In order to separate fully all of these components it is necessary to control the rise in core temperature found during the physical activity.

1-6. Objectives:

The objectives of this investigation were to examine:

i. Whether the increase in core temperature seen during exercise augments the leucocyte responses to exercise.

ii. To what extent the changes in leucocyte count observed under cool and warm conditions, respectively, were mediated by associated increments in plasma GH and cortisol concentrations.

1-7. Hypothesis:

The hypothesis tested in this investigation were that the:

i. Use of a thermal clamping technique during exercise, and the GH and cortisol responses associated with the exercise will have a positive influence on the quantifiable measurers of the immune system.

1-8. Materials and Methods:

This study was completed at the Physiology and Immunology Laboratories of the Defence and Civil Institute of Environmental Medicine at the Canadian Forces Base Toronto. The materials and methods of this investigation were identical to the materials and methods of Chapter 6, with exception to the selective immune investigations.

i. Measures: The same blood sampling schedule as described in Chapter 6 was followed. The samples for immunologic and haematologic analysis were collected in vacutainer tubes which contained exactly 0.4 mL of liquid EDTA (15%) solution, and gently rocked at room temperature until analysis.

ii. Immune Analysis: The immunologic and haematologic analysis was conducted on a Coulter JT Model DA25-6 interfaced with a Coulter DTH2-AS Data Acquisition terminal (Coulter, Hialeah FL, USA). In order to correct for plasma volume shifts experienced during this investigation, all hormonal, immunologic and haematologic values were corrected by the method described by Dill & Costill (1974).

1-9. Results:

i. Effect of Core Temperature Clamping:

Figure 1-1 demonstrates the core temperature responses to the H and C conditions during both C and E trials. Exercise in the H-E led to a progressive rise of rectal temperature (Figure 1-1); as expected, the rectal temperature was somewhat slow to respond, peaking at 39.1 $\pm$ 0.4 °C 15 min after ceasing exercise. In the C-E trial, the core temperature was held to a peak of 37.8 $\pm$ 0.3 ° C at the end of exercise, dropping to 37.5° C during the recovery period ($p<0.05$). Sitting in H-C

closely matched the temperature response to C-E, whereas sitting in C-C led to a steady decrease in core temperature, with a final reading of 36.4 ± 0.6 ° C.

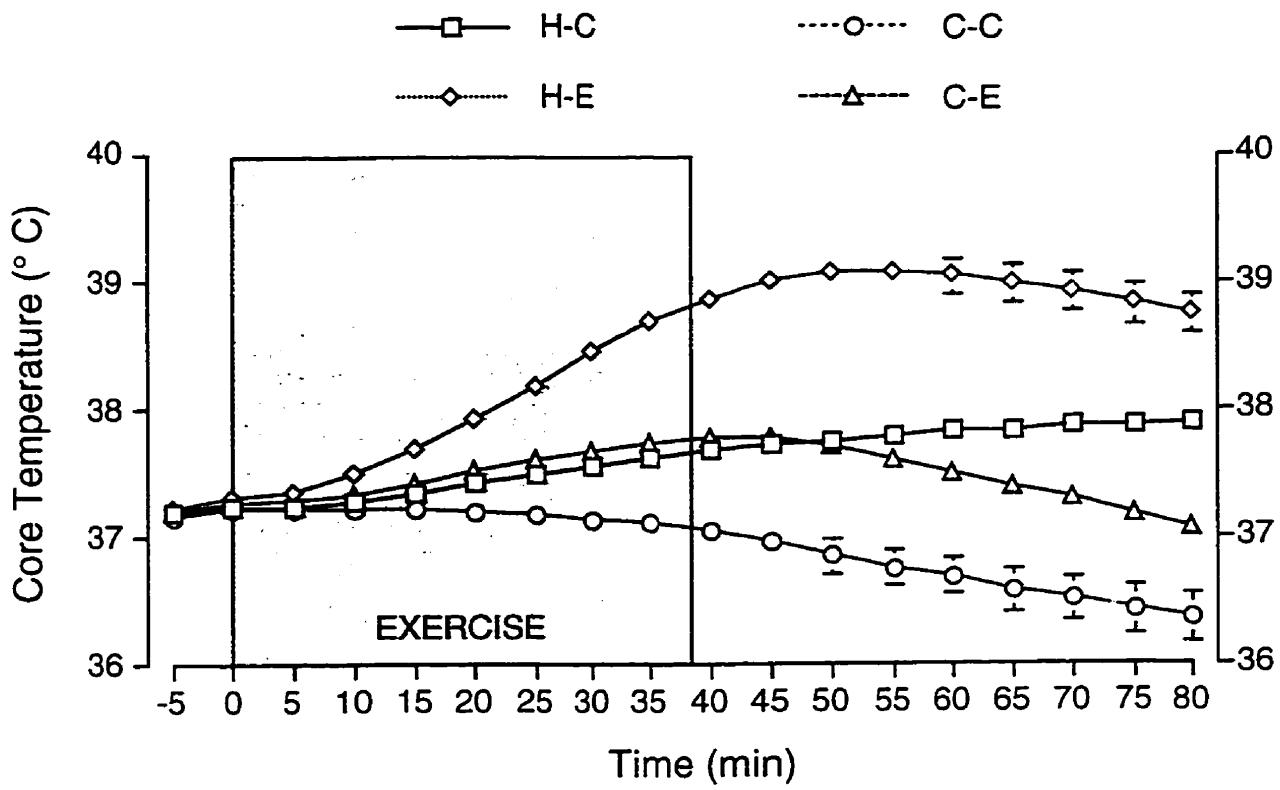

Figure 1-1: Core temperature responses during H-C, H-E, C-C and C-E.

ii. Oxygen Consumption:

Figure 1-2 demonstrates the oxygen uptake during exercise and recovery for each condition. Oxygen consumption during exercise did not differ significantly between the H-E and the C-E conditions, averaging 1.98 ± 0.28 and 1.99 ± 0.24 $L \cdot min^{-1}$, respectively. During the C-C trial, oxygen uptake rose slowly, terminating at an average value of 0.43 $L \cdot min^{-1}$, compared with 0.34 $L \cdot min^{-1}$ in the H-C trial.

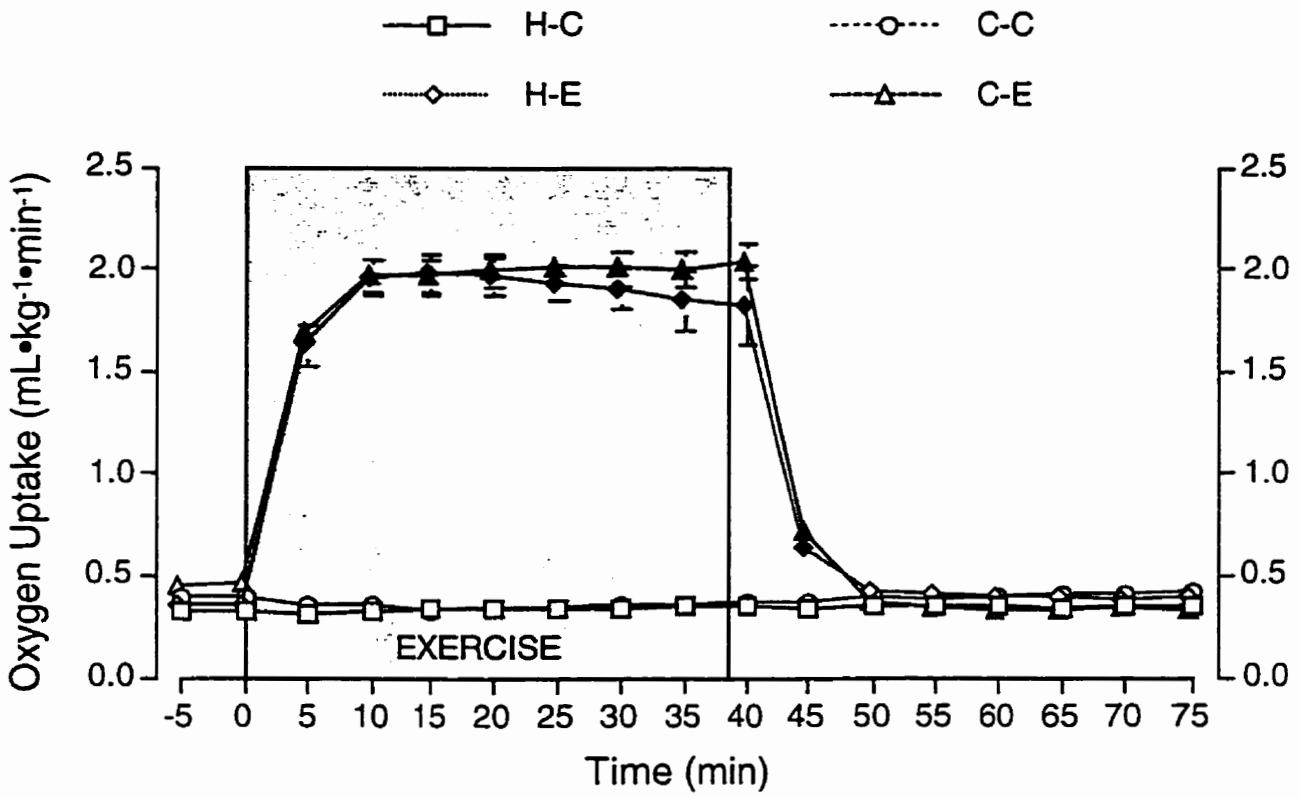

Figure 1-2: *Oxygen uptake during H-C, H-E, C-C and C-E.*

iii. White cell counts:

Figure 1-3 describes the mean white cell responses observed during the two control (H-C, C-C) and the two exercise conditions (H-E, C-E). The total white cell counts at the start of the experimental trials were initially within the expected normal limits of 4.8-10.8 $\bullet$ 10^9 cells$\bullet L^{-1}$ (Walters et al., 1990). From the start of the cycling exercise, there were significant increases in the white cell counts, whether under the H-E or C-E temperature conditions (Figure 1-3), although the two curves differed significantly from each other between 10 min of exercise and 40 min of recovery ($p<0.05$). When exercising in the H-E trial, total white cell count showed a substantial increase to a peak of 9.4 $\bullet$ $10^9 \pm 0.3$ cells$\bullet L^{-1}$. White cell values remained below the ceiling for normality throughout the exercise period, and quickly reached a plateau. This was particularly the

case when exercising under the C-E conditions. The white cell responses were approximately halved when exercising in the C-E trial, with the measured peak count being only $8.1 \bullet 10^9 \pm 0.4$ cells$\bullet L^{-1}$. In the H condition, there was a second rise of total white cell count during the recovery period, but in the C condition there was a rapid reversion to a normal count.

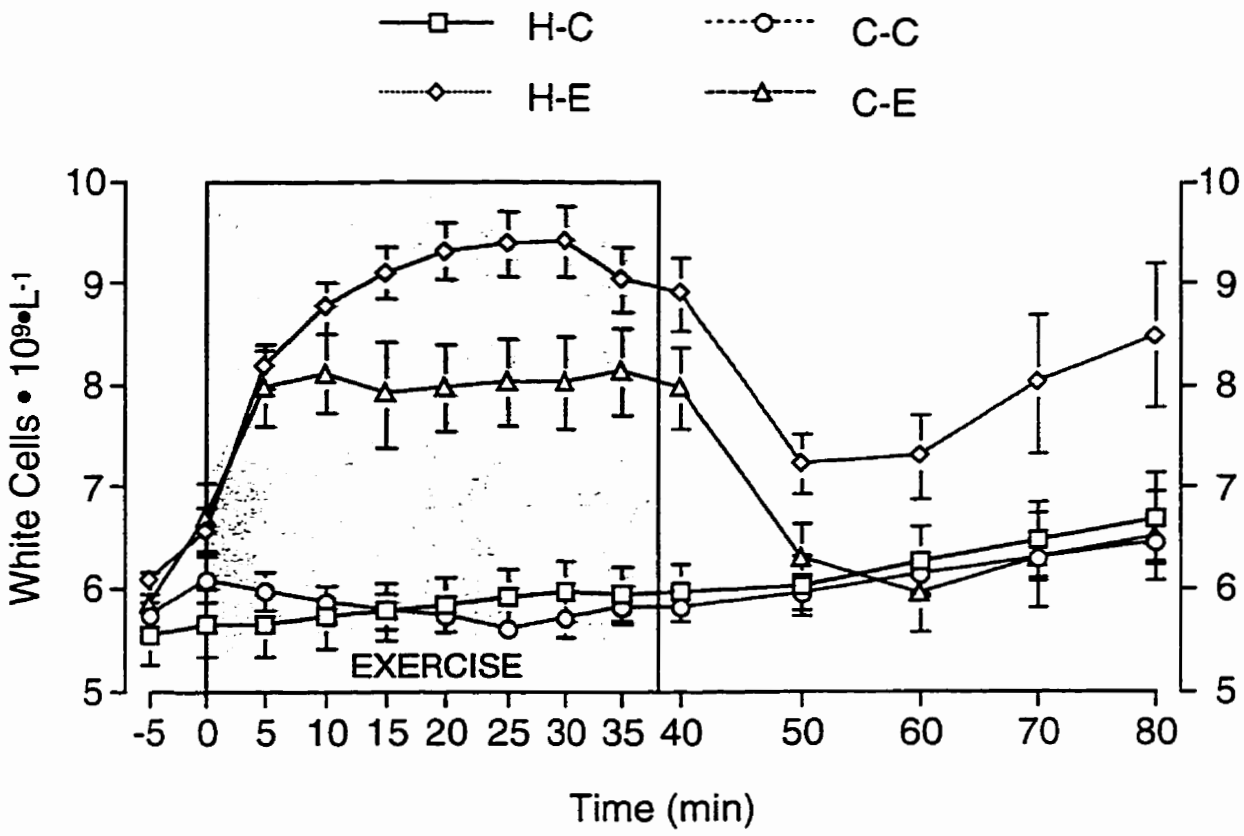

Figure 1-3: White cell count during H-C, H-E, C-C and C-E.

Seated rest in the H-C trial was associated with a small but progressive increase in white cell count above baseline values. This was statistically significant for the last 30 min of the recovery period when core temperature had risen past the 37.5° C point ($p<0.05$). Remaining seated in the C-C environment produced no significant changes. The H-C produced a statistically higher response of white cells above the C-C trial from the 20 min point until the end of the experiment ($p<0.05$).

The white cell response during exercise in the H-E (40 min) was weakly correlated with the corresponding core temperature responses ($r=0.38$; $p<0.05$).

iv. Lymphocytes:

Figure 1-4 describes the mean lymphocyte responses observed during the two control and two exercise conditions. For both exercise trials, lymphocyte counts rose rapidly at the beginning of the exercise period, the increase beginning before there had been any appreciable increase in core temperature. Lymphocyte counts increased significantly with exercise in the H-E trial, peaking above the normal range of 1.2-3.4 $\bullet 10^{9} \bullet L^{-1}$ at 30 min of exercise, and then followed by a subsequent fall to below normal values during the recovery period. After peaking at the 5 min point in the C-E condition, there was a subsequent decline in lymphocyte cell count as the exercise period continued. The two curves differed significantly from 5 min of exercise through the 40 min of post exercise recovery ($p<0.05$). Following the exercise, lymphocyte counts dropped significantly below the initial resting level, although remaining within the normal range; this effect was much larger for the C-E than for the H-E conditions.

Seated rest induced a slight but progressive increase in the lymphocyte count above baseline values which was significant from 50 to 80 min in the H-C conditions. In the C-C condition, there was a small decrease in lymphocyte count, which was only significant from the 25 min to 35 min of exposure.

There was a diminutive correlation between the lymphocyte and core temperature responses found during the first 40 min of the H-E trial ($r=0.35$; $p<0.01$).

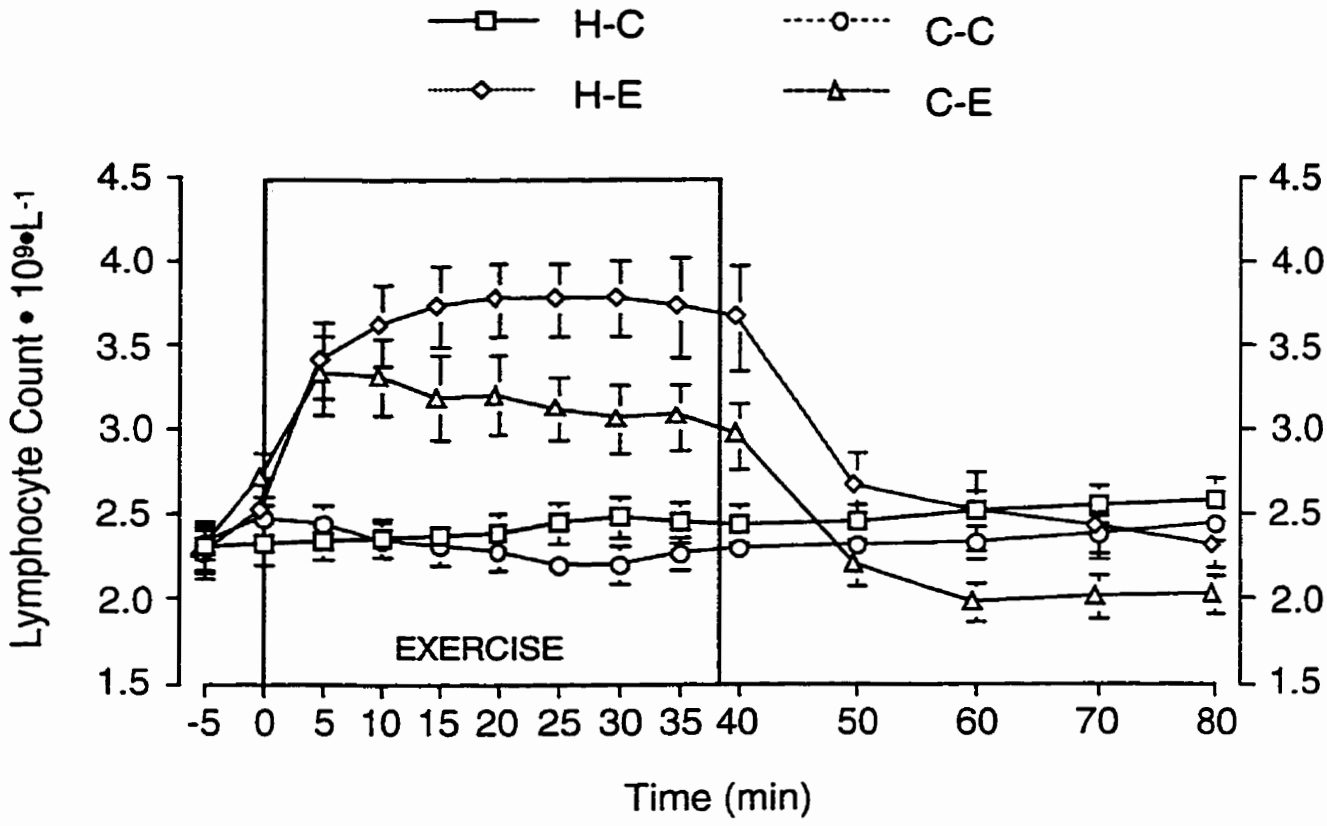

Figure 1-4: *Lymphocyte count in H-C, H-E, C-C and C-E.*

v. Granulocytes:

Figure 1-5 describes the mean granulocyte responses observed during the two control and two exercise conditions. Exercise in the H-E produced a progressive increase in the granulocyte count which already proved significant before the core temperature responses had risen appreciably. During the H-E condition, granulocyte values peaked within the normal range of 1.4-6.5 • $10^9 \cdot L^{-1}$ at 30 min into the exercise period and decreased subsequently, despite the continuing increase in core temperature. Exercise during the C-E environment trial produced a slower increase in granulocyte count with the response becoming statistically significant from 5 min through 40 min of exercise ($p<0.05$). There were also significant differences between the two granulocyte curves, both for the period between 15 min and 30 min of the exercise and during the last 20 min

of the recovery period ($p<0.05$). In this last 20 min segment of the recovery period, there was a steep secondary rise in granulocyte count for the H-E condition.

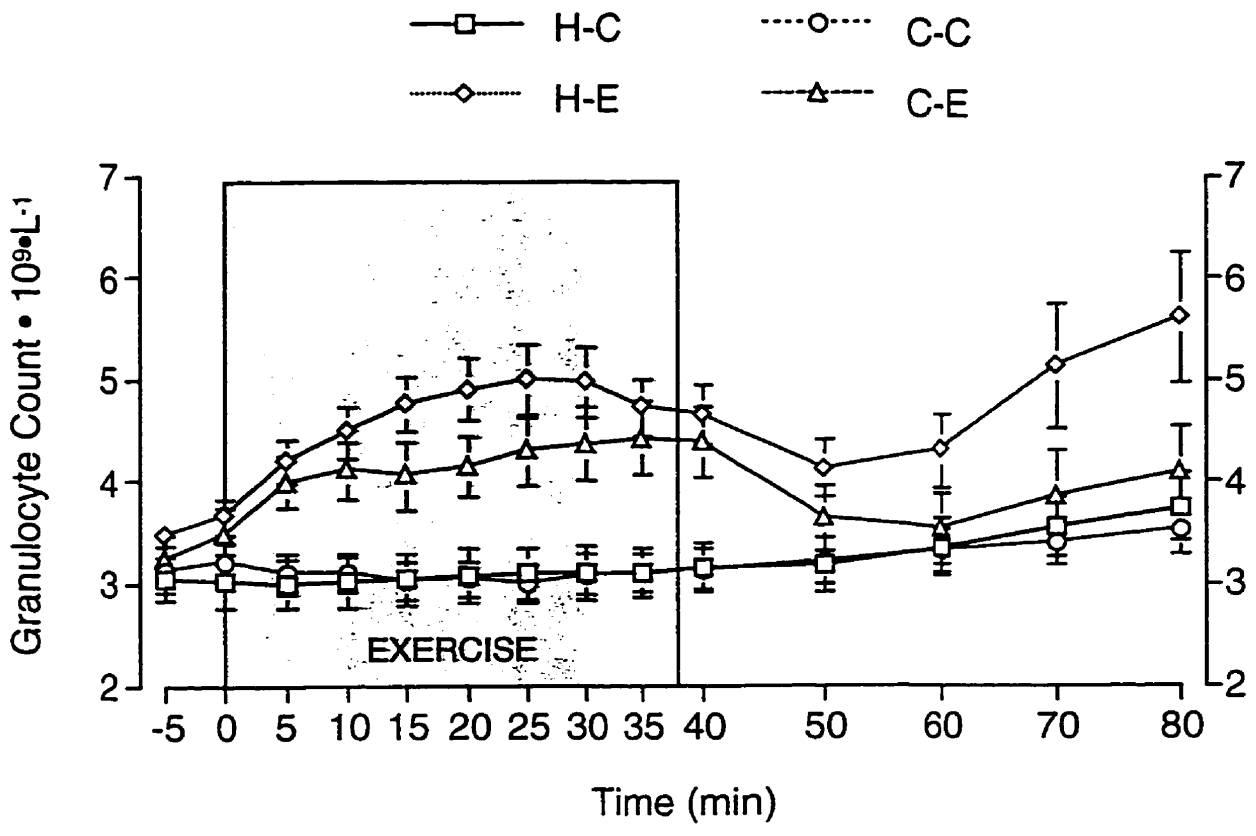

Figure 1-5: Granulocyte count during H-C, H-E, C-C and C-E.

Although the last two samples in the H-C environment trial were significantly increased above the baseline values, there was little change in granulocyte count during the seated rest for either the H-C nor the C-C conditions.

The granulocyte response during the exercise period of the H-E was correlated with the core temperature response, but only to a minor degree ($r=0.31$; $p<0.01$).

vi. Monocytes:

Figure 1-6 describes the mean monocyte responses observed during the two control and two exercise conditions. Before there had been any appreciable increase in the core temperature responses, the monocyte count had risen quickly and exceeded the normal limits of 0.11-0.59 • 10^9 cells•L^{-1} when exercising in both environments.

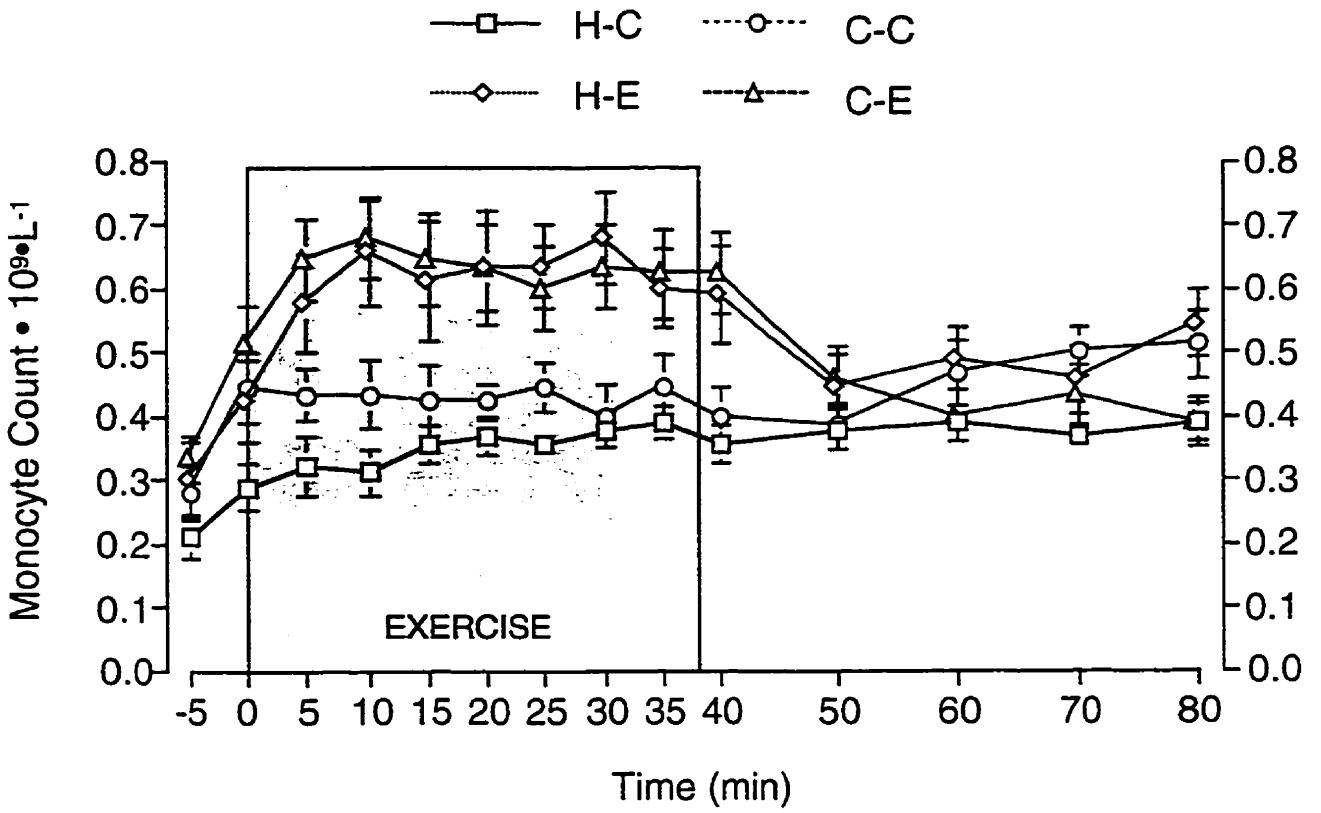

Figure 1-6: Monocyte count responses during H-C, H-E, C-C and C-E.

Exercise in the H-E trial induced a substantial increase in monocyte count with a peak of 0.678 • 10^9 ± 0.07 cells•L^{-1}. In the H-E trial, counts continued to climb during the first 30 min of exercise, but again a down-sloping plateau was observed during the final 10 min of exercise. The C-E produced a smaller initial increase in monocytes, although the peak reading of 0.678 • 10^9 ±

0.06 cells•L^{-1} was comparable. In this case, recovery in the C-E environment was also more rapid, with a drop in monocyte counts to levels below the pre-exercise baseline readings. There were significant differences between the two temperature environments at 20 min to 30 min of exercise and throughout the entire duration of the recovery period.

The control trial in the H-C condition led to a small but progressive increase in monocyte count, which was first significant after quite a small increment of 0.2° C in core temperature after just 20 min of exposure. The monocyte response in the H-C trial remained significant for the entire recovery period. Seated rest in the C-C trial, in contrast, produced a decrease in the monocyte count that was first significant after 60 min, when core temperatures had dropped to 36.6° C.

A very weak and non-significant correlation was found between the monocyte responses during the H-E period and the corresponding core temperature values ($r=0.12$, n.s.).

vii. Platelets:

Figure 1-7 describes the mean platelet responses observed during the two control and two exercise conditions. From the start of the cycling exercise, there were significant increases in the platelet cell counts under both of the H-E and C-C conditions. When exercising in the H-E trial, total platelet count showed a substantial increase to a peak of $338.3 \cdot 10^9 \pm 15.6$ cells•L^{-1} after 35 min of exercise ($p<0.05$). Afterwards the platelet count decreased throughout the recovery period. The platelet responses mirrored those found in the H-E trial, but were approximately halved when exercising in the C-E trial. The platelet count also peaked at the 35 min point of the exercise period in the C-E trial, but only reached a level of $295.1 \cdot 10^9 \pm 22.8$ cells•L^{-1}. As was the case in the H-E trial, the platelet concentration decreased throughout the recovery period. The two platelet curves

differed significantly from each other from the 10 min point of the exercise period and remained statistically different for the 40 min of recovery ($p<0.05$).

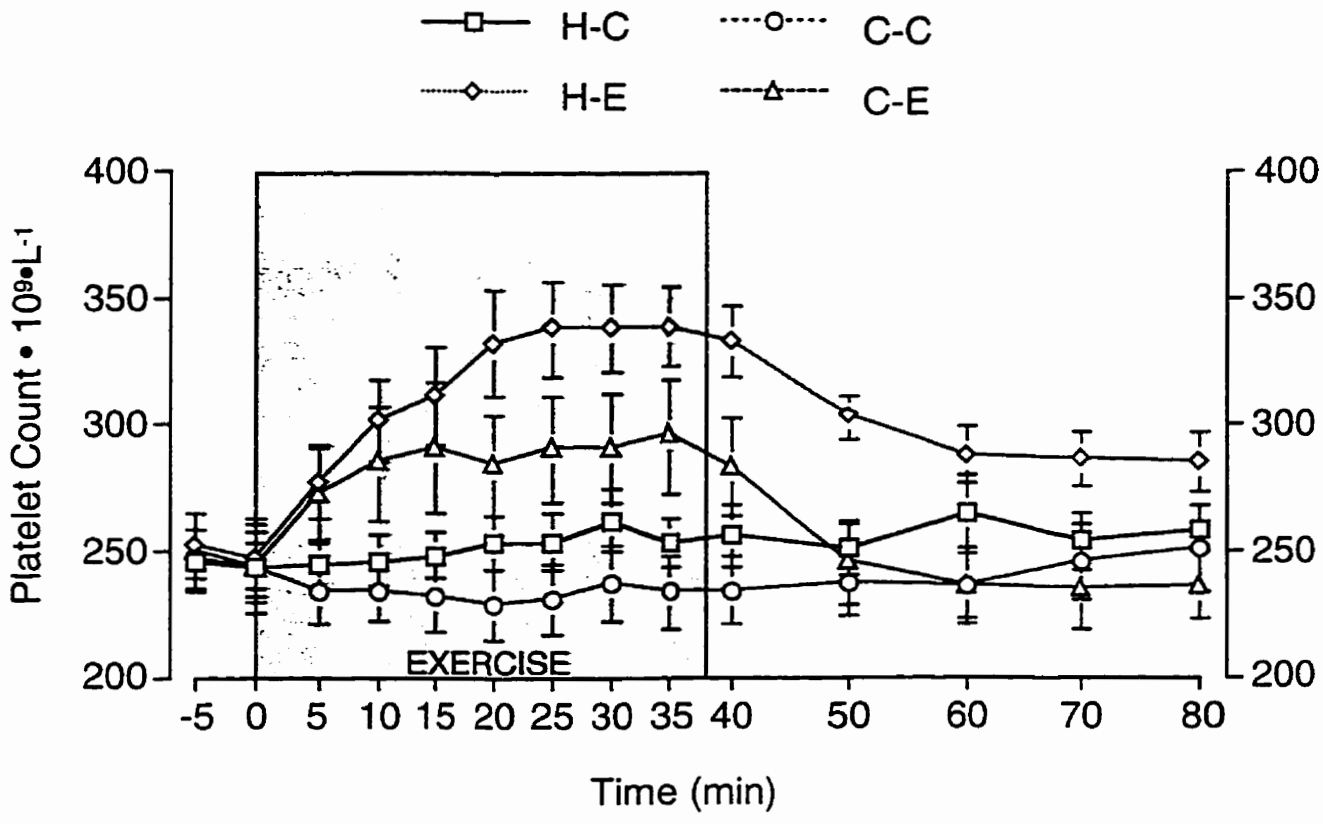

Figure 1-7: *Platelet count during H-C, H-E, C-C and C-E.*

During the C trials, platelet levels in the H-C were significantly elevated above the responses of the platelets in the C-C trial. This difference was significant from 15 min to 60 min ($p<0.05$).

viii. Growth Hormone (GH):

Figure 1-8 accurately describes the Δ GH responses in the control and exercise conditions of H and C. When exercising in the H-E, GH concentrations increased by some 15 $\mu g \cdot L^{-1}$ to a

peak of 19.9 ± 13.2 $\mu g \cdot L^{-1}$, differing significantly from baseline from 10 min through 80 min (Figure 1-8). Exercise in the C-E produced a much smaller increase of GH concentrations, to 11.7 ± 8.9 $\mu g \cdot L^{-1}$; although statistically significant from 15 min through 60 min ($p<0.05$), the peak value was marginally less than the value of 12.1 ± 11.1 $\mu g \cdot L^{-1}$ reached when sitting at rest at a similar core temperature, H-C. When sitting at rest in the C-C, no significant changes of GH concentration were observed.

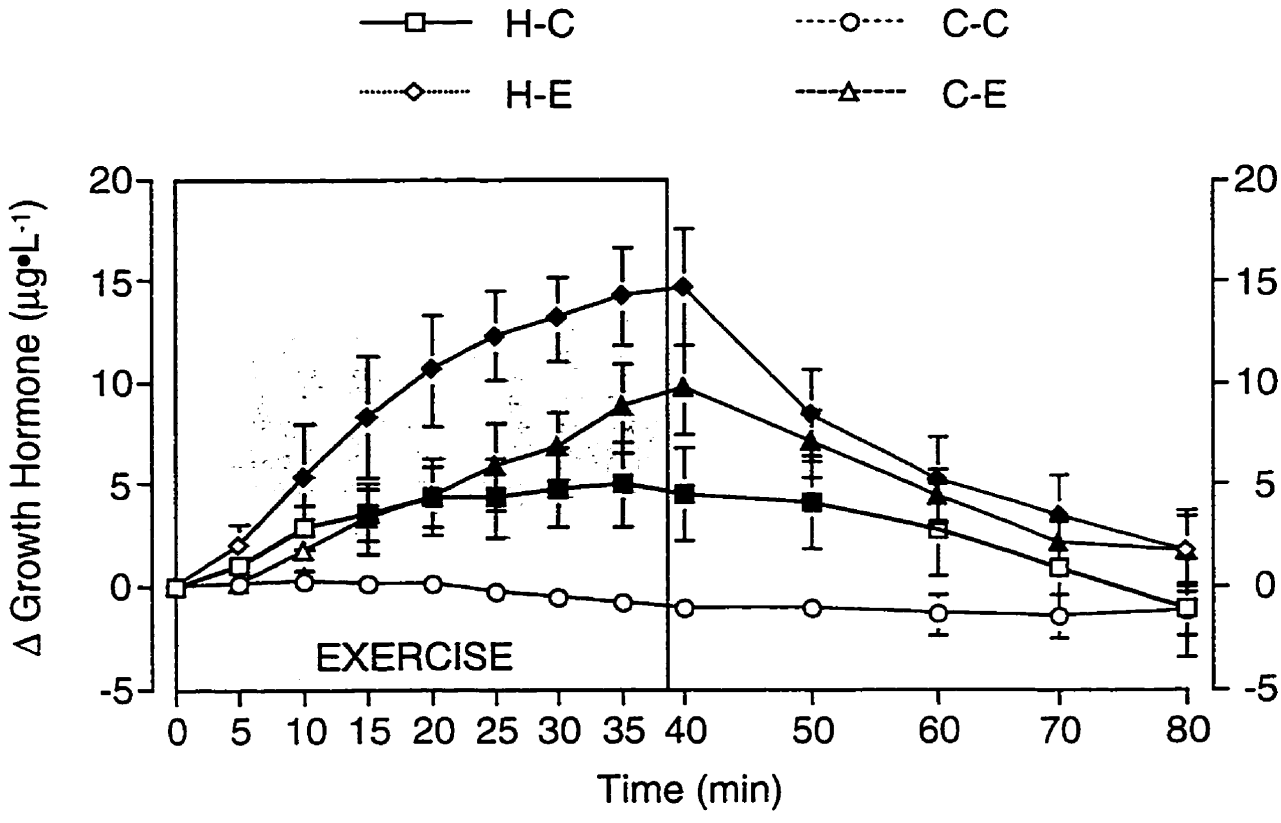

Figure 1-8: *Growth hormone responses during H-C, H-E, C-C and C-E.*

ix. Cortisol:

Figure 1-9 describes the cortisol responses found in the C and E trials during the H and C conditions. When exercising in the H-E, cortisol concentrations rather closely mirrored the

changes in rectal temperature (Figure 1-1). Increments were first significant after 15 min of exercise, and values peaked at 22.2 ± 6.6 $\mu g \cdot dL^{-1}$ 10 min post-exercise, with a subsequent decline in parallel with the decrease in core temperature. When exercising in C-E, there was a much smaller peak of 12.2 ± 4.6 $\mu g \cdot dL^{-1}$, significant from 10 min to 40 min of exercise; however, the curve differed from that for warm conditions at all times except 15 min and 20 min of exercise. Seated rest led to no significant changes of plasma cortisol in either H-C or C-C conditions.

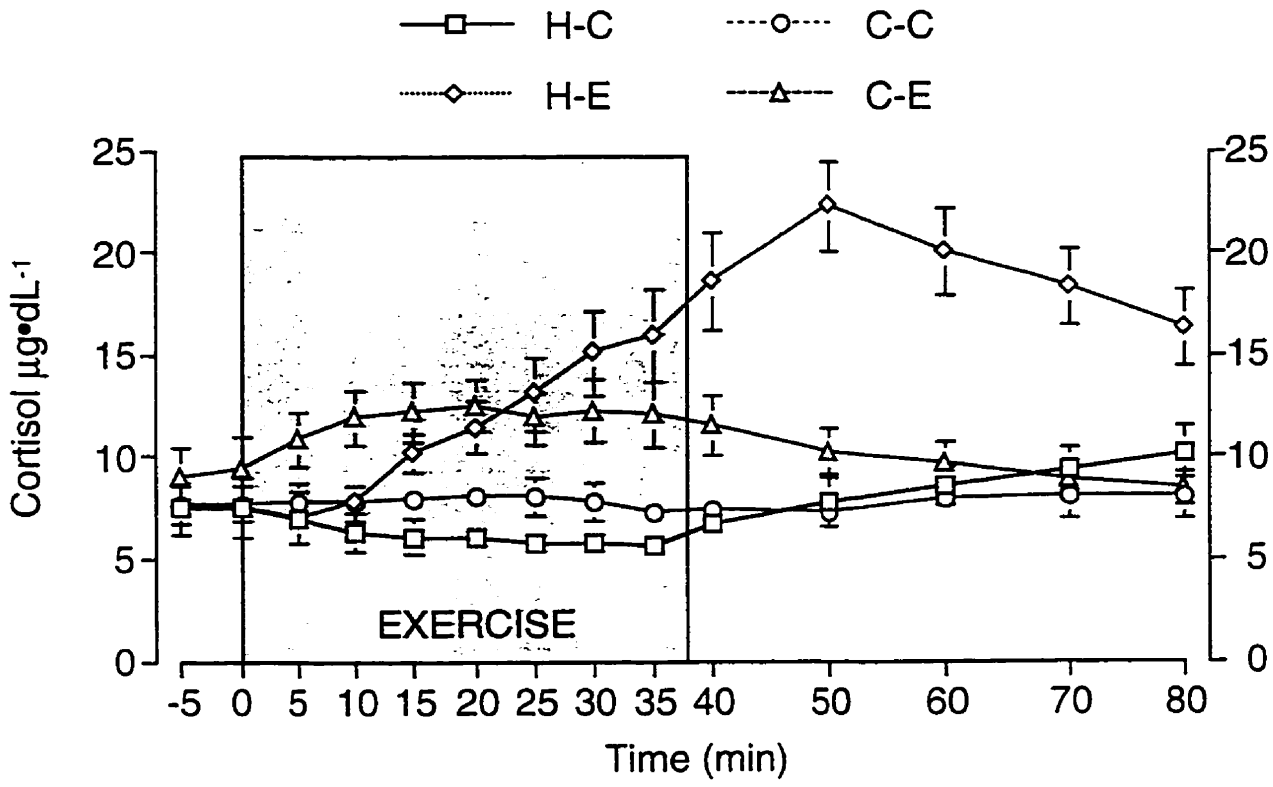

Figure 1-9: Cortisol responses during H-C, H-E, C-C and C-E.

ix. Multiple Regression Analysis: Influence of heat, exercise, GH and cortisol

a) Leucocytosis: A combination of GH and cortisol concentrations and core temperature readings accounted for 77-86 % of the variance in total white cell count under the four experimental

conditions (Table 1-1). When exercising in the H-E condition, all three of these variables demonstrated substantial increments, and all made independent contributions to the description of white cell count; GH and core temperature had positive effects, while cortisol produced a negative effect. When exercising under the C-E condition, there was still a considerable increase in the GH concentration, but no significant association of GH with the leucocyte count was observed. Moreover, in contrast to exercising in the H-E environment, cortisol now had a positive influence upon the white cell count. While sitting in the H-C environment, there was little change in either hormone, and the only significant effect was a decrease in the cell count as the core temperature decreased.

b) Lymphocytes: The hormonal and thermal measurements accounted for 10-93 % of the variance in lymphocyte counts (Table 1-1). When exercising in the H-E trial, lymphocyte counts increased in relation to increments in both GH and cortisol. Exercise under the C-E condition strengthened the association between lymphocyte count and cortisol readings, but changed the effect of GH from a positive to a small negative response. Sitting in the H-C condition eliminated the cortisol effect seen when exercising at a comparable rectal temperature, but it added a small independent thermal effect. Sitting in the C-C environment resulted in no statistically significant regression coefficients.

c) Granulocytes: Between 67 and 93% of the variance in granulocyte counts were described by the hormonal and thermal data (Table 1-1). When exercising in the H-E, cortisol had a strong negative association with granulocyte counts, and temperature demonstrated an independent positive association, while there was no significant effect from the GH response. When exercising under C-E conditions, the smaller increase in cortisol concentrations had a positive influence, while GH also had a positive effect. There was also a negative influence of core temperature. Sitting in the H-C trial showed a large effect of temperature, and a smaller negative influence of GH concentration, while when sitting under the C-C condition, only the negative temperature

coefficient was statistically significant.

d) Monocytes: Hormonal and thermal data accounted for 64 to 83% of the variance in the monocyte counts. When exercising under the H-E, cortisol had a negative association with monocyte count, whereas GH concentration and rectal temperature made positive contributions to the description of the data (Table 1-1). However, when exercising under C-C condition, the only contribution was a significant positive effect of cortisol. The H-E was in contrast with exercise at a similar core temperature, with the heat exposure showing effects from both GH and temperature, but no effect of cortisol. Sitting in the C-C also showed a significant influence of GH, with a negative contribution from core temperature.

Table 1-1:

Multiple regression analysis: effect on immunologic status.

	H-C	H-E	C-E	C-C
a. White Cells:				
R2	0.860	0.830	0.802	0.769
Cortisol	-	-0.55 ± 0.15	0.56 ± 0.08	-
GH	-	0.15 ± 0.03	-	-
Temp.	1.19 ± 0.14	3.79 ± 1.16	-	-0.68 ± 0.13
Resid.	-38.7 ± 5.2	-130.7 ± 42.1	1.19 ± 0.88	31.2 ± 4.8
SEE	0.13	0.51	0.43	0.12
b. Lymphocytes:				
R2	0.851	0.931	0.872	0.095
Cortisol	-	0.080 ± .011	0.391 ± .046	-
GH	0.070 ± 0.01	0.123 ± 0.010	-0.065 ± 0.02	-
Temp.	0.015 ± 0.002	-	-	-
Resid	1.74 ± 0.09	2.73 ± 0.16	-1.07 ± 0.44	2.43 ± 0.09
SEE	0.036	0.186	0.205	0.082
c. Granulocytes:				
R2	0.930	0.668	0.824	0.868
Cortisol	-	-0.398 ± 0.107	0.216 ± 0.045	-
GH	-.048 ± 0.008	-	0.187 ± 0.043	-
Temp.	0.820 ± 0.073	3.31 ± 0.79	-2.70 ± 0.71	-0.484± 0.054
Resid	-27.13 ± 2.71	-116.5 ± 28.9	101.7 ± 26.3	21.1 ± 2.0
SEE	0.064	0.361	0.178	0.057
d. Monoocytes:				
R2	0.827	0.760	0.818	0.640
Cortisol	-	-0.052 ± 0.018	0.073 ± 0.010	-
GH	0.010 ± 0.003	0.015 ± 0.003	-	0.087 ± 0.027
Temp.	0.133 ± 0.025	0.336 ± 0.137	-	-0.254± 0.058
Resid	-4.76 ± 0.94	-11.76 ± 4.98	-0.239 ± 0.107	9.71 ± 2.11
SEE	0.022	0.060	0.052	0.036

1-10. Discussion:

This investigation demonstrated a substantial decrease in total white cell, lymphocyte and granulocyte responses when exercising under conditions where the rise in core temperature was blunted by the C-E condition (Figures 1-3, 1-4, 1-5). These findings agree with the earlier observations of Brenner et al. (1994a), Brenner et al. (1994b) and Severs et al. (1996), where the exercise responses observed at normal room temperature were exacerbated when the environmental temperature was warmer (40° C, 30% relative humidity), particularly if the exercise bout was repeated after a short rest interval that did not allow for complete body cooling.

Although the thermal clamp did allow for a modest rise in body temperature during the exercise period, the degree to which exercise leucocytosis was triggered by the rising core temperature must be evaluated. Figure 1-1 demonstrates this physiologic condition. Sitting in the H-C generated a higher final peak temperature than seen in the C-E condition, yet there was almost no white cell response during the H-C. These findings mirror the results of Severs et al. (1996). These data suggest two different explanations for the observed responses. Either, the rise in core temperature produces a response only when the exposure is accompanied by exercise, or the exercise simply brings the core temperature to a threshold level where the response is finally observed. According to the review of Brenner et al. (1994a), the latter explanation seems most correct, for passive exposures with a sufficient increase in core temperature does generate a substantial leucocytosis. The threshold for this response seems to be a core temperature of around 38° C. Severs et al. (1996) failed to demonstrate a change in leucocytosis with subjects sitting in a warm room that increased their core temperature by 0.7° C. Beisel et al. (1968) and Kappel et al. (1991a) both observed substantial increments of total white cell count when heat exposure in a climatic chamber or a water bath, respectively, had elevated core temperature to 38° C.

The increment of total white cell count reflects increases in several of the leucocyte subsets:

lymphocytes, monocytes and granulocytes. The changes in lymphocyte and granulocyte count are similar to those observed for the total white cell count, but in the case of the monocytes, all of the change seems attributable to the exercise, without an additional effect from the H environment. Although we can report a clear-cut response in the present study, Severs et al. (1996) found that if a second bout of exercise was performed before full recovery from the first, then the monocytosis during and following the second bout of exercise was greater if the environment was warm.

The H-C study gave rise to some increase in the plasma concentration of GH, but exercise under the C-E condition resulted in higher serum GH (Figure 1-8) and cortisol levels than the responses observed in the H-C study at a similar core temperature (Figure 1-9). The concentrations of both hormones were further increased by the combination of exercise and exposure in the H condition.

The backward stepwise multiple regression provides some indication as to the extent to which these hormonal changes may have mediated the observed changes in cell counts. When exercising in the C-E environment, cortisol was associated with increases in the total white cell count and the various subsets. Growth hormone concentration was positively associated with the granulocyte count, although it was negatively related to the lymphocyte count. Hoffman-Goetz & Pedersen (1994) have previously pointed to the high surface density of GH receptors on human mononuclear leucocytes, and suggested that GH may be a further factor modulating immune function. In confirmation of our regression analysis, infusion of GH affected primarily the neutrophil count (Kappel et al., 1993). Temperature had an independent negative association only with granulocyte count.

The responses to sitting in the H-C condition may have been limited by sub-threshold peak temperatures. Nevertheless, core temperature readings were positively associated with white cell, lymphocyte, granulocyte and monocyte counts. Kappel et al. (1991a) have demonstrated that

passive heating induces only minor changes in plasma catecholamine concentrations. Our present investigation found no associations with cortisol, possibly because concentrations of this hormone remained unchanged in the heat (Figure 1-9), but granulocytes showed a small negative association, and monocytes a small positive association with the GH concentrations.

During the total period of observation in this study (80 min), the combination of exercise in the H-E trial consistently reduced the positive association between cortisol and the various cell counts that had been observed when the same exercise was performed under the C-E condition. Furthermore, the associations of cortisol with granulocytes and monocyte counts became negative. An effect of core temperature, the duration of exercise and recovery observations and interactions between hormones may explain conflicting earlier reports on a positive association (McCarthy et al., 1992; Moorthy and Zimmerman, 1978; Nieman et al., 1989), or a lack of association (Eskola et al., 1978; Gimenenz et al., 1986; Hansen et al., 1991; McCarthy et al., 1991; Robertson et al., 1981) between cortisol and white cell or neutrophil counts.

In the present set of experiments, it may have been that, as the core temperature rose, and circulating cortisol concentrations exceeded the capacity of the corticosteroid-binding globulin of approximately 20 $\mu g \cdot dL^{-1}$ (Gray et al., 1993; McCarthy and Dale, 1988), there was an increasing egress of leucocytes into the active muscles (Berk et al., 1990; Gabrielk et al., 1991; Galun et al., 1987; Shephard & Shek, 1994). This process would account for the decreasing cell counts as the exercise period continued.

Under the H condition, the association of cell counts with GH concentrations was also modified, possibly because higher concentrations of GH were reached. Positive associations were seen with white cell count, lymphocytes and monocytes. This response may be due the fact that core temperatures did not rise above the threshold; in fact, temperature now showed a strong independent association with white cell, granulocyte and monocyte, but not with lymphocyte

counts.

Core temperature in itself could provoke a change in cell counts simply by increasing cardiac output, and thus flushing additional cells into the central circulation (Foster et al., 1986; Martin et al., 1982; Muir et al., 1984; Thommasen et al., 1984). The temperature of the H water bath (39° C) was high enough to cause near maximal cutaneous vasodilatation, although this effect would produce a maximal regional blood flow of only 5-6 $L \cdot min^{-1}$ (Rowell et al., 1968). This effect would have been small relative to the increase of cardiac output generated by the working muscles that would likely result in regional blood flow of about 13 $L \cdot min^{-1}$ at an exercise oxygen consumption of 2 $L \cdot min^{-1}$.

It is well known that exercise induces an immediate increase in the secretion of catecholamines that drop sharply when the exercise is stopped. The action of the catecholamines causes a demargination of splenic leucocytes and increases white cell counts found in the central circulation (Brenner et al., 1994b; Crary et al., 1983; Shephard & Shek, 1994; Tonnesen et al., 1987; Tvede et al., 1994). There is a distinct difference of opinions as to whether NE (Brenner et al., 1994a) or EPI (Tonnesen et al., 1987; Tvede et al., 1994) is the more important variable in this connection. The passive elevation of core temperature by warm water immersion leads to only minor changes in the concentration of either EPI or NE (Kappel et al., 1991). It would, therefore, be expected that any catecholamine effect should be observed at the beginning of the exercise period, should disappear quickly when the exercise is halted, and be unaltered by an increase of core temperature. It is likely that the catecholamines probably account for the initial steep rise in white cell, lymphocyte and monocyte counts, as well as the continued elevation of monocyte counts throughout the exercise bout.

1-11. Conclusions:

The technique of clamping core temperature during exercise used in this study allowed us to examine the independent effects of metabolic heat production, and of energy expenditure during exercise, upon both the immune and endocrine systems. Our data suggest that approximately 50 % of the leukocytosis observed during prolonged endurance exercise is attributable to a raised core temperature and associated hormonal changes; if the core temperature is prevented from increasing during exercise, cortisol concentrations are insufficient to influence cell counts.

1-12. Future Study:

Given our present findings, further study should include the analysis of the qualitative and functional characteristics of the immune system under the identical conditions discussed in this investigation.

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IMAGE EVALUATION
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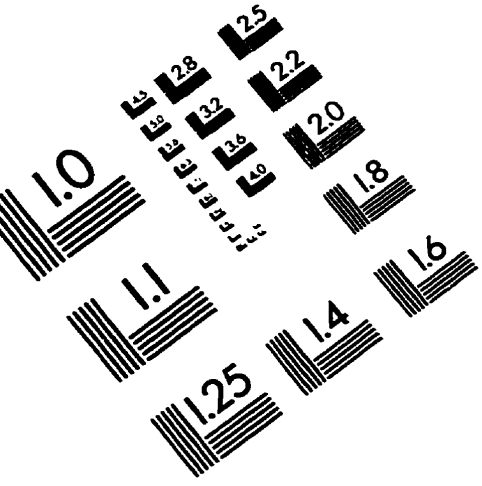

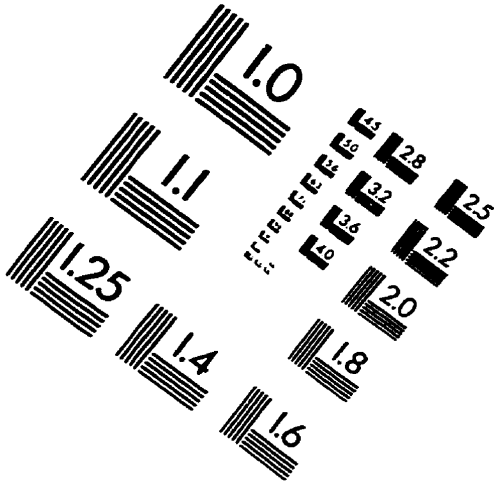

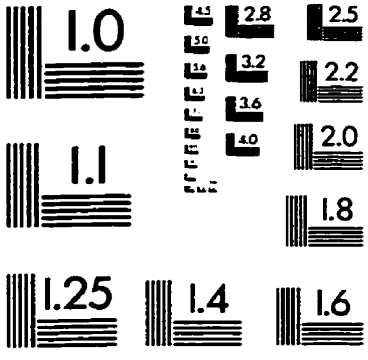

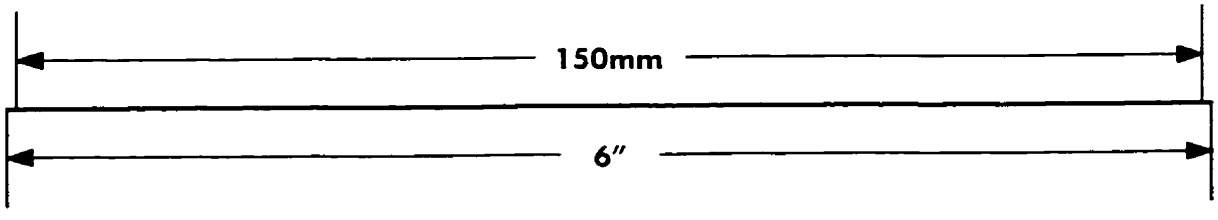

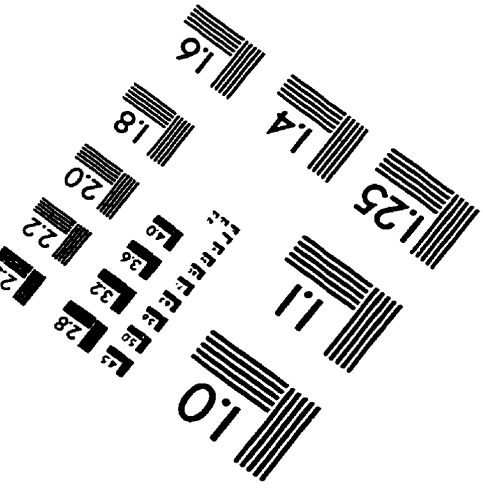

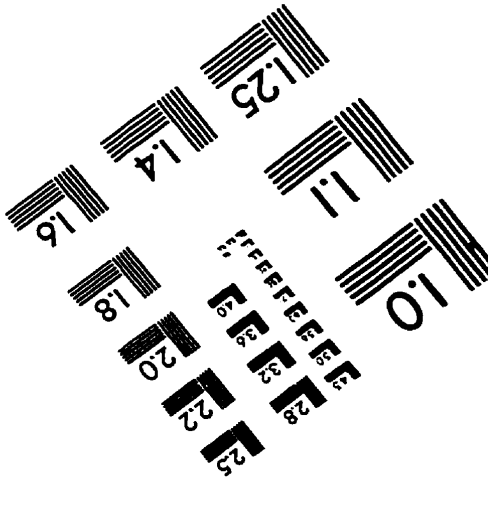

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