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THE INVESTIGATION OF SERUM PROLACTIN CONCENTRATION IN MALES IN RESPONSE TO A STANDARD $\dot{M}VO_2$ TREADMILL STRESS TEST

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Two roads diverged in a wood, and I
I took the one less travelled by,
And that has made all the difference.

Robert Frost

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For this major opus I needed, asked for, and received, help from many people whom I will hereby thank: Dr. Orban for his long-standing patience and guidance. Dr. Hudson for his excellent criticism. Dr. Dionne for his statistical insights as detective, architect and mathematician; Dr. Greenway for performing the biochemical analysis; Bill Montelpare for his computer assistance; the subjects who answered my "MEN WANTED" poster; Bert and Julian who took blood; Pearl and Joanne who measured oxygen; and Ellen who typed all those "unused" lines.

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ABSTRACT

The purpose of this study was to investigate the serum prolactin concentration in males in response to a standard $\dot{M}VO_2$ treadmill stress test.

There were nineteen subjects between the ages of 20 and 29 years. The subjects performed a standard treadmill stress test to self-perceived exhaustion. The stress test provided fourteen stages, each of two minutes duration, speed increasing from 3.0 to 3.75 mph and a grade increasing from 0% to 26% elevation. At each stage, either the grade or the speed changed in order to increase the workload.

Expired gases were collected during the last 30 seconds of every second stage up to the 10th stage, then at every stage thereafter and at the point where exercise was terminated. Venous blood samples for prolactin concentration analysis were collected through an indwelling catheter inserted in the right antecubital vein. Three samples were collected prior to exercise for pre-exercise resting levels. During exercise, blood sampling was done during the last 30 seconds of every second stage and at the point where exercise was terminated. During the 30-minute recovery period blood sampling was done every 6th minute. Pre- and postexercise blood samples were collected and analyzed for lactate and hematocrit. ECG electrodes monitored heart rate during exercise and recovery.

Results showed that the prolactin concentration remained stable for an interval of approximately 20 minutes after the beginning of exercise. Eighteen subjects exceeded the criterion of a 1.8 ng/ml increase in prolactin concentration over pre-exercise levels. A rise in prolactin concentration was observed only after a $\dot{V}O_2$ of about 40 ml/kg/min was reached. Peak prolactin levels, observed after the subjects had stopped exercising, indicated a mean 2.6-fold increase over pre-exercise levels. There was no relationship between the $M\dot{V}O_2$ and the prolactin increment in response to exercise, or between $M\dot{V}O_2$ and peak prolactin concentration.

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CHAPTER 1

THE PROBLEM

1.1

INTRODUCTION

Exercise has became well established as a recreational model with the current popularity of promoting fitness and active lifestyles. Understanding the complex physiological adjustments to exercise requires a knowledge of the influence of hormones. A hormone that has been virtually neglected in this regard is prolactin (PRL). In the last decade the physiology of human prolactin has been intensely investigated, and endocrinologists now appreciate it as being much more than merely a hormone that regulated the mammary gland (Nicoll 1980). It has been suggested that extra-mammary actions of this hormone in synergy with other hormones, relate, in both males and females, to osmoregulation (Berl 1975, Buckman 1973, Horrobin 1971, Re 1979), metabolism and psychic stress, (Berle 1973, Nicoll 1980, Nguyen 1982, Noel 1972, Robyn 1978), and fertility (Boyd 1978, Cowie 1980, Ferland 1980, Frantz 1978, Horrobin 1979, Minuto 1984, Shangold 1981, Tolis 1980, Yen 1978). The important reproductive influence of prolactin is becoming more evident through studies of human hyperprolactinemia. Females with this condition may exhibit amenorrhea or delayed puberty, and males may exhibit hypogonadism and infertility (Boyd 1978, Cowie 1980, Ferland 1980, Frantz 1978, Horrobin 1979, Shangold 1981, Tolis 1980, Yen 1978).
The recent emergence of various sport-related infertility syndromes in males and females makes the prolactin response to exercise topical (Dale 1979, Frisch 1981, 1985, Shangold 1982, Wall 1985, Wheeler 1984).

Prolactin is phylogenetically the oldest polypeptide hormone secreted by the pituitary gland (Turkington 1972). Comparative endocrinology has attributed to prolactin more than 20 different physiologic effects, representing 85 different actions in fish, amphibians, birds and mammals (Nicoll 1972, Turkington 1972, Yen 1978). Frantz (1978) suggests that this range and diversity of actions and target organs, even considering species specificity, is unmatched by any other hormone, and the name "versatilin" was suggested by Nicoll (1980) in view of this great diversity. The most important mammalian effects of prolactin are considered mammotropic and lactogenic (Nicoll 1972). It was not until 1970 that human prolactin was identified and measured in the blood by Frantz (1970) and Hwang (1971). Bioassay and immunoassay have identified human prolactin as a polypeptide separate from growth hormone, despite its having arisen from a common primordial peptide precursor molecule very early in vertebrate evolution (Frantz 1978, Turkington 1972).

The dominant control mechanism for human prolactin secretion is inhibitory (Ben-Jonathan 1980, Clemens 1980, Cowie 1980, Frantz 1978, Ramirez 1977, Yen 1978); dopamine is largely responsible for this inhibition at the hypothalamic level and possibly also the pituitary level. Other neurotransmitters -- noradrenalin, serotonin, and endorphins -- alter prolactin release through inhibitory or stimulatory components (Clemens 1980, del Pozo 1978, Ferland 1980, Frohman 1975, Yen 1978).

The human prolactin response to a variety of exercise conditions in normal, healthy males and non-lactating females has been documented. A number of studies have described a significant rise in blood prolactin levels under a variety of exercise conditions in normal, healthy males and non-lactating females. The results of these experiments are briefly outlined in chronological order.

Noel and associates (1972) reported a two-fold increase in prolactin in both male and female subjects who had run up and down 15 to 30 flights of stairs. Frewin et al (1976) observed an elevation in the plasma prolactin of 10 males after a 20 minute treadmill run at 3.5 mph on a 8.6% grade in a 40° C room. Aakvaag's group (1978) studied prolactin concentration in male army cadets during a gruelling 5-day combat course that included limited sleep, decreased caloric intake, continuous physical activity and military discipline. As the course progressed, a reduction in prolactin was observed. Hagen and Galbo found the prolactin response to exercise enhanced after fasting (Hagen and Galbo 1979). They reported that six men who had fasted for 59 hours and then been subjected to treadmill runs of short duration had a significant rise in plasma PRL levels. Overnight fasting did not result in a PRL change after this same exercise test. Bratusch-Marrain and associates (1979) reported no change in arterial prolactin concentration for three males exercising for 30 minutes at 50% $\dot{M}VO2$. Mayer et al (1980) reported an approximate two-fold increase in prolactin in males who performed a bicycle ergometer test, while maintaining a heart rate of 150 BPM for 20 minutes. Brisson and associates (1980) showed a significant plasma prolactin elevation following a 75% $\dot{M}VO2$ bicycle ergometer test of 30 minutes duration in a group of females with a sports history. No similar elevation was evident after the same test was administered to a group of females without a sports history. Brisson's group (1981) also tested male basketball players on a bicycle ergometer at different workloads, and reported that the blood prolactin changes seemed to be related to the work intensity. Cohen et al had a group of 24 males and females exercise to their maximum predicted heart rate, using the Bruce protocol, and found a significant prolactin change after exercise (Cohen et al 1980).

Johannessen, working with Hagen and Galbo, found that seven males subjected to a 4-day fat diet prior to a 70% $\dot{M}VO_2$ treadmill run to exhaustion had higher prolactin concentrations than those subjected to a 4-day carbohydrate diet prior to the same treadmill protocol (Johannessen, Hagen and Galbo 1981). Prior found running exercise produced a greater rise in serum prolactin levels in nine females than cycling exercise, which suggests that breast motion with running may influence the prolactin increase (Prior et al 1981). Shangold observed exercise-induced increases in plasma prolactin in six female runners after a 30-minute outdoor running session at their accustomed pace (Shangold et al 1981). Moretti investigated four male and four female professional runners performing treadmill exercise to "maximum aerobic capacity" and found an increase in prolactin in response to maximal effort (Moretti et al 1981). Moretti later investigated plasma levels of prolactin, before and after bicycle exercise in six subjects for 8 minutes at a constant heart rate of 170 BPM, and found an increase in prolactin (Moretti et al 1982). Similarly, Moretti's third investigation found an increase in plasma prolactin in eight male professional athletes who exercised on a bicycle ergometer at a constant workload of 80% of their maximum heart rate (Moretti et al 1983). Nguyen's experiment combined mental and physical stress. The exercise component was composed of a 20-minute outdoor run of 50% to 70% $\dot{M}VO_2$, following a period of mental stress. His results showed a prolactin rise during exercise for those subjects who had previously attended a lecture, but a prolactin decrease during exercise for those subjects who had previously written an examination (Nguyen et al 1982). Bazzarre and Royster studied ten male marathon runners over a 2-hour submaximal treadmill run. They reported a sustained rise in prolactin and a bimodal pattern of peaks. Their subjects had ingested either a high carbohydrate, high fat, or control diet meal prior to the treadmill run (Bazzarre and Royster 1983).

Jezová et al carried out two experiments on "untrained" males and found plasma prolactin significantly increased in both after 20 minutes of "submaximal exercise" on a bicycle ergometer (Jezová 1982, 1983).

The results from these studies showed that prolactin increased with exercise over time. There were many non-exercise-related factors included in the studies and many variations in blood sampling intervals, which may have interfered with the interpretation of the prolactin response to exercise.

CHAPTER 2

REVIEW OF THE LITERATURE

2.1

SOURCE AND CHEMISTRY OF PROLACTIN

In the anterior pituitary gland, human PRL is synthesized and secreted by special acidophilic cells, called lactotropes. The lactotrope is unique in that its intrinsic secretory drive is manifest, unless the cell is inhibited (Thorner 1980). It depolarizes spontaneously in the presence of calcium and PRL is released (Tindal 1974). Although lactotropes normally constitute at least one third of the anterior pituitary gland cell population, the PRL content in the gland is relatively small, approximately 135 ng/gland (Yen 1978). The probable explanation for the disparity between pituitary content and cell population is related to the fact that PRL has a high turnover rate and is stored in small quantity (Yen 1978). Prolactin is continuously secreted in small amounts with additional episodic bursts during sleep, or emotional, or physical stress (Boyd 1978). Stimulation of PRL secretion is influenced by a variety of exogenous and endogenous stimuli (Cowie 1980, Frohman 1975). Those of an exogenous nature include the tactile sucking stimuli, thermal stimuli, and stimuli causing pain and stress. Endogenous stimuli arise from nutritional and metabolic factors and blood hormone concentrations. Tolis (1980) reports that extrapituitary production of PRL from other brain areas, placental membranes and neoplastic tumors seems to occur. Horrobin suggests there could be a number of forms of PRL, and that PRL isolated from the pituitary may differ in some respects from blood PRL, advancing the possibility of a pro-hormone (Horrobin 1978). PRL in plasma and amniotic fluid may exist in more than one form, the most frequently-occurring variety being monomeric PRL; however, dimeric and other forms do occur (Horrobin 1978).

PRL consists of a single polypeptide chain with 198 amino-acid residues and an apparent molecular weight of about 21,000 to 23,000 daltons (Cowie 1980, Lewis 1971). The mean "basal" level of prolactin for men and women of all ages has been found to be 4.7 ng/ml and 8 ng/ml, respectively (Frantz 1978). A normal range for men and women is from 1 to 25 ng/ml (Boyd 1978, Frantz 1978, Noel 1980, Tolis 1980, Wise 1980). Mean basal levels, depend on the assay, and the range is consistently higher for women, due to estrogen sensitization of the pituitary, causing more prolactin secretion (Boyd 1978, Ferland 1980, Yen 1978). The half-life of prolactin in the blood is 10 to 15 minutes (Frantz 1978, Turkington 1972, Yen 1978). Inactivation of prolactin occurs in the liver and the kidney, and current literature describes the likelihood of an intracellular degradation at the target site (Boyd 1978, Josefsberg 1979, Kahn 1976, Kolata 1978, Turkington 1972).

2.2

REGULATION OF PROLACTIN SECRETION

2.2.1

HYPOTHALAMIC AND NEUROTRANSMITTER INFLUENCE

Prolactin secretion is controlled by stimulatory and inhibitory hypothalamic components, and by various neurotransmitters that alter PRL release (Boyd 1978, Clemens 1980, Cowie 1980, Ferland 1980, Frohman 1975). The release of prolactin is controlled primarily by inhibition. The inhibition-mediating substance, termed prolactin-inhibitory factor (PIF) is liberated into the pituitary portal vessels by the hypothalamus (Cowie 1980, Ferland 1980, Frantz 1978, Yen 1978). The chemical identity of PIF is still under

investigation, but there is strong evidence implicating hypothalamic dopamine as one of several prolactin-inhibiting factors (Ben-Jonathan 1980, Cowie 1980, Frantz 1978, Horrobin 1978, Yen 1978). In addition, dopamine can act directly on the pituitary to decrease prolactin secretion (Frantz 1978). There is also evidence to suggest prolactin stimulation by hypothalamic prolactin-releasing factor (PRF) (Boyd 1978, Cowie 1980). The PRF physiological chemical identity is also unknown, although thyrotropin-releasing hormone (TRH) and vasoactive intestinal peptide (VIP) are prime candidates (Boyd 1978, Clemens 1980, Cowie 1980, Meites 1979, Yen 1978). The influence of various neurotransmitters on prolactin secretion has been studied. Serotonin and its precursors, tryptophan and 5-hydroxytryptophan have been found to release prolactin (Cowie 1980, del Pozo 1978, Frohman 1975, Lancranjan 1977, Woolf 1977). The endogenous opiates -- enkephalins and endorphins -- stimulate prolactin secretion by increasing serotonin and decreasing dopamine (Meites 1979, Tolis 1978). They may act, therefore, as modifiers of both hypothalamic PRF and PIF (Meites 1979). Gamma-amino butyric acid (GABA) induces release of prolactin through a dopamine blockade (Cavagni 1977, Clemens 1980, Cowie 1980, Masala 1978, Yen 1978). However, the physiological role and mechanism of prolactin regulation by these neurotransmitters needs further investigation and clarification (Cowie 1980, Yen 1978). It has been suggested that PRFs act to effect acute prolactin release, as in the response to stress, whereas inhibition of PIF is the mechanism for a slower, delayed prolactin secretion, as in response to suckling (Cowie 1980).

The episodic discharge of PRL during sleep, and emotional and physical stress, is probably mediated by extrahypothalamic brain centers (Boyd 1978). The limbic system may play a role and in animals is being investigated in this respect (Boyd 1978, Mepham 1976, Moore 1980, Tindal 1974).

2.2.2

AUTOREGULATION

Prolactin lacks a specific peripheral hormone-producing target organ through which a feedback loop can interact with the pituitary and hypothalamus (Boyd 1978, Cowie 1980, Yen 1978). For this reason, a "short-loop" feedback mechanism has been suggested as the physiological route for prolactin to control its own secretion (Boyd 1978, Smith 1980, Melmud 1980, Moore 1980, Tolis 1980). Through this route, increased concentrations of serum prolactin cause more dopamine to be released into hypophyseal blood, which then inhibits prolactin release from the anterior pituitary and lowers the serum prolactin concentration (Moore 1980, Smith 1980).

2.2.3

CIRCADIAN CYCLE

One to two hours after the onset of sleep, prolactin levels begin to rise, peaking between 3 a.m. and 5 a.m. The levels decline rapidly upon arising, with the lowest levels occurring between 10 a.m. and noon (Boyd 1978, Delitala 1976, Nokin 1972, Sassin 1973, Tolis 1980, Yen 1978). The nocturnal prolactin increase may be serotonin-mediated through an overriding of dopamine effects, since a serotonin-blocking agent blunted this rise in healthy subjects (Boyd 1978, Cowie 1980, Smith 1980, Tolis 1980, Yen 1978).

2.2.4

OTHER FACTORS AFFECTING PROLACTIN SECRETION

The major tranquilizers and opiates increase prolactin release through a dopamine receptor blockade (Frantz 1978, Tolis 1978, Yen 1978). Insulin-induced hypoglycemia causes a significant rise in serum prolactin (Copinschi 1974, Noel 1972, Okajima 1980, Wakabayaski 1980, Woolf 1977) and controversy exists regarding endorphins as the possible release mechanism. Thyroxine decreases the prolactin response to TRH (which is a PRF) at the pituitary level, resulting in decreased prolactin release (Yen 1978).

The dietary manipulation experiments, combined with prolonged running, done by both Johannessen and Bazzarre, presented conflicting results. Johannessen et al reported higher PRL concentrations during the run in the subjects who had followed the fat-enriched diet, while Bazzarre and Royster found a higher PRL concentration in the subjects who had followed the high-carbohydrate diet (Johannessen et al 1981, Bazzarre and Royster 1983).

The recent study by Wheeler et al found mean "basal" levels of PRL significantly lower in male long-distance runners, compared to sedentary control subjects, although the levels remained within the "physiological range" (Wheeler et al 1984).

Heat stress has been found to cause an elevation in serum PRL. Aldercrutz subjected 6 males to a 90° C sauna for 20 minutes and found a 1400% rise in plasma PRL (Aldercrutz et al 1976). Mills and Robertshaw observed a 53% elevation in plasma PRL in six males subjected to 45° C for 90 minutes (Mills and Robertshaw 1981).

The importance of the ionic environment; namely, of CA++ and HCO3- for the in vitro synthesis and release of PRL, is reported by Hafez and Thorner (Hafez 1975, Thorner 1980). Thorner's in vitro studies suggest that PRL secretion from the anterior pituitary lactotrope is a calcium-mediated process and that dopamine may inhibit calcium influx into the lactotrope, thereby reducing PRL secretion. Similarily, if calcium influx is high, PRL secretion is enhanced (Thorner 1980). Bicarbonate ion concentration also alters prolactin release, by affecting calcium concentration (Hafez 1975, Thorner 1980).

2.3

PROLACTIN RECEPTORS

Although confirmed PRL receptors have been detected only in human mammary gland tissue, there is indirect evidence of receptors elsewhere in the human: the ovary and the adrenal cortex (Carter 1977, McNatty 1974). The prolactin-binding site has always been considered to be on the target cell surface membrane because large, charged polypeptide molecules are not the kind that can penetrate membranes and enter cells (Kolata 1978). However, Josefsberg has recently found intact prolactin in rat liver cytosol in the Golgi apparatus (Josefsberg 1979). This may be an intracellular hormone and receptor degradation site (Josefsberg 1979, Kahn 1976, Kolata 1978). This internalization into its target cells suggests

a functional intracellular role for prolactin involving cellular reactions and metabolism (Catt 1979, Josefsberg 1979, Kolata 1978, Rillema 1980).

Posner and Costlow have both suggested that high serum prolactin levels coincide with a high concentration of receptors (Costlow 1976, Posner 1974). Costlow found an increased number of liver prolactin receptors in rats during pregnancy, a time when prolactin concentration is elevated. He also found that removal of the pituitary resulted in a dramatic decrease of liver prolactin receptors (Costlow 1976). Posner injected male rats with prolactin and subsequently found their liver receptor levels to be increased (Posner 1975). The current theory, in contradiction to Costlow and Posner, suggests that elevated concentrations of the homologous hormone is probably responsible for the down-regulation of receptors (i.e., an inverse relationship exists between the serum prolactin concentration and the membrane receptor concentration) (Catt 1979, De Meyts 1976, Gustafsson 1980, Kahn 1976, Kolata 1978, Lesniak 1976).

The intracellular mediator, or second messenger, possibilities for prolactin appear to be tissue-variable and include calcium ions, cyclic nucleotides, active protein kinase, prostaglandin, polyamines, and Na/K concentrations (Falconer 1977, Horrobin 1979, Rillema 1980). The subsequent effects of prolactin may involve:

1. An increased level of cGMP synthesis and reduced level of cAMP synthesis;
2. An increased intracellular concentration of potassium and reduced level of sodium;
3. An enhanced rate of prostaglandin synthesis; and
4. Stimulation of polyamine synthesis.

At least two second messengers -- protein kinase and Na/K ATPase -- are reported to be critically dependent on the presence of calcium (Horrobin 1979, Rillema 1980).

2.4

BIOLOGIC ACTIONS

Lactogenesis and lactation involves prolactin in a multihormonal interaction with pituitary, ovarian and thyroid hormones, prolactin being the key requirement for lactogenesis (Ferland 1980, Frantz 1978, Yen 1978). The important reproductive influence of prolactin is becoming more evident through studies of human hyperprolactinemia. Females with this symptom may exhibit menstrual irregularities, amenorrhea or delayed puberty, and males may exhibit impotence, hypogonadism and infertility (Boyd 1978, Cowie 1980, Ferland 1980, Frantz 1978, Horrobin 1979, Shangold 1981, Tolis 1980, Yen 1978). In many females hyperprolactinemia is associated with a hyperandrogen state, polycystic ovarian syndrome, and hirsutism. This suggests that prolactin may stimulate adrenal androgens (Higuchi 1984, Luciano 1984).

The high concentration of prolactin (100,000 ng/ml) in amniotic fluid has led Yen to suggest that during the "aquatic" phase of human development it may play an osmoregulatory role (Boyd 1978, Yen 1978). A similar role at the kidney level in adult humans has been investigated and has resulted in conflicting reports. Horrobin et al injected sheep prolactin intramuscularly into five healthy males, and found that renal retention of water, sodium and potassium resulted. The plasma concentration of sodium and plasma

osmolality rose, suggesting a movement of water into cells (Horrobin 1971). Buckman and Peake studied the effect of changing serum osmolality on endogenous prolactin secretion by administering both hypotonic and hypertonic fluids at different times to eleven healthy volunteers (four males and seven females). They observed that the hypertonic fluid caused an increase in serum prolactin concentration, which could then influence renal retention of water. Conversely, a hypotonic fluid caused a decrease in serum prolactin concentration which could then influence the observed diuresis (Buckman 1973). Berl used an intravenous injection of TRH to increase serum prolactin in five subjects, but found no significant alteration in sodium or water excretion (Berl et al 1975).

Re et al investigated the possibility that prolactin is able to stimulate the secretion of aldosterone by the adrenal cortex, rather than acting directly on the kidney, to promote sodium reabsorption (Re et al 1979). Elevated serum prolactin was induced with TRH injection in eight healthy women, but the authors were unable to find any change in plasma renin or aldosterone. When they studied patients with hyperprolactinemia and failed to find hyperaldosteronism, they stated that chronic elevation of prolactin had little effect on plasma aldosterone (Re et al 1979).

Several researchers have attempted to explain the controversy regarding the osmoregulatory role of prolactin in the human kidney. Their arguments are that 1) vasopressin contaminated the prolactin used for injection; 2) TRH-induced, endogenous prolactin elevation failed to alter sodium and water excretion; and 3) there is a lack of evidence regarding aldosterone stimulation by prolactin (Dellman 1974, Holland 1977, Yen 1978). In a recent review, Horrobin suggested that vasopressin and prolactin have independent kidney functions,

both being necessary for optimal antidiuretic response. He postulated that vasopressin enhances cAMP formation while prolactin enhances another second messenger, a prostaglandin or polyamine, and the antidiuretic response requires a combination of the two (Horrobin 1980).

Berle's metabolic study reported the effects on carbohydrate and lipid metabolism in healthy women after an intravenous injection of prolactin. An increase in β-hydroxybutyrate, acetoacetate and FFA was observed as well as a significant fall in pyruvate and lactate (Berle 1973). Prolactin has been implicated in the suppression of lipoprotein lipase activity in adipose tissue and its increased activity in mammary glands during lactation in rats (Cowie 1980, Zinder 1974).

2.5

PROLACTIN AND EXERCISE

Twenty experiments that have investigated the plasma PRL changes observed when men and women are subjected to a variety of exercise conditions were available to this author.

Noel et al were the first to measure plasma PRL after an exercise stress test. After having run up and down 15 to 30 flights of stairs as rapidly as possible, his eight female and twelve male subjects all had statistically significant elevations in plasma prolactin. Their pooled results yielded a mean pre-exercise PRL value of 13.2±1.8 ng/ml, a mean PRL value of 19.1±1.8 ng/ml one minute after cessation of exercise, and a mean value of 22.0±2.1 ng/ml 15 minutes after cessation of exercise. (Noel found the female response was higher at every point, but not statistically different from the male response, hence the pooled results) (Noel et al 1972).

Frewin and associates measured plasma PRL in ten 25-year-old males after a treadmill run of 20 minutes at 3.5 mph on an 8.6% grade with a room temperature of 40° C and also 10° C. There was a significant PRL elevation after exercise for the subjects in the 40° C room. The researchers concluded that the observed PRL elevation was essentially an exercise-induced effect, as non-exercised control subjects in the 40° C room did not have an increase in PRL levels. It was reported that only one of the ten exercised subjects had an increase in prolactin of considerable magnitude, but when considered as a group the overall rise in prolactin was barely significant at the 5% probability level. At 10° C the changes in prolactin with exercise were not significant (Frewin et al 1976).

Aakvaag's group studied the prolactin response in 22 well-conditioned male army cadets, subjected to a gruelling 5-day combat course under strong military discipline and punishment. The course took place at 500 m altitude in rainy weather and temperatures ranging from 5° C to 15° C. The cadets underwent extremely hard, continuous physical strain, plus a caloric deficiency of 7,000 to 10,000 kcal/day and sleep deprivation ranging from no organized sleep to 3- and 6 hour periods of sleep. Blood sampling was done before the course started and every morning during it. The elevated PRL levels at the start of the course were attributed to psychic stress and apprehension. The subsequent reduction in PRL observed as the course progressed, was postulated to be a dopamine inhibition of PRL, since dopamine, elevated during stress, is a well-established PIF (Aakvaag et al 1978).

Hagen and Galbo noted an enhanced PRL response to exercise after fasting in six males subjects (Hagen and Galbo 1979). They found that the subjects, who had fasted for 59 hours and then been subjected to short treadmill runs of 50% MVO2 for 10

minutes and 100% $\dot{V}O_2$ for 5 minutes, had a significant rise in plasma PRL levels, 7.3±2 ng/ml (pre-exercise), and 16.0±6 ng/ml (postexercise). However, the same exercise performed after just an overnight fast failed to elicit a significant change in PRL levels. Johannessen, working with Hagen and Galbo in 1981, reported on the PRL response to exercise following fat-enriched or carbohydrate-enriched diets (Johannessen, Hagen and Galbo 1981). The exercise protocol involved a prolonged treadmill run requiring 70% $\dot{V}O_2$. The subjects ran 30-minute bouts, separated by 10-minute rest periods, to exhaustion. The seven males in this study had a mean $\dot{V}O_2$ of 56 ml/kg/min, which for their age range, 24 to 29 years, is considered fit. Blood sampling was done before exercise, after every 30-minute run, at exhaustion, and after 30 minutes of recovery. The mean pre-exercise PRL value of approximately 12 ng/ml rose to a peak value of about 28 ng/ml after 106±5 minutes of exercise for the subjects given the high carbohydrate diet. The subjects given the diet high in fat had an increase in PRL from a mean pre-exercise value of approximately 8 ng/ml, to a peak value of about 39 ng/ml after 64±6 minutes of exercise (Johannessen, Hagen and Galbo 1981).

The study by Bazzarre and Royster also investigated the effect of a high fat or high carbohydrate diet on the PRL response to a 2-hour treadmill run requiring between 65% and 75% of maximum heart rate (Bazzarre and Royster 1984). The ten male marathon runners performing this exercise had blood samples taken before exercise, every 20 minutes during the run and 20 minutes after exercise termination. A bimodal pattern of PRL peaks was observed, one peak at 40 minutes of running and the other at 100 minutes, the latter peak being higher. The response of the subjects given the high carbohydrate diet was an increase in PRL concentration from a mean pre-exercise value of 8.9±4.4 ng/ml to 16.4±10.0 ng/ml at 100 minutes, compared to the response of subjects given a high fat diet of 8.1±3.2 ng/ml and 12.0±3.6 ng/ml, respectively (Bazzarre and Royster 1984).

Mayer et al had 10 male volunteers between 22 and 39 years old perform a bicycle ergometer test, maintaining a heart rate of 150 BPM for 20 minutes. Blood sampling was done before exercise and at 10-minute intervals for 60 minutes. PRL concentration rose from a mean pre-test value of approximately 7 ng/ml to a peak value of 13 ng/ml at termination of exercise (Mayer et al 1980).

Bratusch-Marrain et al had 3 males aged 21 to 42 years perform exercise at 50% $\dot{M}VO_2$ for 30 minutes as part of a study on hepatic disposal of endogenous PRL. (The mode of exercise was not specified.) Through catheters in a right hepatic vein and a brachial artery, simultaneous blood sampling was carried out prior to the onset of exercise and at intervals during and after exercise. No change in arterial PRL concentration was observed (Bratusch-Marrain et al 1979).

Cohen's group studied 24 subjects (twenty male and four female) with a mean age of 51 years, using the Bruce protocol of treadmill exercise (Cohen et al 1980). The subjects exercised to maximum predicted heart rate, myocardial ischemia or symptom limitation under this protocol. Blood samples were taken before exercise and immediately upon cessation of exercise. The pooled results gave a mean group pre-exercise value of 8.0 ± 4.1 ng/ml and a mean group immediate postexercise value of 10.6 ± 9.5 ng/ml (Cohen et al 1980).

Brisson's group studied seven females who had a sports history and five who did not (Brisson et al 1980). They performed at 75% $\dot{M}VO_2$ on a bicycle ergometer for 30 minutes. Blood sampling was done before exercise, every 15 minutes during exercise and for the 45 minutes recovery period. Only the subjects with a sports history had a significant elevation in plasma prolactin concentration, the peak at exercise termination being 48 ng/ml. Brisson's group studied the PRL

response to exercise in male athletes the following year (Brisson et al 1981). Their mean $\dot{M}VO2$ was 56.2±6.4 ml/kg/min. They performed three different workloads, 55%, 70% and 85% of $\dot{M}VO2$, on a bicycle ergometer for 20 minutes. These workloads were equivalent to an oxygen consumption of 30.9, 39.3, and 47.8 ml/kg/min, respectively. Blood sampling was done before, during and after exercise. Brisson reported that the PRL response to exercise seemed to relate to the work intensity in that no PRL increase was seen for the 55% $\dot{M}VO2$ workload, a slight increase was seen for the 70% $\dot{M}VO2$ workload and PRL almost doubled for the 85% workload. The peak PRL value for the latter two workloads occurred at exercise termination (Brisson et al 1981).

Prior's group studied nine women performing either 5 km run or 5 km of stationary bicycling (Prior et al 1981). The run was performed wearing a sport brassiere and also not wearing one, while the cycling was done wearing a brassiere in order to test the hypothesis that breast motion may play a role in the PRL increase seen with exercise. Blood sampling was done before exercise and at the termination of exercise. The results showed that PRL increased more with running than with cycling. Pooled data showed pre-exercise PRL levels of 8.4±2.9 ng/ml and immediate postexercise PRL levels of 11.9±5.8 ng/ml (Prior et al 1981).

Shangold and associates studied the PRL concentration changes in six female recreational runners after 30 minutes of running at their customary pace (Shangold et al 1981). Blood sampling before exercise revealed a mean group value for PRL of 17.0±2.7 ng/ml and the 60-second post exercise mean group value was 42.5±8.9 ng/ml. The decreased renal and hepatic blood flow during exercise, affecting the metabolic clearance of prolactin, was seen as a contributing factor to the observed PRL increase with exercise (Shangold et al 1981).

Moretti carried out three different experiments in his investigation of the PRL response to exercise (Moretti 1981, 1982, 1983). In 1981 Moretti et al studied eight professional middle-distance runners, four males and four females (Moretti et al 1981). Their 15 minutes of exercise was a treadmill protocol, consisting of a 6-minute warm-up period (2 minutes at 6 km/h, 2 minutes at 9 km/h and 2 minutes at 12 km/h), followed by a treadmill speed of 15 km/h until "maximum capacity" was reached. Blood samples were collected before exercise, when maximum capacity was reached, and at 15, 30 and 45 minutes of recovery. The results showed an acute rise in PRL during exercise, the peak being reached approximately 15 minutes into the recovery phase. The mean pre-exercise PRL value was 15±2 ng/ml for the females, 12±3 ng/ml for the males. The mean postexercise value was 74±11 ng/ml and 69±12 ng/ml, respectively (Moretti et al 1981). Moretti's next experiment, in 1982, tested six subjects (their sex was not stated) on a bicycle ergometer at 80% of their maximum heart rate (i.e., 170 BPM for 8 minutes). Blood sampling was done 20 minutes before exercise and immediately before the start of exercise for basal levels, as well as immediately upon cessation of exercise and at 5, 15, 30, 45 and 60 minutes of recovery. The results showed a mean PRL value of 1.9±0.8 ng/ml immediately before the start of exercise and a mean peak PRL value of 25.5±7.4 ng/ml at 15 minutes of recovery. (These are the results for the subjects given a saline infusion during the experiment. Moretti, subsequently used a pyridoxine infusion during an experiment with identical protocol, to study its involvement in PRL secretion control). The 1983 study by Moretti's group consisted of subjecting eight professional male athletes to a constant bicycle workload equal to 80% of their maximum heart rate for 18 to 20 minutes. Blood sampling was done before exercise, at exercise termination and during the 60 minute recovery period. The results showed that PRL rose from a mean basal level of 6.1±1.1 ng/ml to a mean peak

level of 19.5±1.9 ng/ml by the termination of exercise. (These results are also from the subjects given saline infusion during the experiment. Subsequently Moretti used naloxone infusion during an experiment with identical protocol to study its involvement in exercise-induced PRL release.)

Nguyen's study (1982) reported on the PRL response to exercise following mental work. Eighteen male volunteers, classified as moderately fit, and between 19 and 21 years old, performed a 20-minute outdoor run at 50% to 70% MVO2. Blood sampling was done 15 minutes before exercise started and again 15 minutes after exercise stopped. The results showed that for the ten subjects who had attended a lecture (non-stressful mental work) prior to the exercise session, PRL rose from a mean pre-exercise level of 3.7±0.2 ng/ml to a postexercise level of 10.9±1.6 ng/ml. However, for the eight subjects who had written an examination (mental work associated with emotional stress) prior to the exercise session, PRL fell from a mean pre-exercise level of 13.3±2.4 ng/ml to a postexercise level of 5.5±0.3 ng/ml. Nguyen concluded that previous emotional stress could inhibit PRL response to exercise (Nguyen et al 1982).

Jezová's two experiments also found PRL levels increased after submaximal exercise on a bicycle ergometer (Jezová et al 1982, 1983). The first experiment involved eleven untrained male volunteers aged 21 to 24 years, who exercised on a bicycle ergometer at four workloads of ever-increasing intensity for 20 to 22 minutes, depending on the physical fitness of the subject. Blood samples were taken at before exercise, at termination of exercise and at 10, 30, 60, and 90 minutes of recovery. The results showed a mean pre-exercise PRL value of 4.5±1.1 ng/ml and an immediate postexercise value of 7.4±1.5 ng/ml. The peak PRL value of 8.4±2.0 ng/ml was reported 10 minutes into the recovery interval. The second experiment

similarly involved twelve untrained male volunteers, aged 20 to 23 years, exercising on a bicycle ergometer. Three workloads of increasing intensity were performed, each one being of 6 minutes duration. Blood sampling was done 30 minutes before exercise, immediately prior to exercise, immediately at cessation of exercise and at 10, 30, and 60 minutes into recovery. The results showed a mean pre-exercise PRL level of 4.5 ng/ml, which rose to approximately 7.5 ng/ml by the cessation of exercise, and reached a peak value of approximately 8.5 ng/ml 10 minutes into recovery.

In a recent study De Meirleir and associates (1985) reported that the exercise-induced prolactin release is related to anaerobic threshold (AT). They studied the PRL response to exercise conditions below and above AT. The below-threshold test involved ten males exercising on a bicycle ergometer for 1 hour at an average workload intensity corresponding to 44% to 55% of their $\dot{M}VO_2$. Venous blood samples for PRL assay were drawn from an indwelling arm catheter before exercise and at 10 and 30 minutes of exercise, and 60 minutes after cessation of exercise, with simultaneous arteriolar samples for lactate assay being taken from the earlobe. The same subjects were tested again with exercise conditions above AT; with a maximal test on the same bicycle ergometer, using graded increases in workload and a duration of 20 minutes. Blood samples for prolactin and lactate assay were drawn as described above, at the start of exercise, at 15 minutes into exercise and at cessation of exercise or exhaustion. The results for the below-threshold test showed that the PRL levels were not significantly different from the control test (controls had rested for 1 hour in bed and walked around for 1 hour) and no systemic increase in lactic acid (i.e., above 4 mmol/L) had occurred. For the above-threshold test, a sharp rise in PRL occurred parallel to an accelerated increase in lactic acid. The PRL rise continued after cessation of exercise, peaking at

around 5 minutes of recovery. The researchers concluded that exercise intensity must be such that AT is reached to induce any increase in PRL levels. De Meirleir's study was published June 1985, when this thesis was in press. This abstract is included here, but his results are excluded from Section 2.6.

2.6

PATTERN OF PROLACTIN RESPONSE TO EXERCISE

Seventeen experiments have shown that the blood prolactin concentration rose in response to exercise over time. There were also many non-exercise-related factors included in these studies, which may have interfered with the interpretation of the prolactin response to exercise. These factors included fasting, diet, temperature and sleep deprivation.
Two authors, Bratusch-Marrain (1979) and Brisson (1980 for the females with no sports history) reported no rise in prolactin concentration in response to exercise and Aakvaag (1978) reported reduced prolactin levels during the 5-day combat course his subjects experienced.

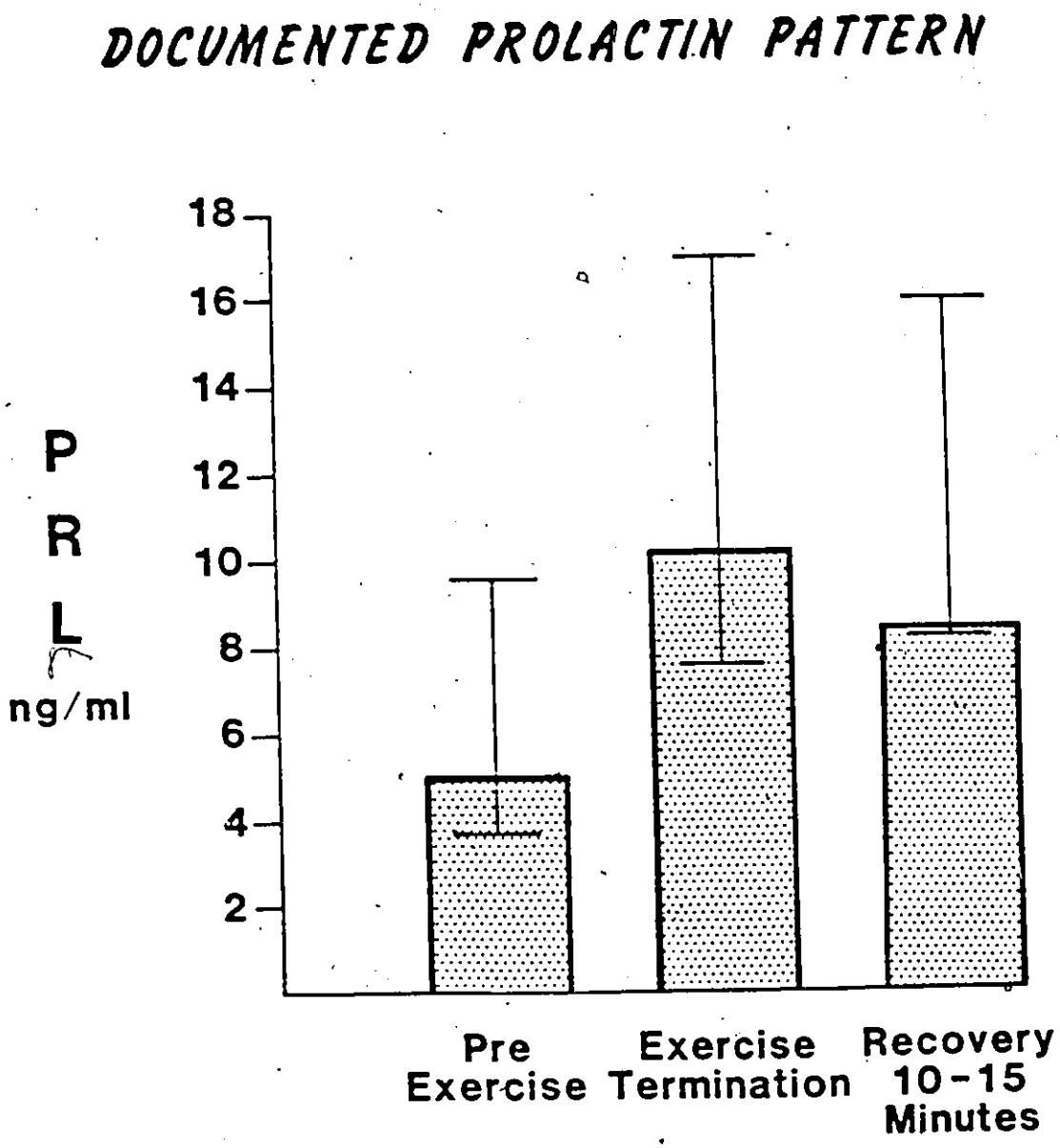
There was a variation of blood sampling intervals cited in these 19 studies reporting on the PRL response to exercise over time. Blood sampling during exercise was done in only six of the studies. Four of these had an exercise duration 30 minutes (Bratusch-Marrain 1979, Mayer 1980, Brisson 1980, 1981) and the other two were prolonged treadmill runs of 1 to 2 hours' duration (Johannessen 1981, Bazzarre 1983). The reports of PRL increase from the short-duration studies indicated that the onset of PRL rise occured at the onset of exercise, with the peak concentration at the point of exercise termination. Johannessen's study also reported the peak PRL concentration at the point of exercise termination, but

Bazzarre observed a bimodal pattern of PRL peaks, one occurring at 40 minutes and the other at 100 minutes of the 120 minute treadmill run.

The other studies used only pre- and postexercise blood sampling in the experimental protocol. No samples were taken during exercise to identify the timing of the onset of prolactin increase. When blood sampling was done only pre-exercise and upon immediate cessation of exercise, peak PRL values were reported at exercise cessation (Cohen 1980, Hagen and Galbo 1979, Prior 1981, Shangold 1981). No recovery samples were available to indicate that an even higher level may have been reached during recovery. When the first post-exercise sample was taken 5 to 15 minutes into recovery, peak PRL values were reported as having occurred during recovery (Jezová 1983, 1982, Moretti 1982, 1981, Nguyen 1982, Noel 1972). An awareness of the blood sample timing and frequency is important for interpreting the results of the studies of PRL response to exercise conditions over time. Extrapolation, not actual measurement, may be inaccurately describing the PRL response to exercise.

The pattern of prolactin response to exercise has been compiled from a review of the literature. The studies used in this compilation were limited to those with male subjects and an exercise duration of 30 minutes or less. The reported blood sampling times were before exercise, at cessation of exercise, and at 10 to 15 minutes of recovery. The five studies meeting these criteria are outlined in Appendix A. Using the median PRL concentration levels and the range, a bar diagram was compiled. It describes the documented pattern of PRL response to exercise as a PRL increase during exercise, with the peak concentration occurring at exercise termination or early in recovery, followed by a gradual decline in concentration thereafter. Figure 2.6 presents this documented PRL pattern.

FIGURE 2.6



MEDIAN AND RANGE LEVELS ARE DEPICTED

2.7

RATIONALE FOR THE STUDY

The numerous factors not related to exercise that occurred in the studies reporting on the PRL response to exercise, eg., fasting, diet, temperature, and sleep deprivation may interfere with the interpretation of the PRL response to exercise. Blood sampling during exercise, carried out in only 6 studies, was deemed to be a salient feature that should be included in the investigation of the prolactin response to exercise. In reviewing this literature, it was apparent that little is known about the PRL response to exercise in relation to the physical capacity or fitness of the subjects. The recent identification of various sport-related infertility syndromes in males and females makes the PRL response to exercise a topical subject.

It was therefore considered relevant to study the response of serum PRL concentration in males, to a standard $\dot{M}VO_2$ treadmill stress test to self-perceived exhaustion. The results may provide information regarding the kinetics of PRL and oxygen consumption, and the influence of physical fitness on the PRL response to the stress test.

2.8 PURPOSE OF THE STUDY

Within the context of this study three questions are raised, and each serves as a working hypothesis. The questions are stated as follows:

1. Would the present study concur with the pattern of PRL response described in the review of literature: that is, PRL increase during exercise, with the peak occurring at exercise termination or early in recovery, followed by a gradual decline in concentration thereafter?

2. Would an increase in the rate of oxygen consumption during exercise be accompanied by an increase in PRL concentration?

3. Would there be a relationship between PRL increment and $\dot{M}VO2$?

The purpose of the study is to provide evidence to answer these questions.
It is noted here that the PRL increment means the change from pre-exercise to peak value and that the $\dot{M}VO2$ is volitional $\dot{M}VO2$.

CHAPTER 3
RESEARCH METHODOLOGY

3.1

INTRODUCTION

This investigation was exploratory. It was performed in order to examine the serum prolactin concentration in males in response to a standard $\dot{M}VO_2$ treadmill stress test. An attempt was made to discover if differences in physical fitness as indicated by $\dot{M}VO_2$ would affect the prolactin response. This chapter outlines the testing environment and instrumentation, the subject selection and orientation, the experimental protocol and the method of data analysis.

3.2

TESTING ENVIRONMENT AND INSTRUMENTATION

The subjects were tested in the Kinanthropology Laboratory 306, at the University of Ottawa. The personnel present during each testing session included the author, the thesis advisor and two laboratory technicians. A Quinton (Seattle, Washington) treadmill with Quinton Instruments Treadmill Control was used. The ECG monitoring equipment used was Avionics Stress Test Monitor Cardioguard MODEL 2900B (Montreal, Quebec). The physiograph model used was Physiograph Four-8 Narco Bio-systems, Inc. (Houston, Texas).

The Respironic, Speak-Easy Stress Test Mask was the model used for expired air collection in the Tissot Tank for volume measurement. The Godart Oxygen analyzer and the Godart Capnograph CO2 analyzer (Stratam - B.V., Bilthoven, Holland) were used for the expired air analysis. The other lab technician, hired from Riverside Hospital's intravenous team, was responsible for the installation of the anticubital vein catheter and all the blood collection procedures. He used an 18G x 1 1/4" Abbocath.T catheter with a 20-inch length of plastic tubing leading to a 3-way stopcock. Sterile saline was infused after each blood collection to keep the catheter patent and replace fluid losses.

3.3 SUBJECT SELECTION AND ORIENTATION

The subjects in this study were 19 human male volunteers ranging in age from 20 to 29 years, and included a set of twins. They were answering a poster advertisement requesting volunteers for an $\dot{M}VO_2$ study including blood analysis for hormone levels. They were in good health, asymptomatic for any disease and had no history of discomfort during exertion. They were required to complete the health appraisal form in Appendix B and the recreation activity questionnaire in Appendix C. The physical characteristics of the subjects are outlined in Appendix D.

The subjects were required to attend an orientation session at least one week prior to their test day at the Kinanthropology Laboratory at the University of Ottawa, so that they could become familiar with the experimental environment and requirements. The purpose of the study was outlined and the test procedures and risks involved explained fully. The

subjects were informed that for the actual MVO2 test they would be encouraged to complete as many stages as possible and would be asked to continue to their self-perceived maximum effort and point of exhaustion. Furthermore, no subject would be forced to continue for any reason, and at his signal, the exercise portion of the experimental protocol would be terminated immediately. The subjects were then allowed to complete Stages 1 to 5 of the MVO2 treadmill test as a practice run. They wore the face mask, ECG electrodes and had the brachial catheter taped to their right arm. This practice run simulated the actual experimental conditions and allowed the subjects to experience how the apparatus, tubes and wires would affect their performance. It also served to reduce anxiety on test day in that all procedures, except the insertion of the brachial catheter, were experienced by each subject. Heart rate was monitored during the practice run, so that if any ECG abnormalities became apparent during or after this exercise, the subject could be asked to withdraw from the experiment.

The subjects were encouraged to ask questions to clarify any points that were unclear to them.

After this orientation session, the subjects who wished to volunteer to participate in the experiment were required to sign the informed consent form in Appendix E.

Each subject then performed the Canada Fitness Step Test in order for the author to evaluate his general physical fitness. (Standardized Test of Fitness 1981). These results are recorded in Appendix D, under Calculated Aerobic Capacity.

3.4

EXPERIMENTAL PROTOCOL

3.4.1

PRE-EXERCISE RESTING STATE

The individual testing sessions were scheduled for 8:30 a.m. or 10:30 a.m. (fifteen subjects were tested at 8:30 a.m. and four at 10:30 a.m.). Each subject arrived at the laboratory following an overnight fast. The resting state, consisting of 45 minutes of bed rest, began immediately. The catheter was inserted into the antecubital vein of the right arm. Three 5.0 ml blood samples were collected to determine the pre-exercise serum prolactin concentration. The first sample was taken 15 minutes after the insertion of the catheter and the two subsequent samples at 15-minute intervals. A 1.5 ml sample was also taken and divided for the pre-exercise lactate and hematocrit analysis.

The ECG electrodes were attached and heart rate monitoring was begun 5 minutes prior to the start of the $\dot{M}VO_2$ test.

3.4.2

EXERCISE STATE: TREADMILL $\dot{M}VO_2$ TEST AND PARAMETERS RECORDED

Each subject performed the exercise protocol outlined in Table 3.4.2, continuing for as many stages as possible until he signalled his self-perceived maximum effort and point of exhaustion. At this point the $\dot{M}VO_2$ test was terminated.

TABLE 3.4.2

MVO2 TREADMILL TEST PROTOCOL

MEDIUM HIGH INTENSITY PROGRAMME
JETTÉ M, DEPARTMENT OF KINANTHROPOLOGY
UNIVERSITY OF OTTAWA

STAGE	DURATION (minutes)	SPEED (mph)	GRADE (%)
1	2	3.0	2.5
2	4	3.0	5.0
3	6	3.0	7.5
4	8	3.5	7.5
5	10	3.5	10.5
6	12	3.5	12.0
7	14	3.5	14.0
8	16	3.5	16.0
9	18	3.75	16.0
10	20	3.75	18.0
11	22	3.75	20.0
12	24	3.75	22.0
13	26	3.75	24.0
14	28	3.75	26.0

HEART RATE MONITORING

Heart rate was carefully monitored throughout the treadmill $\dot{V}O_2$ test. Heart rate was recorded in the final 10 seconds of every minute during the stress test and at the point when the subject signalled self-perceived exhaustion.

BLOOD SAMPLING

A blood sample was taken in the final 30 seconds of every 2nd stage, (i.e., every 4th minute). The final exercise blood sample was taken when the subject signalled self-perceived exhaustion.

EXPIRED GAS COLLECTION

Expired gases were collected during the final 30 seconds of every 2nd stage, (i.e. every 4th minute), up to the 10th stage. Thereafter they were collected in the final 30 seconds of every stage and at the point when the subject signalled self-perceived exhaustion.

3.4.3

POSTEXERCISE RECOVERY STATE

The total recovery time lasted 30 minutes for all subjects. The postexercise cool-down period was the first 6 minutes of recovery. It began immediately after the subject signalled exhaustion and the $\dot{V}O_2$ test was terminated. The treadmill was gradually lowered to 0% grade and a 1.5 mph walking speed for a 1- to 2-minute cool-down walk. Heart rate monitoring

was continued during this time. The subject was then allowed to leave the treadmill and walk around the lab for several minutes, accompanied by a second person. After approximately 5 minutes of recovery, the subject returned to a chair positioned on the treadmill for the HR monitoring and blood sampling. At the 6th minute of recovery a 6.5 ml blood sample was collected and divided; 5.0 ml was used for prolactin assay and 1.5 ml for post exercise lactate analysis and hematocrit reading. HR was monitored for 1 minute at the 6th minute interval.

The subject stayed sitting for the remainder of the recovery period. At the 12th minute of recovery a 5.0 ml blood sample for prolactin was taken and heart rate was recorded for 1 minute. This series was repeated at the 18th, 24th and 30th minute. At termination of the 30-minute recovery period the technician removed the catheter and applied pressure over the area for 5 minutes. The ECG electrodes were also removed. The subjects were then offered orange juice and bananas ad libitum in view of their overnight fast. They were allowed to shower and afterward returned to the lab to have the catheter site examined and taped by the technician. They reported to the author how they felt and were allowed to leave the laboratory if everything was satisfactory.

3.5 BLOOD SAMPLING PROCEDURES

All blood samples were collected through the brachial catheter into a syringe and transferred into the appropriate vacutainer. An equal volume of sterile saline was then infused to keep the catheter patent and to replace lost blood volume. All blood samples for prolactin consisted of 5.0 ml.

They were transferred into a nonheparinized vacutainer and allowed to stand at room temperature until clotting occurred. They were then centrifuged at 2000 to 3000 RPM for 10 minutes and the serum was frozen at -16°C for future analysis.
Two 1.5 ml blood samples were collected for lactate assay and hematocrit reading. The pre-exercise sample was taken in conjunction with the second pre-exercise prolactin sample and the postexercise sample with the 6th minute recovery prolactin sample. These samples were tranferred to a heparinized vacutainer. For future lactate assay, 0.5 ml was deproteinized with 1.0 ml perchloric acid, centrifuged at 3000 RPM for 10 minutes and the supernatant was frozen at -16°C. The remaining blood sample was placed in ice water in the refrigerator at -5°C for use in subsequent hematocrit readings.

3.6

ASSAY PROCEDURES

Dr. D. Greenway at the Biochemistry Laboratory of the Ottawa General Hospital carried out the prolactin assay and the lactate assay.
Prolactin was assayed with the Amersham Prolactin RIA Kit (Amersham Corporation, Oakville, Ontario). This method uses an antibody against human prolactin raised in sheep. Its cross reactivity with human growth hormone is 0.2%; no detectable cross reactivity is seen with HPL, LH, FSH, HCG and TSH. This method employs the second antibody technique for separating free and bound radioactivity. All the serum prolactin samples for each subject were assayed in duplicate in the same batch. The reproducibility of the Prolactin RIA Kit is summarized in Table 3.6.1.

TABLE 3.6.1

REPRODUCIBILITY OF THE PROLACTIN RIA KIT

CONTROL	A	B	C	D
WITHIN ASSAY				
Mean of 40 duplicate pairs < 10 (ng/ml)	4.5			
Mean of 10 replicates (ng/ml)		17.0	38.9	75.3
Standard Deviation	0.6	0.7	1.4	1.8
Coefficient of variation (%)	13.1	4.3	3.5	2.4
BETWEEN ASSAY				
Mean of duplicates from 20 separate assays (ng/ml)		16.6	38.9	74.1
Standard Deviation		.95	1.8	4.3
Coefficient of variation (%)		5.7	4.6	5.8

A is the within assay precision calculated using the duplicate pair results applied in the formula:

$$SD = \sqrt{\frac{\Sigma d^2}{2n}}$$

d = difference between pairs

n = number of pairs

B, C, D are the results supplied by the manufacturer.

Dr. Greenway suggested excluding the first PRL sample after catheterization, from mean pre-exercise value, in an attempt to reduce any anxiety-induced high PRL reading at that sampling time. Therefore, any reference to pre-exercise PRL levels throughout the text refers to the last two samples prior to exercise.

The prolactin assay precision at the level of less than 10 ng/ml was established at 0.6 (Table 3.6.1).

The criterion for PRL concentration change was established at the 99.8 percentile, on advice from Dr. R.W. Hudson (personal communication 1985). As is customary, the errors of measurement were assumed to be normally distributed. In this framework, two observations, X1 and X2, from separate assays are considered different whenever they are at least three SD apart. For example: $X2 > X1 + 3\ SD$

$X2 < X1 - 3\ SD$

This restriction can be interpreted as follows: an observation is either below the 0.2 percentile or above the 99.8 percentile on the distribution of X1, which is assumed to be normal $(\overline{X1}, SD^2)$. Applying this interpretation to the data from the 40 duplicate pairs, ± 3 SD or 1.8 ng/ml was the criterion for minimal PRL concentration increase.

Lactate was assayed by the Boehringer-Mannheim GmbH Diagnostica Test - Combination kit method (Dorval, Quebec). This method is a spectrophotometric procedure based on the increase of NADH following the lactate dehydrogenase reaction.

3.7

DATA ANALYSIS

Due to the exploratory nature of this study, and with three questions raised as working hypotheses, descriptive analysis of the data was an important feature. The Sign test was employed to determine the statistical significance of the number of subjects showing a PRL concentration increase. The Least Square Method was used to derive a mathematical expression for the best fit to relate $\dot{V}O_2$ to PRL. Regression analysis was applied to determine if a relationship existed between PRL increment and $M\dot{V}O_2$ or between peak PRL and $M\dot{V}O_2$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1

OVERALL PERFORMANCE AND PHYSIOLOGICAL CHARACTERISTICS

In the context of this study, overall performance refers to the pre-exercise, exercise and recovery states. The subjects are grouped for presentation of overall performance to simplify the descriptive analysis and graphics. The subjects in each group are related to each other by having stopped at the same stage of the MVO2 test, and they therefore shared a common duration and intensity of exercise. The number given to the groups correspond to the stage achieved on the MVO2 test. The seven groups in Table 4.1.1 result from this relationship.

TABLE 4.1.1

SUBJECT GROUPING FOR OVERALL PERFORMANCE

GROUP	NUMBER OF SUBJECTS	DURATION OF EXERCISE (min)	DURATION OF RECOVERY (min)
8	3	16	30
9	3	18	30
10	2	20	30
11	2	22	30
12	4	24	30
13	4	26	30
14	1	28	30

Two different graphs are used to demonstrate the PRL kinetics during the overall performance. Figure 4.1.1 presents the overall performance in minutes, from the beginning of exercise, to the end of the 30-minute recovery period. This graph demonstrated the general profile for PRL kinetics. Because the subjects stopped the $\dot{M}VO2$ test at their self-perceived maximum effort, or point of exhaustion, the range of exercise duration for the seven groups was 16 to 28 minutes. The exercise time common to all subjects in this graph was the first 16 minutes. At this point, three subjects stopped exercising and started their recovery period, while the other sixteen subjects continued exercising. For this reason, in this graph there cannot exist a common delineation between exercise and recovery in the PRL profiles.

Figure 4.1.2 presents the overall performance delineating the point at which exercise is terminated and recovery is begun. Focusing on this point, which was common to all subjects although it occurred at different times, provided a clear separation between the PRL kinetics during exercise and during recovery. In this graph, the 16 minutes prior to exercise termination and the 30 minutes of recovery are common to all seven groups.

FIGURE 4.1.1

OVERALL PERFORMANCE IN REAL TIME

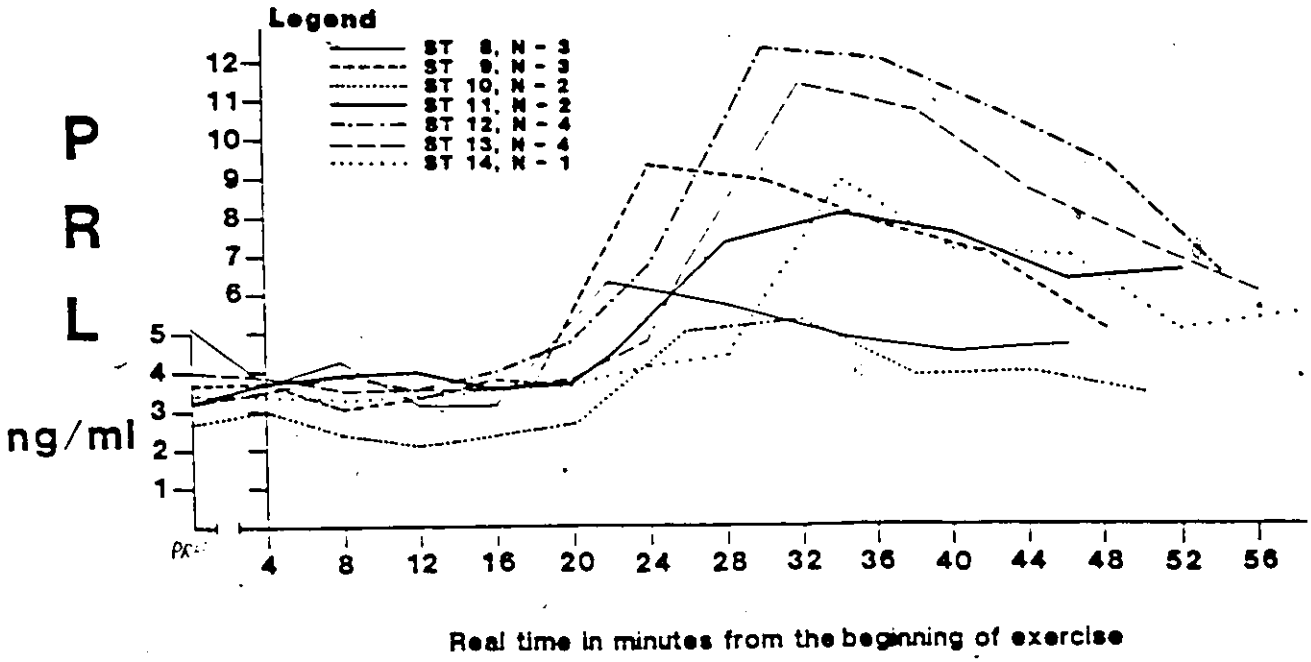
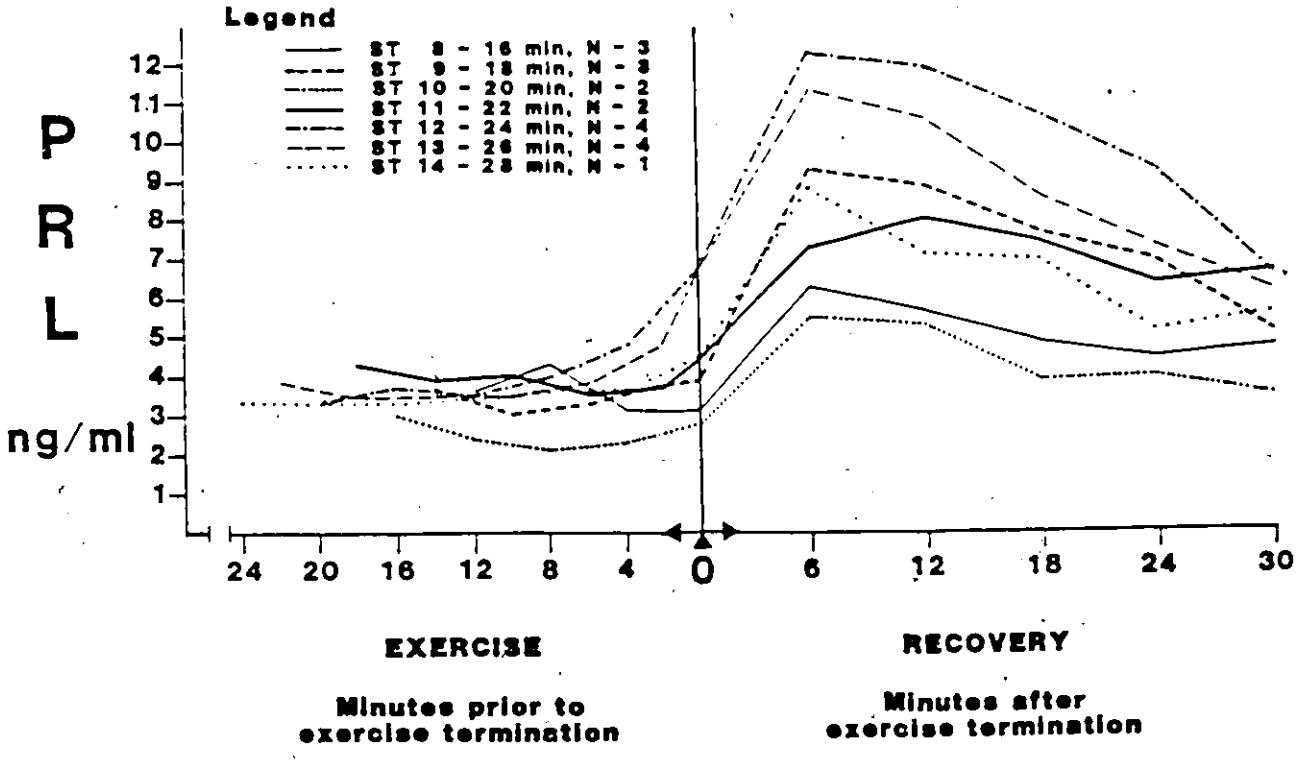


FIGURE 4.1.2

OVERALL PERFORMANCE DELINEATING EXERCISE AND RECOVERY



Certain features of PRL kinetics were observed during the overall performance.

Figure 4.1.1 revealed a consistent pattern of stable PRL concentration for all groups, for a duration in the order of 20 minutes after the start of exercise, with an acute rise thereafter. The onset of this rise appears to have occurred during exercise for those groups who exercised beyond the 20-minute mark, and during recovery for those who had already stopped exercising prior to or approximately at the 20-minute mark. Some fluctuation in PRL concentration was observed during this time, but not above the 1.8 ng/ml criterion. Peak PRL concentration, calculated from the beginning of exercise, occurred in the range was 22 to 34 minutes.

From Figure 4.1.2 a number of observations can be made. All peak PRL values occurred after the subjects had stopped exercising, at a mean time of 8 ± 3 minutes after the termination of exercise. When the onset of a rise in PRL concentration occurred during exercise, it appeared just prior to exercise termination. Only Group 12 and 13 showed this onset, the other five groups did not exhibit a rise in PRL during exercise. At the end of the 30-minute recovery stage the PRL concentration was still elevated above the pre-exercise levels.

The statistics describing the physiological characteristics of the nineteen subjects are presented, using means and standard deviations, in Table 4.1.2. In this table the increment is the pre-exercise prolactin value subtracted from the peak value. Appendix F provides the physiological characteristics, and Appendix G provides the raw data for all parameters observed.

TABLE 4.1.2

PHYSIOLOGICAL CHARACTERISTICS OF THE SUBJECTS

PARAMETER	MEAN	SD
Age (years)	25	2.9
Weight (lbs)	170	19.0
Height (ft/in)	5.8	.4
$\dot{M}VO2$ (ml/kg/min)	49	7.5
MHR (BPM)	183	9.2
Prolactin (ng/ml)		
pre-exercise	3.7	1.2
1st 16 minutes	3.5	1.0
exercise termination	4.9	2.4
peak	9.8	5.5
increment	6.1	5.3
ratio (peak)/(pre)	2.6	
30 minutes recovery	5.6	2.6
Lactate (mg/100 ml)		
pre-exercise	9.3	4.5
postexercise 6 minutes	66.3	17.8
Hematocrit		
pre-exercise	43	2.0
postexercise 6 minutes	47	2.0

The magnitude of the PRL response in this study was a mean 2.6-fold increase in peak over pre-exercise levels. Seven other studies had also reported at least a doubling in PRL concentration in response to exercise (Brisson 1980, Johannessen 1981, Moretti 1981, 1982, 1983, Nguyen 1982, Shangold 1981).

The mean pre-exercise value for PRL, 3.7±1.2 ng/ml, with a range of 1.0 to 7.2 ng/ml, is within the normal "basal" PRL concentration range for males (Frantz 1978, Noel 1972). The mean PRL concentration for the first 16 minutes of exercise was 3.5±1.0 ng/ml and the mean PRL concentration at the termination of exercise was 4.9±2.4 ng/ml. The mean PRL concentration at 30 minutes of recovery was 5.6±2.5 ng/ml. The mean peak PRL concentration was 9.8±5.5 ng/ml. All peak PRL values in this study occurred after the subjects stopped exercising, at a mean time of 8±3 minutes after termination of exercise. Peak PRL concentration calculated from the beginning of exercise occurred at a mean time of 30±5 minutes, with a range of 22 to 34 minutes.

Other authors reporting PRL peak in recovery were De Meirleir 1985, Jezová 1982, 1983, Noel 1972, Moretti 1981, 1982, 1983, and Nguyen 1982. The PRL increment was a mean value of 6.1±5.3, with a range of 0.0 to 20.7 ng/ml. One subject failed to show any increase in PRL concentration, but the other eighteen all satisfied the criterion of 1.8 ng/ml change in PRL concentration. Using a Sign test, a highly significant number ($p \leq 0.001$) of subjects showed a PRL concentration increase.

The range of PRL increment in this study is in accord with the increments reported by Noel 1972, Frewin 1976, Mayer 1980, Brisson 1981, Prior 1981, Jezová 1982, 1983, Nguyen 1982, Moretti 1982, 1983, Cohen 1980, and Bazzarre 1983. Three studies showed a mean increment beyond this range. Johannessen reported a mean PRL increment of 27 ng/ml for his

male subjects following a fat-enhanced diet (Johannessen 1981); Shangold and associates reported a 25 ng/ml mean PRL increment for the six trained women in their study (Shangold et al 1981), and Moretti reported a mean PRL increment of 60 ng/ml (pooled results) for the four men and four women professional middle distance runners that his group studied (Moretti et al 1981).

The mean $\dot{V}O_2$ in this study was 49±7.5 ml/kg/min, with a range of 36 to 62 ml/kg/min. According to Jetté's norms for this medium-high intensity stress test, fifteen of the subjects fall into the average and above average fitness category (Jetté 1982; Appendix I).

The mean maximum heart rate was 183±9.2 BPM, and the range was 167 to 195 BPM. The mean heart rate response for each stage is lower in this study, up to ST 12, than the norms reported by Jetté (Jetté 1982; Appendix I). Those norms were not age grouped, which may explain the higher value.

The mean pre-exercise lactate level measured in this study was 9.3±4.5 mg/100 ml, with a range of 5.1 to 19.5 mg/100 ml. The mean post exercise lactate level was 66.3±18 mg/100 ml, with a range of 33.8 to 101.0 mg/ml. Expressed in mmol/L, the mean lactate level pre-exercise was 1.0±0.5 and the mean 6 minute postexercise level was 7.4±2.0.

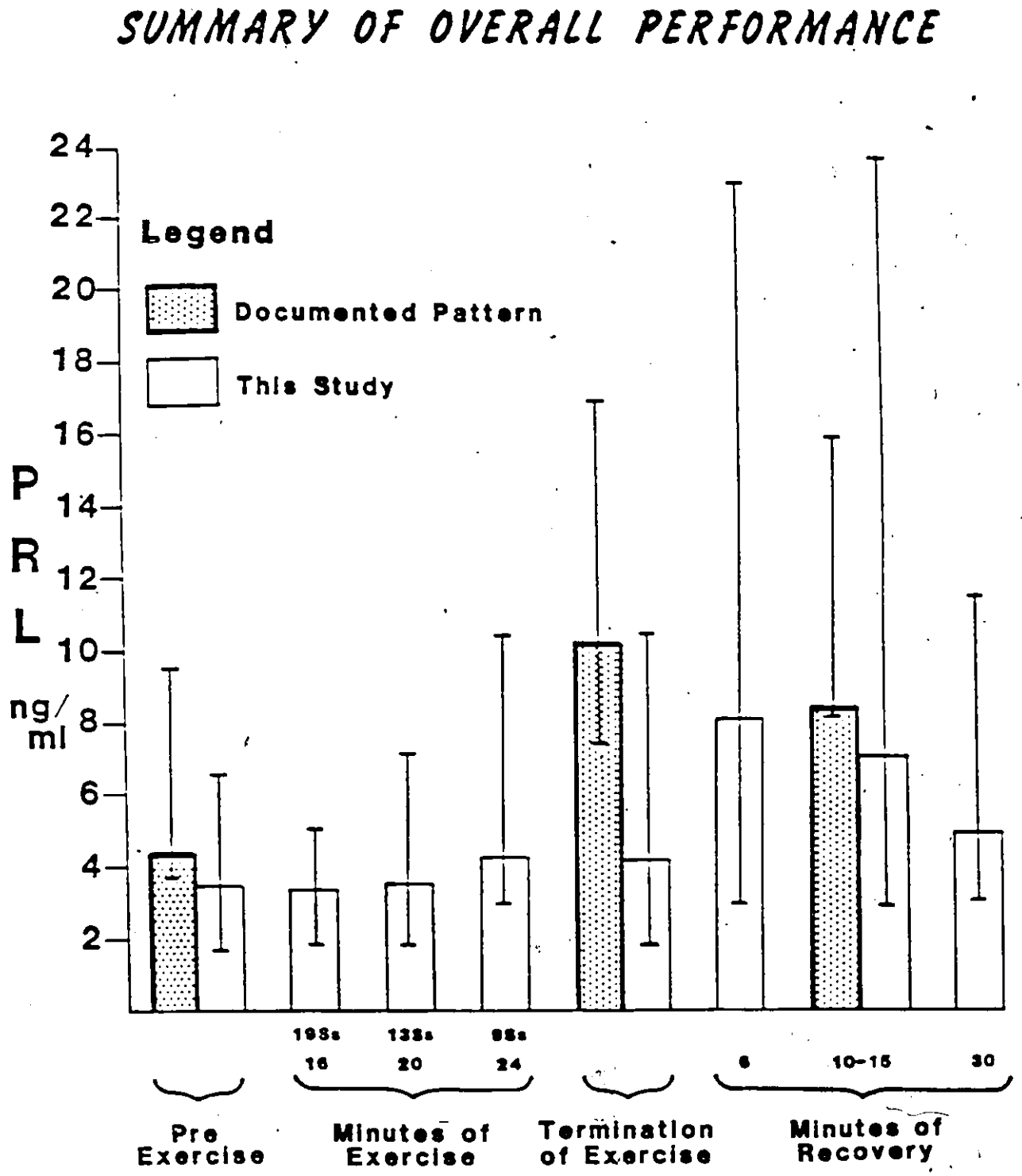
The mean pre-exercise hematocrit was 43±2.0 and the mean 6 minute postexercise hematocrit was 47±2.0.

SUMMARY OF OVERALL PERFORMANCE

The blood sampling frequency in this study, consisting of 3 pre-exercise samples, one sample every 4th minute during exercise, one at termination of exercise and one sample every 6th minute during the 30-minute recovery period, made available a minimum of twelve blood samples per subject. (The subjects continuing on the treadmill beyond 16 minutes had more than 12 samples). This frequent monitoring for PRL changes in the blood was responsible for revealing the PRL kinetics during the overall performance. The nature of this PRL response is summarized in Figure 4.1.3.

Figure 4.1.3 presents the median values and the range from the results in Figure 4.1.1, the overall performance graph. It also shows the documented PRL pattern previously described in Figure 2.6 to permit comparison. From this comparison, evidence is provided for the first working hypothesis. The results of this study concur with the documented pattern of PRL increase during exercise, with the peak occurring at exercise termination or early in recovery, followed by a gradual decline thereafter. The three common intervals for PRL measurement show an overlap in the range of concentration, even taking into account the differences in experimental protocol. The median PRL values in this study are lower, however, the general PRL pattern is not contradictory to the documented one. The frequency of blood sampling in this study was responsible for a more global and refined description of the PRL response. It revealed the stability of PRL concentration during exercise, for a duration in the order of 20 minutes, and the subsequent acute rise to peak in the recovery period.

FIGURE 4.1.3


SUMMARY OF OVERALL PERFORMANCE
Legend
Documented Pattern
This Study
PRL ng/ml
24
22
20
18
16
14
12
10
8
6
4
2
195s
16
135s
20
85s
24
5
10-15
30
Pre Exercise
Minutes of Exercise
Termination of Exercise
Minutes of Recovery

4.2

PROLACTIN KINETICS DURING THE $\dot{V}O_2$ TEST

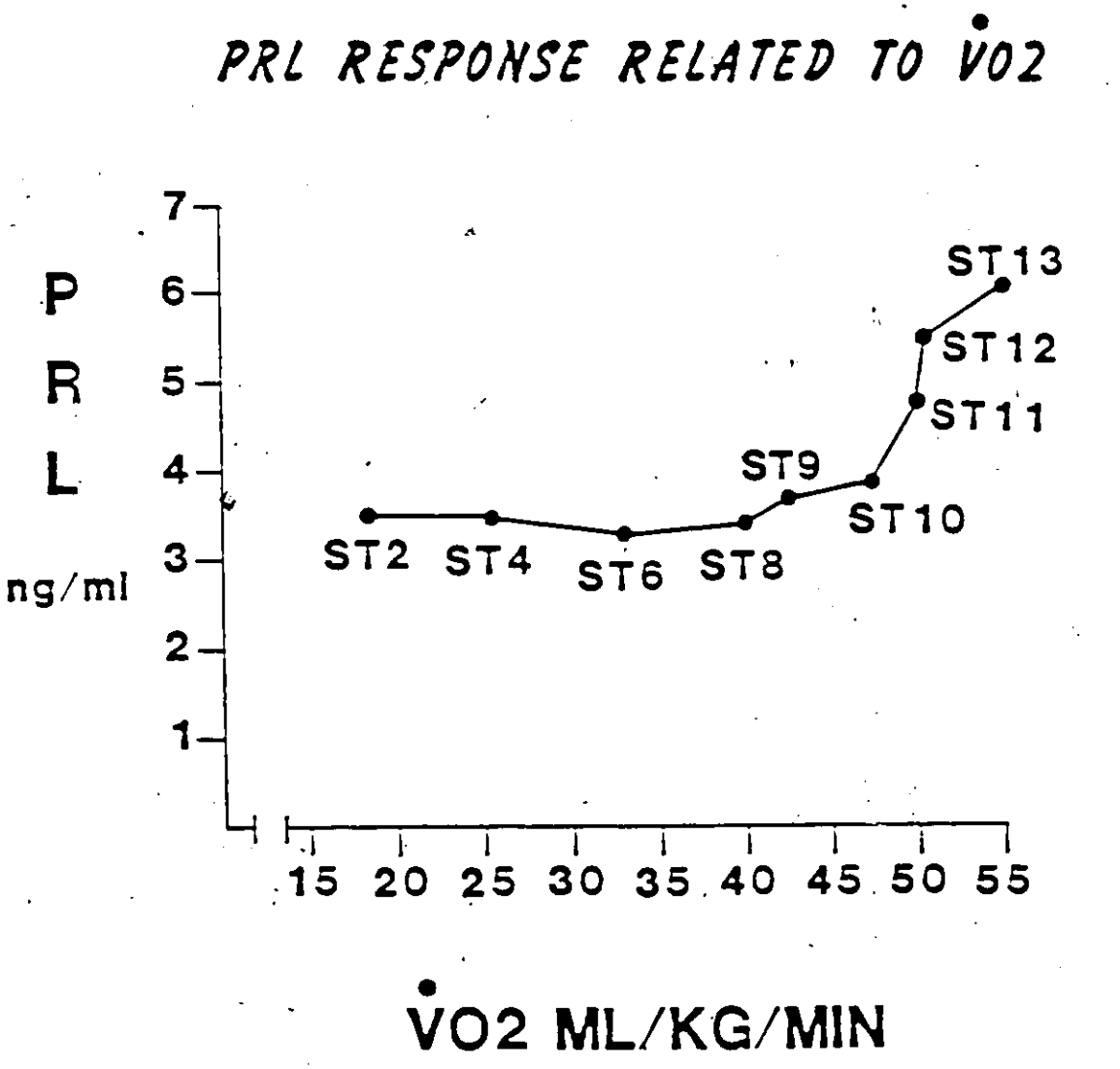
The prolactin kinetics occurring during the $\dot{V}O_2$ test that were related to the duration of exercise and the rate of oxygen consumption are presented in this section. The results were examined globally and at a more detailed level. The PRL concentrations used are the mean absolute values. Figure 4.2.1 provides the global description of prolactin kinetics during the $\dot{V}O_2$ test. Each point on the graph represents the stage of the $\dot{V}O_2$ test, the minutes of exercise completed, the mean PRL concentration and the mean volume of oxygen consumption for the number of subjects completing that stage. The means and standard deviations for the parameters described in Figure 4.2.1 are presented in Appendix I.

A detailed description of the prolactin kinetics related to the duration of exercise and to the volume of oxygen consumption is possible by a stage-wise graphing of the $\dot{V}O_2$ test. All subjects who completed the same stages of the test were grouped together. Six groups result from this division:

1. 19 subjects completed ST 1-8
2. 16 subjects completed ST 1-9
3. 13 subjects completed ST 1-10
4. 11 subjects completed ST 1-11
5. 9 subjects completed ST 1-12
6. 5 subjects completed ST 1-13

In this context, Figure 4.2.2 presents the six graphs that describe the prolactin kinetics in response to exercise of varying duration, and Figure 4.2.3 presents six graphs that describe the prolactin response to an increasing volume of oxygen consumption.

FIGURE 4.2.1



Legend

ST 2 - 4 min, N - 19
ST 4 - 8 min, N - 19
ST 6 - 12 min, N - 19
ST 8 - 16 min, N - 19
ST 9 - 18 min, N - 16
ST 10 - 20 min, N - 13
ST 11 - 22 min, N - 10
ST 12 - 24 min, N - 9
ST 13 - 26 min, N - 5

FIGURE 4.2.2

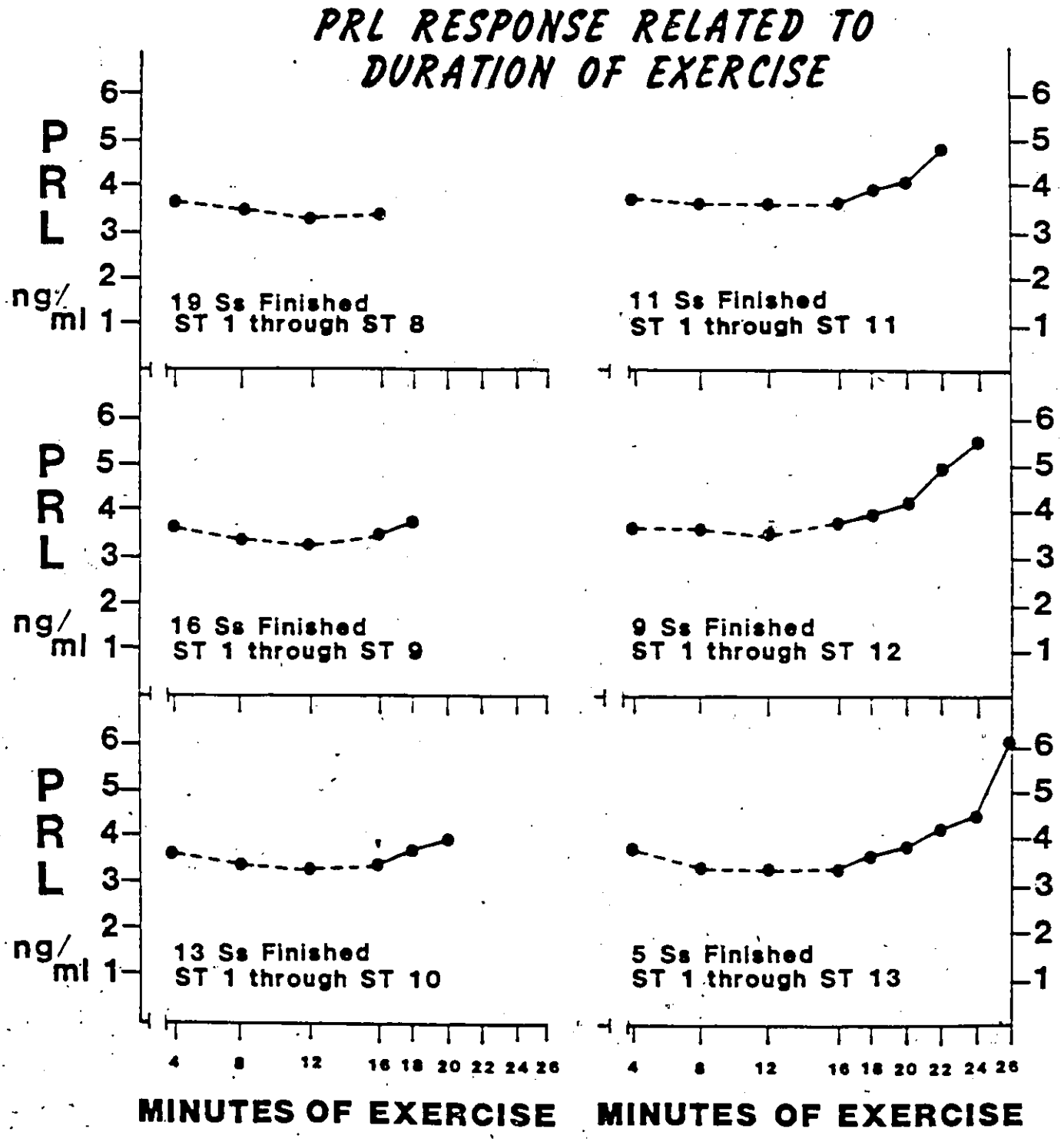
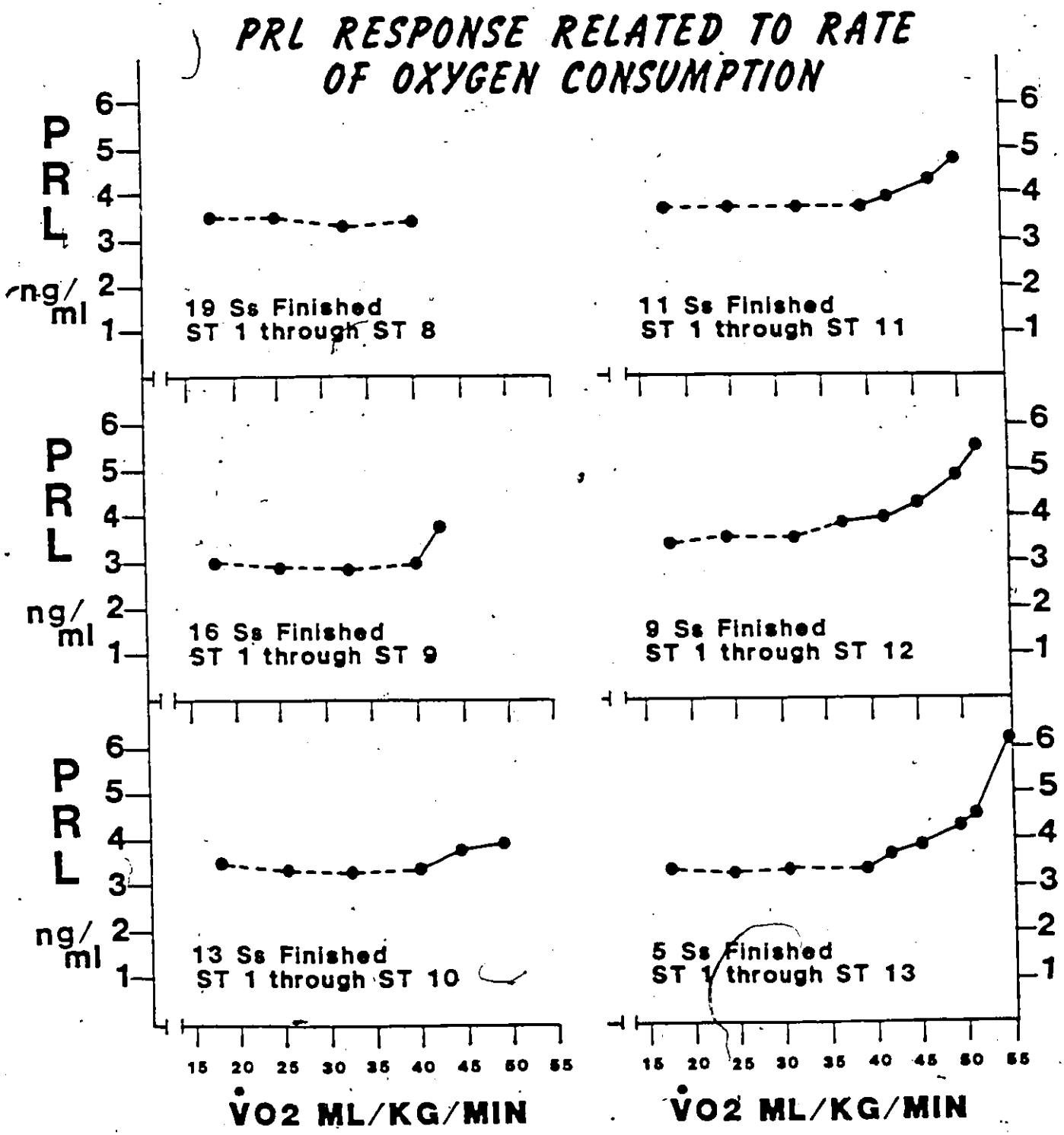

PRL RESPONSE RELATED TO DURATION OF EXERCISE
PRL ng/ml
19 Ss Finished ST 1 through ST 8
11 Ss Finished ST 1 through ST 11
16 Ss Finished ST 1 through ST 9
9 Ss Finished ST 1 through ST 12
13 Ss Finished ST 1 through ST 10
5 Ss Finished ST 1 through ST 13
4 8 12 16 18 20 22 24 26
MINUTES OF EXERCISE
MINUTES OF EXERCISE

FIGURE 4.2.3


PRL RESPONSE RELATED TO RATE OF OXYGEN CONSUMPTION
PRL ng/ml
19 Ss Finished
ST 1 through ST 8
11 Ss Finished
ST 1 through ST 11
16 Ss Finished
ST 1 through ST 9
9 Ss Finished
ST 1 through ST 12
13 Ss Finished
ST 1 through ST 10
5 Ss Finished
ST 1 through ST 13
15 20 25 30 35 40 45 50 55
V̇O2 ML/KG/MIN

Observations during the $\dot{V}O_2$ test revealed that there was a rise in PRL concentration only after the $\dot{V}O_2$ exceeded 40 ml/kg/min. The highest PRL concentration during exercise was seen with the highest $\dot{V}O_2$. There was a consistent pattern of an initial stable PRL concentration, for a time period in the order of 20 minutes, followed by a gradual increase. The highest PRL concentration during exercise was seen with the longest duration of exercise.
Two parameters of PRL response were identified that had not been identified before. These were the $\dot{V}O_2$ at which PRL began to rise and the time at which this rise occurred. This parameter identification suggested that a threshold or triggering mechanism had to occur for a PRL concentration increase to be observed.

These results provided evidence for the second working hypothesis. An increase in the rate of oxygen consumption was accompanied by an increase in PRL concentration but only after the $\dot{V}O_2$ reached the order of 40 ml/kg/min.
In Figure 4.2.3 the trend of increasing PRL concentration with increasing $\dot{V}O_2$ was most apparent for the subjects completing ST 1 through ST 12 and ST 1 through ST 13. Using the Least Square Method on the mean absolute PRL and $\dot{V}O_2$ values from the graph for the five subjects completing ST 1 through ST 13, a mathematical expression for best fit can be obtained to relate $\dot{V}O_2$ and PRL. The third degree equation obtained through this method is given by:

$$PRL = -0.23905 + 0.41633\ \dot{V}O_2 - 0.01508\ \dot{V}O_2^2 + 0.00017\ \dot{V}O_2^3$$

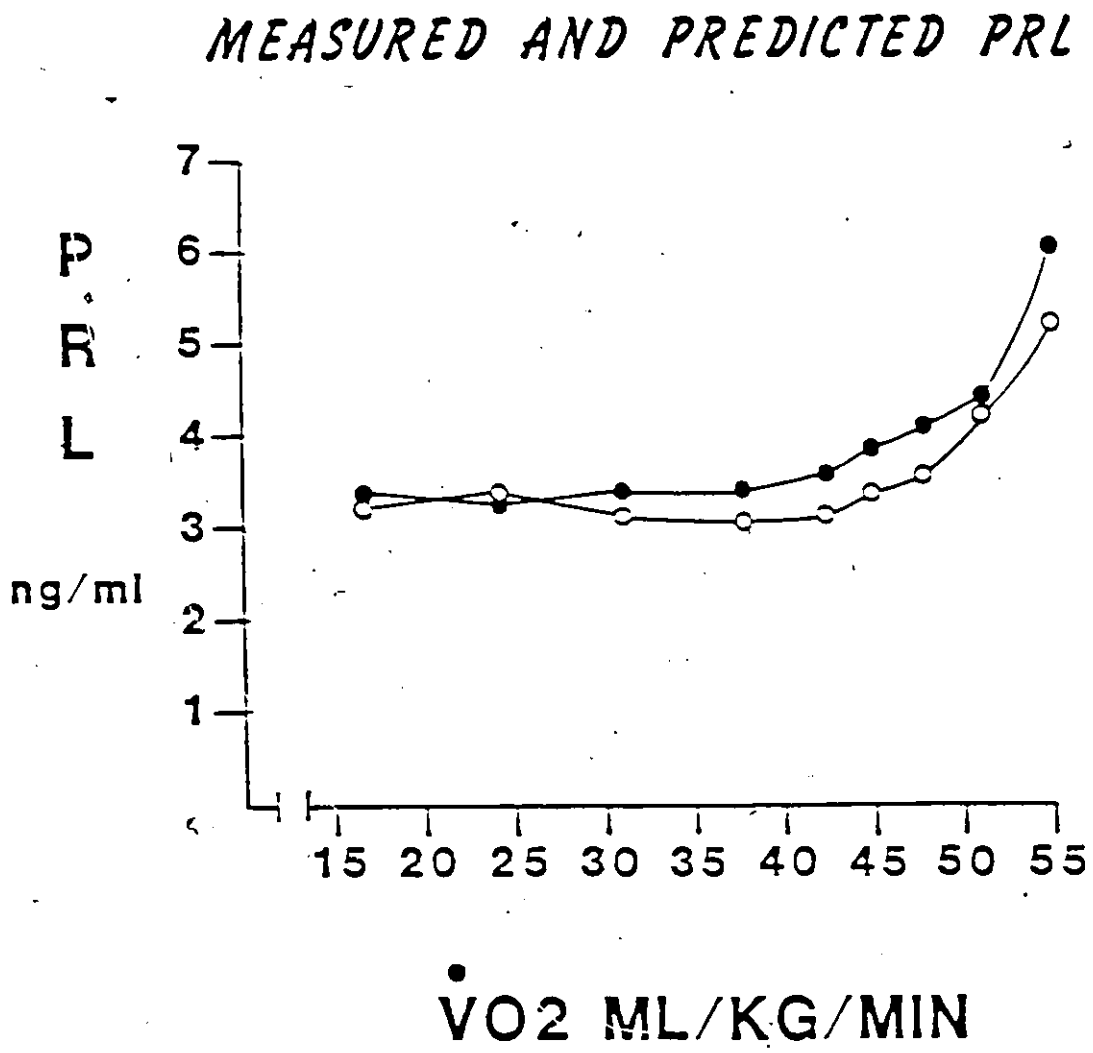
Although inferential tests would suggest significant linear, quadratic and cubic components, the expression is better integrated as a descriptive device, since with such a small number of subjects, the above coefficients are known to be very unstable. However, it should be noted that the pairs of

coordinates each represents the mean of five subjects rather than single measurements on individuals, thereby increasing the stability of these points. This mathematical expression is provided as a methodology, not as an exact relationship between PRL and $\dot{V}O2$. Since the relationship is strong, it was considered worth noting:

R-squared of the linear component = 0.55,
R-squared of the quadratic component = 0.34,
R-squared of the cubic component = 0.08.

Using the above mathematical expression to predict PRL from $\dot{V}O2$ provides a formal relationship between the two variables and has empirical validity as observed in Figure 4.2.4. This figure provides the measured PRL values for the five subjects completing ST 1 through ST 13 from Figure 4.2.3 and the predicted PRL values from the mathematical expression. The mathematical expression methodology provided here requires a larger sample in order to be used for other than a descriptive device.

FIGURE 4.2.4


MEASURED AND PREDICTED PRL
PRL
ng/ml
7
6
5
4
3
2
1
15 20 25 30 35 40 45 50 55
V̇O2 ML/KG/MIN
Legend
Measured PRL
Predicted PRL

4.3

PROLACTIN INCREMENT AND $\dot{M}VO_2$

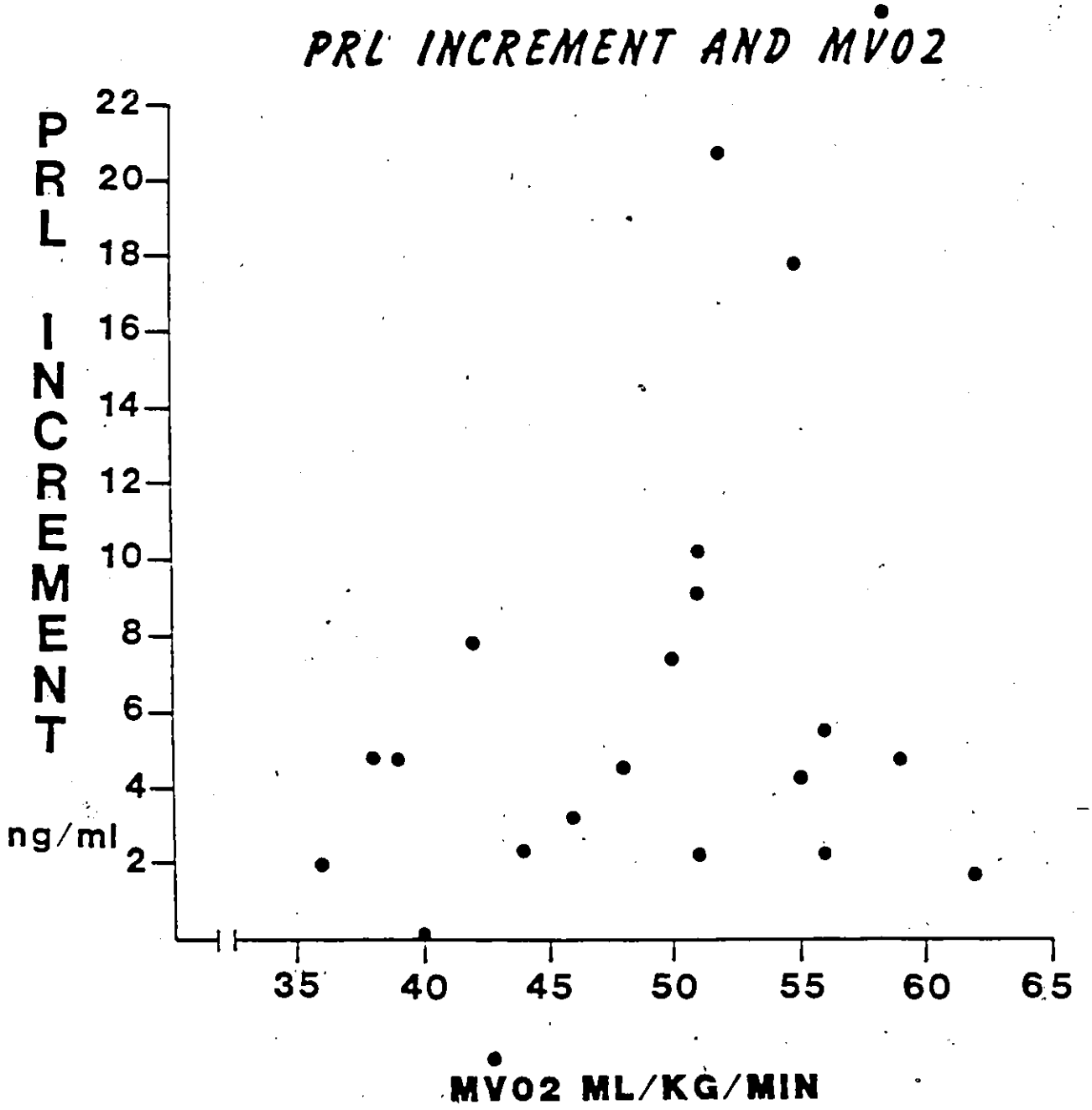
The third question under investigation in this study was whether there would be a relationship between PRL increment and $\dot{M}VO_2$. The $\dot{M}VO_2$ and PRL increment for each of the nineteen subjects is presented in Figure 4.3. The results of the regression analysis performed on this data is presented in Table 4.3. This analysis showed that no significant relationship exists between PRL increment and $\dot{M}VO_2$. The same type of relationship was expected between peak PRL and $\dot{M}VO_2$ given that the PRL values pre-exercise and for the first 20 minutes of exercise were relatively stable between and within individuals and the addition of this "constant" to PRL increment results in the peak value. Therefore, regression analysis was also performed on the peak PRL and $\dot{M}VO_2$ data for the nineteen subjects and these results are presented in Table 4.3.

TABLE 4.3

REGRESSION ANALYSIS RESULTS

PARAMETERS	COMPONENT	R-SQUARE	RELATIONSHIP
$\dot{M}VO_2$ and PRL INC	linear	0.0533	N.S.
	quadratic	0.1241	N.S.
	cubic	0.0555	N.S.
$\dot{M}VO_2$ and peak PRL	linear	0.0238	N.S.
	quadratic	0.1131	N.S.
	cubic	0.0400	N.S.

FIGURE 4.3


PRL INCREMENT AND MV02
PRL INCREMENT
ng/ml
22
20
18
16
14
12
10
8
6
4
2
35
40
45
50
55
60
65
MV02 ML/KG/MIN

4.4

DISCUSSION OF THE PROLACTIN RESPONSE TO EXERCISE

The treadmill stress test induced alterations in PRL concentration. The initial stability of PRL concentration during exercise had not been reported in the literature. While this thesis was in press (June, 1985), De Meirleir's group published its results, which define very precisely the intensity of exercise required to induce an increase in PRL levels as anaerobic threshold (AT)(De Meirlier et al June, 1985). They demonstrated that submaximal exercise of an intensity that did not increase systemic levels of lactic acid (4 mmol/L was their criterion) did not alter PRL levels. This precise definition enables the initial stable PRL concentration seen in this study to be explained as reflecting a subthreshold exercise intensity. Only when the AT intensity was reached was a PRL increase induced and the acute rise to peak in recovery observed. The duration of exercise is therefore not the factor that induces PRL increase. In the treadmill stress test protocol used for this study, the intensity of exercise reflecting a $\dot{V}O_2$ of 40 ml/kg/min was only reached by a duration in the range of 20 minutes (Table 3.4.2). Therefore, the identification of the two parameters, the $\dot{V}O_2$ at the onset of PRL increase and the time of this onset, may only be identifying the more subtle parameter of anaerobic threshold.

Similarly, the study by Brisson et al in 1981 that reported that the PRL response seemed to be related to work intensity (i.e., a 55% $M\dot{V}O_2$ workload failed to induce a PRL increase, whereas an 85% $M\dot{V}O_2$ workload resulted in almost doubled PRL concentration), may have been preliminary evidence to define AT as the work intensity necessary to trigger PRL increase. By definition, maximal exercise to exhaustion reaches anaerobiosis, so all the subjects in this study reached the

intensity of exercise defined by De Meirleir. The mean postexercise lactate in this study was in excess of the 4 mmol/L criterion De Meirleir used for anaerobiosis. This study lends support to the proposal that exercise intensity above AT increases PRL concentration. It also demonstrates that frequent blood sampling during exercise and recovery is necessary for a refined profile of the PRL response to exercise. The pre- and immediate postexercise sampling performed in most previous studies is not adequate to pinpoint the onset of the PRL rise during exercise or the peak concentration in recovery. $\dot{M}VO_2$, considered to be a measure of physical fitness, was not related to the PRL increment, thus demonstrating that the level of fitness of the subject did not influence the PRL increment in response to exercise.

4.5

LIMITATIONS

The major limitation of the study lies in the sample itself, since it included only respondents willing to undergo the procedures outlined in the methodology. It was not a random sample of the population, the sample size was small and there was no control group. Time and financial constraints were largely responsible for these limitations. Under the limitations of this study, the following conclusions are drawn.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

1. The general pattern of PRL response to exercise in this study is not contradictory to that described in the review of literature. A more refined pattern was revealed due to the frequency of blood sampling. It was stable PRL concentration for the first twenty minutes of exercise, peak concentration early in recovery and a gradual decline thereafter.

2. In this progressive exercise test to exhaustion, the PRL concentration did not increase before an intensity reflecting a $\dot{V}O_2$ of 40 ml/kg/min had been reached.

3. There was no relationship between the $M\dot{V}O_2$ of the nineteen male subjects and their PRL increment in response to exercise.

From these conclusions, the following recommendations for further research are made:

1. The use of fixed and progressive workloads, as well as variations induration and intensity, when examining the PRL response to exercise.

2. The incorporation of anaerobic threshold and lactic acid measurements into the protocol of future studies.

3. Frequent blood sampling should be done during exercise and recovery, and specifically before and after anaerobic threshold.

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APPENDICES

APPENDIX A

STUDIES USED TO COMPILE DOCUMENTED PROLACTIN PATTERN

STUDY	REPORTED MEAN PRL CONCENTRATION (ng/ml)		
	Pre Exercise	Termination Of Exercise	10-15 min Recovery
Brisson 1981 N = 8 (85% MVO2)	9.5	17.0	16.0
Nguyen 1982 N = 18	3.7		10.9
Mayer 1980 N = 10	7.0	13.0	
Jezová 1982 N = 11	4.5	7.4	8.4
Jezová 1983 N = 12	4.5	7.4	8.5

APPENDIX B

HEALTH APPRAISAL FORM

NAME: _____

AGE : _____ HEIGHT: _____ WEIGHT: _____

B.P.: _____ ECG : _____ MEDICATIONS: _____

_____

	YES	NO	IF YES, GIVE DETAILS
1. Do you smoke?			
2. Have you had any chest pain within the past year?			Describe
3. Do you suffer any chest pain or discomfort: at rest, when exercising in any form, e.g., climbing stairs?			
4. Is there any history of Heart Disease in the family?			Who, and at what age?
5. Have you ever suffered from: Lung Disease, Rheumatic Heart Disease, Heart Failure, Heart Attack, High Blood Pressures, Angina, Any other Heart Problem?			
6. Have you ever had Diabetes, Thyroid trouble, Anemia, Arthritis, Slipped Disc, Chronic Knee or Foot Disorders?			
7. Have you had or are you now suffering from any serious disorder not mentioned above?			

When did you last receive a medical examination?

Personal physician:
NAME: _____
ADDRESS: _____
_____

SIGNATURE: _____
DATE: _____

APPENDIX C

RECREATION ACTIVITY QUESTIONNAIRE NAME: _____ DATE: _____

The purpose of this questionnaire is to gain information regarding your usual leisure time physical activity. Please confine your answers to the last 2-week period so they will be seasonally appropriate.	TIME actually spent in last 2 weeks HRS MIN	INTENSITY LIGHT slight change from normal	MEDIUM some perspiration above normal breathing	HEAVY heavy perspiration heavy breathing	ORGANIZED LEAGUE YES NO	COMPETITIVE YES NO
WALKING FOR EXERCISE						
JOGGING (SHORT STRIDE)						
RUNNING (LONG STRIDE)						
BICYCLING						
HOME EXERCISE (PUSH/SIT-UP)						
EXERCISE CLASSES						
WEIGHT TRAINING						
YOGA						
GOLF (WALKING)						
RAQUETBALL						
SQUASH						
TENNIS						
BASEBALL						
SOFTBALL						
RUGBY						
SOCCER						
SWIMMING						
OTHER ACTIVITIES						
_____						
_____						

APPENDIX D

PHYSICAL CHARACTERISTICS OF THE SUBJECTS

SUBJECT	AGE (years)	WEIGHT (lbs)	HEIGHT (ft/in)	CALCULATED AEROBIC CAPACITY ml/kg/min
IL	28	227	6.3	42
GS	26	149	5.5	57
SR	20	158	5.7	56
SM	25	150	5.6	47
BL	27	166	5.10	46
MBRO	28	163	6.0	45
DW	27	150	5.10	48
SL	21	171	6.0	57
LG	27	188	6.0	53
MS	26	195	6.1	55
DD	29	177	5.9	--
GG	22	175	6.0	57
AH	24	152	5.6	57
TD	23	159	5.10	54
JH	24	172	6.0	54
DM	26	165	6.0	57
MBRA	20	151	6.0	49
KF	29	175	6.2	--
PL	23	165	5.10	58

APPENDIX E

CONSENT FORM

I, _____ the undersigned, agree to participate in the thesis research entitled "The Investigation of Serum Prolactin Concentration in Males in Response to a Standard MVO2 Treadmill Stress Test".

This will involve my performance of one exercise test on a treadmill. The treadmill speed will be incrementally increased, but always remain at a walking pace, and the treadmill grade will gradually change similarly. I will be required to continue the exercise to my maximum ability although I realize I can stop the test at any time.

Blood samples will be withdrawn from a vein in my arm by means of a venous catheter that has been inserted by qualified medical personnel.

There are certain personal risks accompanying an exercise effort to maximum. They include nausea, light-headedness, muscle pain and cramps, chest discomfort and extremely rarely, cardiac failure.

I do not expect any money or other material reward from my participation in this study. I realize that all the results of this study will be kept confidential by the researchers.

In signing this consent form, I acknowledge that I have had the test procedure and potential risks explained and have asked any questions I wanted.

Subject: _____

Witness: _____

Date: _____

APPENDIX F

PHYSIOLOGICAL CHARACTERISTICS OF THE SUBJECTS

SUBJECT	PEAK PRL (ng/ml)	*PRL INCREMENT (ng/ml)	$\dot{V}O_2$ (ml/kg/min)	MHR (BPM)
IL	7.1	2.0	36	178
SR	3.0	-0.7	40	167
GS	9.1	2.4	44	176
SM	8.3	4.9	39	185
BL	11.8	7.9	42	195
MBRO	8.3	4.9	38	187
DW	5.8	3.2	46	173
SL	5.1	2.3	56	187
MS	6.6	4.9	59	164
LG	11.9	7.2	50	173
TD	8.1	4.6	48	193
AH	6.4	4.1	55	189
DD	23.8	20.7	52	187
GG	14.0	10.1	51	185
JH	5.8	2.9	51	195
DM	12.7	9.1	51	176
MBRA	23.0	17.7	55	195
KF	6.5	2.4	62	195
PL	8.9	5.4	56	184

* Increment calculated as the mean pre-exercise value (2 samples as described on page 37) subtracted from the peak PRL value.

APPENDIX G

RAW DATA FOR ALL OBSERVED PARAMETERS

This appendix supplies all the raw data measurements for each individual subject.
An asterisk (*) indicates those subjects tested at the 10:30 a.m. sessions.

PRE EXERCISE DATA FOR SUBJECT IL

PRL			LACTATE	HR	HEMATOCRIT	BP
5.3	5.0	5.2	15.3	78	40	118/80

EXERCISE DATA FOR SUBJECT IL

	PRL	02	HR
ST 2	3.6	16.1	107
ST 4	3.6	24.2	127
ST 6	3.5	31.6	157
ST 8	3.2	36.2	178

RECOVERY DATA FOR SUBJECT IL

	PRL	LACTATE	HR	HEMATOCRIT
6 min	6.7	70.2	100	45.5
12 min	7.1		88	
18 min	5.7		94	
24 min	4.4		90	
30 min	5.6		92	

PRE EXERCISE DATA FOR SUBJECT SR

PRL		LACTATE	HR	HEMATOCRIT	BP
4.9	3.4	6.4	64	43	

EXERCISE DATA FOR SUBJECT SR

	PRL	02	HR
ST 2	2.6	20.0	107
ST 4	4.3	27.7	125
ST 6	2.6	40.0	145
ST 8	2.7	40.3	167

RECOVERY DATA FOR SUBJECT SR

	PRL	LACTATE	HR	HEMATOCRIT
6 min	3.0	37.8	94	48
12 min	2.9		100	
18 min	2.8		87	
24 min	3.3		89	
30 min	3.4		85	

PRE EXERCISE DATA FOR SUBJECT GS

PRL			LACTATE	HR	HEMATOCRIT	BP
8.6	7.2	6.1	7.7	63	39.5	120/90

EXERCISE DATA FOR SUBJECT GS

	PRL	02	HR
ST 2	4.5	20.6	101
ST 4	5.1	26.4	135
ST 6	3.3	36.5	163
ST 8	3.5	43.6	176

RECOVERY DATA FOR SUBJECT GS

	PRL	LACTATE	HR	HEMATOCRIT
6 min	9.1	65.7	98	43.5
12 min	7.3		97	
18 min	6.1		93	
24 min	5.7		90	
30 min	5.1		90	

PRE EXERCISE DATA FOR SUBJECT SM

PRL			LACTATE	HR	HEMATOCRIT	BP
3.2	3.5	3.3	6.9	80	44.4	110/70

EXERCISE DATA FOR SUBJECT SM

	PRL	02	HR
ST 2	3.8	17.8	115
ST 4	2.6	24.0	132
ST 6	3.0	31.9	161
ST 8	3.2	37.9	173
ST 9	2.9	39.1	185

RECOVERY DATA FOR SUBJECT SM

	PRL	LACTATE	HR	HEMATOCRIT
6 min	8.3	54.9	105	49.0
12 min	7.2		105	
18 min	6.5		100	
24 min	6.7		98	
30 min	5.5		96	

PRE EXERCISE DATA FOR SUBJECT BL

PRL			LACTATE	HR	HEMATOCRIT	BP
5.6	4.5	3.2	19.4	58	46	118/78

EXERCISE DATA FOR SUBJECT BL

	PRL	02	HR
ST 2	4.0	15.2	100
ST 4	4.2	23.4	139
ST 6	3.5	31.2	164
ST 8	3.8	41.4	191
ST 9	4.9	41.7	195

RECOVERY DATA FOR SUBJECT BL

	PRL	LACTATE	HR	HEMATOCRIT
6 min	11.4	101.0	112	50
12 min	11.8		107	
18 min	10.4		100	
24 min	8.9		94	
30 min	5.0		92	

PRE EXERCISE DATA FOR SUBJECT MBRO

PRL			LACTATE	HR	HEMATOCRIT	BP
3.6	3.4	3.3	7.7	70	41	

EXERCISE DATA FOR SUBJECT MBRO

	PRL	02	HR
ST 2	2.9	16.5	91
ST 4	2.2	22.2	111
ST 6	3.4	33.6	144
ST 8	4.3	36.8	182
ST 9	3.3	38.4	187

RECOVERY DATA FOR SUBJECT MBRO

	PRL	LACTATE	HR	HEMATOCRIT
6 min	8.3	68.4	104	46.5
12 min	7.7		90	
18 min	6.6		88	
24 min	5.4		86	
30 min	4.9		82	

PRE EXERCISE DATA FOR SUBJECT DW

PRL			LACTATE	HR	HEMATOCRIT	BP
2.4	2.6	2.5	6.3		41.5	102/76

EXERCISE DATA FOR SUBJECT DW

	PRL	02	HR
ST 2	4.2	17.5	89
ST 4	3.1	27.5	111
ST 6	2.5	35.4	133
ST 8	2.5	43.4	155
ST 10	1.9	46.4	173

RECOVERY DATA FOR SUBJECT DW

	PRL	LACTATE	HR	HEMATOCRIT
6 min	4.8	36.5	90	45.5
12 min	5.8		88	
18 min	4.2		81	
24 min	4.5		85	
30 min	3.6		83	

PRE EXERCISE DATA FOR SUBJECT SL

PRL			LACTATE	HR	HEMATOCRIT	BP
2.3	2.3	3.2	6.6	55	44	132/84

EXERCISE DATA FOR SUBJECT SL

	PRL	02	HR
ST 2	1.7	21.5	96
ST 4	1.7	26.7	115
ST 6	1.7	37.2	145
ST 8	2.2	41.8	164
ST 10	3.5	55.8	187

RECOVERY DATA FOR SUBJECT SL

	PRL	LACTATE	HR	HEMATOCRIT
6 min	5.1	33.8	94	47
12 min	4.8		100	
18 min	3.6		84	
24 min	3.4		90	
30 min	3.3		75	

PRE EXERCISE DATA FOR SUBJECT MS

PRL			LACTATE	HR	HEMATOCRIT	BP
1.5	2.3	1.0	15.3	66	45	140/80

EXERCISE DATA FOR SUBJECT MS

	PRL	02	HR
ST 2	2.8	20.6	100
ST 4	2.2	28.5	109
ST 6	3.3	37.7	123
ST 8	3.4	44.4	141
ST 10	3.6	54.8	155
ST 11	4.6	58.7	164

RECOVERY DATA FOR SUBJECT MS

	PRL	LACTATE	HR	HEMATOCRIT
6 min	6.6	76.5	94	48.5
12 min	5.4		88	
18 min	3.0		85	
24 min	3.0		85	
30 min	3.1		78	

PRE EXERCISE DATA FOR SUBJECT LG

PRL		LACTATE	HR	HEMATOCRIT	BP
4.0 - 4.8	4.5	5.1	55	43	110/74

EXERCISE DATA FOR SUBJECT LG

	PRL	02	HR
ST 2	5.7	16.6	88
ST 4	5.5	27.8	115
ST 6	4.7	36.1	141
ST 8	3.5	42.0	153
ST 10	3.7	48.8	169
ST 11	4.2	49.8	173

RECOVERY DATA FOR SUBJECT LG

	PRL	LACTATE	HR	HEMATOCRIT
6 min	8.0	82.4	96	49
12 min	10.7		91	
18 min	11.9		91	
24 min	9.8		90	
30 min	10.2		85	

PRE EXERCISE DATA FOR SUBJECT TD

PRL			LACTATE	HR	HEMATOCRIT	BP
3.9	3.4	3.6	7.4	74	41	144/110

EXERCISE DATA FOR SUBJECT TD

	PRL	02	HR
ST 2	2.7	20.4	97
ST 4	3.2	24.9	111
ST 6	3.3	29.4	130
ST 8	3.4	32.8	144
ST 10	3.1	40.9	173
ST 12	3.3	48.3	187

RECOVERY DATA FOR SUBJECT TD

	PRL	LACTATE	HR	HEMATOCRIT
6 min	8.1	74.7	111	45.5
12 min	6.7		102	
18 min	6.4		102	
24 min	5.0		98	
30 min	4.4		107	

PRE EXERCISE DATA FOR SUBJECT AH

PRL			LACTATE	HR	HEMATOCRIT	BP
2.9	2.2	2.4	7.4	66	46	123/82

EXERCISE DATA FOR SUBJECT AH

	PRL	02	HR
ST 2	2.9	20.5	100
ST 4	3.5	27.2	120
ST 6	2.8	33.2	138
ST 8	3.0	46.1	155
ST 10	3.1	51.3	180
ST 12	3.4	54.7	189

RECOVERY DATA FOR SUBJECT AH

	PRL	LACTATE	HR	HEMATOCRIT
6 min	6.1	78.8	102	49
12 min	6.1		110	
18 min	6.4		98	
24 min	5.3		87	
30 min	3.7		82	

PRE EXERCISE DATA FOR SUBJECT DD

PRL			LACTATE	HR	HEMATOCRIT	BP
5.1	3.5	2.7	7.7	65	42.5	124/82

EXERCISE DATA FOR SUBJECT DD

	PRL	02	HR
ST 2	3.2	18.0	79
ST 4	3.4	25.4	94
ST 6	3.8	32.6	114
ST 8	4.3	38.8	145
ST 10	7.2	43.9	168
ST 12	9.5	51.8	187

RECOVERY DATA FOR SUBJECT DD

	PRL	LACTATE	HR	HEMATOCRIT
6 min	20.9	86.9	100	49.5
12 min	23.8		102	
18 min			96	
24 min	19.6		98	
30 min	10.8		93	

PRE EXERCISE DATA FOR SUBJECT GG

PRL			LACTATE	HR	HEMATOCRIT	BP
6.1	3.9	3.8	6.8	70	43.5	120/62

EXERCISE DATA FOR SUBJECT GG

	PRL	02	HR
ST 2	4.6	19.9	90
ST 4	5.0	22.7	95
ST 6	4.0	33.8	125
ST 8	5.1	41.2	141
ST 10	5.8	47.0	167
ST 12	10.5	51.4	185

RECOVERY DATA FOR SUBJECT GG

	PRL	LACTATE	HR	HEMATOCRIT
6 min	14.0	77.9	102	48
12 min	11.3		100	
18 min	8.8		95	
24 min	7.6		87	
30 min	7.6		83	

PRE EXERCISE DATA FOR SUBJECT JH

PRL			LACTATE	HR	HEMATOCRIT	BP
2.3	2.9	2.8	19.5	68	41	118/80

EXERCISE DATA FOR SUBJECT JH

	PRL	02	HR
ST 2	2.4	15.8	94
ST 4	2.2	23.7	109
ST 6	2.8	33.0	123
ST 8	2.8	32.0	147
ST 10	3.2	41.0	173
ST 12	3.0	47.1	187
ST 13	5.2	51.3	195

RECOVERY DATA FOR SUBJECT JH

	PRL	LACTATE	HR	HEMATOCRIT
6 min	5.8	59.9		45
12 min	5.3			
18 min	4.6			
24 min	4.8			
30 min	3.9			

PRE EXERCISE DATA FOR SUBJECT MBRA

PRL			LACTATE	HR	HEMATOCRIT	BP
4.8	5.5	5.0	6.8	69	41	122/76

EXERCISE DATA FOR SUBJECT MBRA

	PRL	02	HR
ST 2	5.2	15.7	87
ST 4	5.4	27.0	102
ST 6	4.6	29.7	127
ST 8	4.8	38.1	154
ST 10	4.8	43.2	173
ST 12	5.5	45.2	186
ST 13	8.0	54.5	189

RECOVERY DATA FOR SUBJECT MBRA

	PRL	LACTATE	HR	HEMATOCRIT
6 min	23.0	65.7	118	47.5
12 min	20.2		118	
18 min	17.3		113	
24 min	13.2			
30 min	11.5			

PRE EXERCISE DATA FOR SUBJECT DM

PRL	LACTATE	HR	HEMATOCRIT	BP
5.0 3.7 3.5	2.8	50	40	110/68

EXERCISE DATA FOR SUBJECT DM

	PRL	O_2	HR
ST 2	2.7	17.9	67
ST 4	2.1	21.8	85
ST 6	1.8	27.7	108
ST 8	1.9	37.4	123
ST 10	2.5	41.8	151
ST 12	4.3	47.4	167
ST 13	6.9	50.6	176

RECOVERY DATA FOR SUBJECT DM

	PRL	LACTATE	HR	HEMATOCRIT
6 min	12.7	77.9	90	44
12 min	10.9		83	
18 min	8.3		83	
24 min	6.8		83	
30 min	5.2		73	

PRE EXERCISE DATA FOR SUBJECT KF

PRL			LACTATE	HR	HEMATOCRIT	BP
5.2	4.3	3.9	9.6	60	43	

EXERCISE DATA FOR SUBJECT KF

	PRL	02	HR
ST 2	5.1		84
ST 4	4.2		101
ST 6	4.7	33.1	128
ST 8	4.2	46.6	150
ST 10	4.7	54.4	165
ST 12	6.2	60.6	187
ST 13	6.5	61.8	195

RECOVERY DATA FOR SUBJECT KF

	PRL	LACTATE	HR	HEMATOCRIT
6 min	4.0	59.9	95	48
12 min	6.2		96	
18 min	4.7		96	
24 min	4.8		85	
30 min	4.0		94	

PRE EXERCISE DATA FOR SUBJECT PL

PRL			LACTATE	HR	HEMATOCRIT	BP
3.9	3.7	3.2	8.5	63	43	

EXERCISE DATA FOR SUBJECT PL

	PRL	O2	HR
ST 2	3.4	16.8	83
ST 4	3.3	22.1	102
ST 6	3.3	32.3	113
ST 8	3.4	38.6	141
ST 10	3.6	47.2	167
ST 12	3.6	54.9	182
ST 14	4.4	55.9	184

RECOVERY DATA FOR SUBJECT PL

	PRL	LACTATE	HR	HEMATOCRIT
6 min	8.9	50.9	90	47
12 min	7.1		83	
18 min	7.0		73	
24 min	5.1		75	
30 min	5.6		69	

APPENDIX H

MEDIUM - HIGH INTENSITY STRESS TEST
20 - 29 YEARS AGE GROUP NORM

FITNESS CATEGORY	$\dot{V}O_2$ (ml/kg/min)	FREQUENCY THIS STUDY
Excellent	59	2
Above Average	48-58	9
Average	38-47	6
Below Average	28-37	2
Poor	27	0

MEAN HR AND $\dot{V}O_2$ RESPONSE TO EXERCISE TREADMILL TEST

STAGE	JETTE'S NORMS	THIS STUDY	$\dot{V}O_2$ NORMS	$\dot{V}O_2$ THIS STUDY
ST 2	116 ± 13	93 ± 11	19.0 ± 2.2	18.2 ± 2.0
ST 4	138 ± 15	113 ± 15	24.6 ± 2.6	25.2 ± 2.3
ST 6	160 ± 17	136 ± 17	32.3 ± 2.9	33.2 ± 2.6
ST 8	177 ± 14	156 ± 17	38.7 ± 3.6	40.0 ± 3.9
ST 10	184 ± 13	169 ± 9	45.3 ± 2.9	47.3 ± 5.3
ST 12	186 ± 10	184 ± 7	53.4 ± 3.9	51.8 ± 5.4

NORMS FROM:
Jetté M.
Department of Kinanthropology
University of Ottawa

APPENDIX I

PARAMETERS DESCRIBED IN FIGURE 4.2.1
(MEAN ± SD)

STAGE / MIN COMPLETED	NUMBER OF SUBJECTS	MEAN PRL CONCENTRATION ng/ml at each stage	MEAN VO2 AT EACH STAGE
ST2 / 4	19	3.5±1.0	18.2±2.0
ST4 / 8	19	3.5±1.2	25.2±2.3
ST6 / 12	19	3.3±0.8	33.2±2.6
ST8 / 16	19	3.4±0.8	40.0±3.9
ST9 / 18	16	3.7±1.0	43.0±4.6
ST10 / 20	13	3.9±1.4	47.3±5.3
ST11 / 22	11	4.8±1.9	49.9±5.3
ST12 / 24	9	5.5±2.8	51.8±5.4
ST13 / 26	5	6.1±1.6	55.0±4.5