University of Alberta

The Nadir in Serum Glucose as a Predictor of Anaerobic Threshold

by

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ABSTRACT

The nadir in serum glucose as a predictor of anaerobic threshold and the associated hormonal response of insulin, glucagon and cortisol were examined in male cyclists completing a graded exercise test on a cycle ergometer. Blood samples were collected in the fasted state at rest and during the last minute of each workload for examination of glucose, lactate, insulin, glucagon and cortisol concentrations. There was no significant difference between the power outputs of lactate threshold and the nadir in serum glucose. There was a significant difference and correlation between the nadir in serum glucose and ventilatory threshold, with the ventilatory measure being approximately one power output greater than the glucose nadir. Insulin was lower and glucagon higher after the nadir in serum glucose ($p<0.05$). In conclusion, a nadir in serum glucose occurs in a predictable fashion in relation to other measures of anaerobic threshold that are associated with the insulin and glucagon response.

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AT	Anaerobic Threshold
ATP	Adenosine Triphosphate
GT	Glucose Threshold
HGP	Hepatic Glucose Production
LT	Lactate Threshold
PO	Power Output
rpm	Revolutions per minute
VCO_2	Rate of Carbon Dioxide expiration/production
V_E	Ventilation
V_E/VO_2	Ventilatory Equivalent for Oxygen Consumption
V_E/VCO_2	Ventilatory Equivalent for Carbon Dioxide Production
VO_2	Rate of Oxygen consumption
VO_{2max}	Maximal Oxygen Consumption
VT	Ventilatory Threshold
w	Watts

CHAPTER 1

INTRODUCTION

1.1 Introduction

Arguably, one of the most controversial topics in sport science over the last 25 to 30 years has been establishing the existence and measurement of the anaerobic threshold (AT) (Svedahl and Macintosh, 2003). It has been reasonably accepted that the anaerobic threshold is a physiological phenomenon that can be validly and reliably measured using both blood lactate measurements and ventilatory parameters (Wasserman et al., 1973; Prud'homme et al, 1984; Bhambhani and Singh, 1980; Wasserman, 1984; Wasserman et al, 1991; Weltman et al, 1989; Weltman et al, 1990). It has also been found that the response of blood glucose concentration to exercise of increasing intensity may be a useful means to help predict the anaerobic threshold (Northius, Halvorsen & Leon, 1995; Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). From the onset of graded, acute exercise, glucose concentration steadily decreases reaching a low point followed by a sharp increase (Northius et al, 1995). This observation of a momentary drop has been termed the "nadir" (Northius et al, 1995; Wasserman et al, 1984) or minimum (Simoes et al, 1999; Ribeiro et al, 2004) of blood glucose. In relation to anaerobic threshold, it has been termed the glucose threshold (GT) (Simoes et al, 1999). The finding of the predictability of AT from the glucose nadir has a practical application to field testing and monitoring of athletes but also adds to the further understanding of glucose kinetics during graded exercise.

Exercise of increasing intensity is characterized by a point where blood lactate levels rise significantly above resting levels as a result of the increased rate of demand for adenosine triphosphate (ATP) that requires an acceleration of the involvement of anaerobic glycolysis (MacDougall, 1977; Wasserman, 1984; Skinner & McLellan, 1980). This shift, termed the anaerobic threshold, has been defined as the exercise VO_2 above which anaerobic metabolism supplements aerobic metabolism with increasing lactate production at the site of cellular anaerobiosis (Wasserman et al, 1999). Several studies have supported the validity and reliability of using changes in blood lactate concentration during graded exercise to measure AT and have termed this the lactate threshold or LT (Wasserman et al, 1999; Skinner & McLellan, 1980; Neary et al, 1985).

In spite of the many examinations of AT there has been minimal study on the response of blood glucose to incremental exercise. Hartley et al, (1972) observed that plasma glucose levels increased from rest to maximal work and a decrease in glucose was recorded at moderate intensity which is not further commented on by the researchers. It has been suggested that muscle glucose uptake does not change as a linear function of work rate (Cooper et al, 1989; Winder et al, 1983), but this has not been adequately investigated in response to incremental exercise. The interest in determining a possible glucose threshold was realized in the unexpected observations of Northius et al (1995), who found a relationship between the changes in blood lactate and blood glucose during incremental exercise. This observation lead to the suggestion that a glucose threshold could be measured and used to indicate AT and therefore be a further predictor of the anaerobic threshold. Recently, Simoes et al (1999; 2003) and Ribeiro et al (2004) have shown that blood glucose responses are positively correlated to lactate responses and can

accurately indicate AT. These findings support the validity of a glucose threshold, but this research did not establish an exercise testing protocol nor further examine the physiological basis of this phenomena. It would be prudent to at least understand the response of the adrenergic and pancreatic hormones to the glucose nadir during graded exercise (Simoes et al, 2003; Ribeiro et al, 2004).

It has been suggested that the increased liver glucose output and decreased blood glucose uptake by the exercising muscle in response to intense exercise is partly due to the interaction of the effects of cortisol, glucagon, catecholamine, and insulin (Ribeiro et al, 2004; Simoes et al, 2003; Wasserman et al, 1991). Cortisol response during exercise has been shown to be influenced by intensity. Circulating levels of cortisol during low and moderate intensity remains stable or declines with changes associated with the psychological stress response to a work situation (Terjung, 1979; Davies and Few, 1973). In contrast, intense bouts of exercise stimulate an increase in plasma cortisol that is thought to be partly a protective effect against hypoglycemia (Wasserman et al, 1984; Terjung, 1979; Sutton, 1978; Tharp, 1975; Shephard and Sidney, 1975). From the onset of a graded exercise test to a mild submaximal workload, plasma glucagon concentration gradually increases while the concentration of the catecholamines remains approximately stable (Galbo et al, 1975; Galbo, 1983; Winder et al, 1983). As the intensity of exercise increases from a moderate submaximal to a maximal intensity, plasma glucagon concentration increases and can stimulate the enzymes of liver glycogenolysis thus increasing plasma glucose levels (Galbo et al, 1975; Wolfe et al, 1975; Hargreaves et al, 1997, Watt & Hargreaves, 2002). Similarly there is an increase in the plasma catecholamine concentration during graded exercise to a maximum which increases

hepatic glucose production while at the same time decreasing skeletal muscle uptake (Kreisman et al, 2000; Marliss et al, 1991). Antagonistic to the actions of glucagon and the catecholamines, insulin can lead to an inhibition of the enzymes of glycogenolysis, decreasing plasma glucose levels (Winder, 1985). Therefore, during intense exercise, insulin secretion declines and this allows an adequate hepatic glucose production (Winder, 1985; Issekutz, 1980). These observations have been extensively examined during prolonged exercise although they have not been acutely examined during specific graded exercise protocols in conjunction with the assessment of the proposed blood glucose threshold (Galbo et al, 1975; Wolfe et al, 1975; Hargreaves et al, 1997; Watt & Hargreaves, 2002; Kreisman et al, 2000; Marliss et al, 1991; Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004)

It is generally accepted that the observed workload where lactic acid production exceeds its removal is an identifier of anaerobic threshold and occurs as a result of the shift towards increased contribution of anaerobic glycolysis to total energy supply (MacDougall, 1977; Wasserman, 1984). Recently the threshold of blood glucose has been identified as an accurate predictor of metabolic AT, although the reason for this relationship remains speculative (Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). Therefore, it was the aim of this thesis to provide further evidence of the existence of a nadir of serum glucose that corresponds to lactate and ventilation thresholds, and investigate some of the glucoregulatory hormone responses associated with these thresholds during graded exercise testing.

1.2 Purpose and Hypothesis

The purpose of this study was to investigate the response of serum glucose levels and the associated hormones glucagon, insulin and cortisol to incremental exercise. The response of glucose was defined by the concentration of glucose in serum observed at workloads of increasing intensity. The research question asked if serum glucose levels reached a nadir which occurred at the same workload as other indicators of anaerobic threshold such as lactate threshold and ventilatory threshold using a continuous graded exercise test protocol. It was hypothesized that there was a nadir of serum glucose which occurs in a predictable fashion to lactate and ventilation thresholds. It was further hypothesized that the occurrence of glucose threshold would be mirrored by an increased concentration of glucagon and cortisol, and a decreased concentration of insulin amidst the changing metabolic demands leading up to and beyond anaerobic threshold.

1.3 Significance of Study

The ability to provide an additional predictor of AT through glucose threshold could alleviate deficiencies in current determination methods. Observation of a lactate threshold is known to be delayed due to time constraints of muscular lactate reaching vascular space and can result in overestimation of workload determination of AT (Skinner & McLellan, 1980). Ventilatory and lactate methods of threshold determination are linked via chemo-receptor responses to changes in anaerobically produced hydrogen ion concentration and increased CO_2 production and thus the limitations are the same for these two parameters (Skinner & McLellan, 1980; Wasserman et al, 1999). Using heart rate methods for AT prediction have questionable validity and reproducibility (Foster et al, 1998).

Due to the relatively recent awareness of the glucose threshold, there is a lack of data describing the mechanisms that underlie glucose threshold as an accurate predictor of AT. Only a few research groups (Simoes et al, 1999, 2003; Ribeiro et al 2004) have examined the existence of a glucose threshold that corresponds to anaerobic threshold but no underlying hormonal or metabolic mechanisms of glucose control were investigated. It is clear that glucagon, insulin, and cortisol play an important role in glucose homeostasis during exercise (Wasserman et al, 1999; Galbo et al, 1975; Wolfe et al, 1986; Kreisman et al, 2000; Marliss et al, 1991; Hargreaves, 1997; Winder, 1985; Watt & Hargreaves, 2002), and these need to be investigated in association with the glucose threshold.

The significance of this study to the research community extends to the further understanding of blood glucose kinetics during graded exercise.

1.4 Delimitations

For this study, 22 healthy males between 19 and 34 years of age, considered healthy and active, were recruited. A group with these demographic characteristics limits the generalizability of the results. For example, it does not allow us to explore varying responses of trained versus untrained individuals (Kjaer et al, 1986) or gender on glucose uptake (Marliss et al, 2000; Jurkowski et al, 1981). The number of participants was based on the effort to draw a correlation between a glucose threshold and anaerobic threshold. The three known studies that established glucose concentration as an accurate predictor of AT used 11, 15 and 8 subjects (Simoes et al, 1999; Simoes et al 2003; Ribeiro et al, 2004, respectively). The findings of Simoes et al (1999, 2003) and Ribeiro et al (2004) and previous pilot work suggested that there was a low likelihood that there

would be a significant difference between glucose and anaerobic threshold, therefore a large sample size was not required to determine the relationship. Research on the hormones associated with blood glucose often use sample sizes of 5 to 13 (Lavoie et al, 1997; Weltan et al, 1998; Watt and Hargreaves, 2002) and sample sizes of 10 participants in research involving the measurement of AT are common and have been shown to be large enough to establish a statistically significant relationship between the predictor variable and AT (Beaver et al, 1986; Wasserman, 1984).

A graded test was performed on a Monark cycle ergometer using a standardized exercise test to exhaustion. Workloads were 3 minutes in length which allowed for the delay of diffusion of lactate from muscle to blood and ensured that steady state was achieved (Skinner & McLellan, 1980).

1.5 Limitations

Participants were volunteers from within Edmonton and were not randomly selected from a greater population. Thus one limitation is that the external validity was limited to only healthy males between 19 and 34 years of age.

All subjects were provided with prior experience to the testing environment although a second limitation was there could still have been varying levels in anxiety which could have altered adrenergic activity and possibly hepatic glucose production. This was reduced by performing a familiarization session to the lab that included a graded exercise test to determine maximal oxygen consumption on a separate day prior to experimental testing. It was also assumed that blood samples from the antecubital vein of the arm are an accurate representation of the body's glucose response to graded exercise. Nutritionally, the meal replacement drink provided to each participant may have been a

limitation as all were given the same quantity preceding the threshold test regardless of body mass. This may have influenced resting glucose and hormonal measures.

A final limitation was that due to varying levels of fitness the length of the exercise test varied between participants. This could have influenced the extent of hormone response.

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CHAPTER 2

REVIEW OF LITERATURE

2.1 Exercise Metabolic Pathways

The storage of metabolites for energy generation in humans is primarily divided into three groups. First are small amounts of intracellular ATP and phosphocreatine, which are used quickly in response to immediate high intensity activity. The second and third groups are glycogen and triglycerides (carbohydrates and fats), which provide energy throughout the duration of activity. Glycogen is broken down to glucose when needed. Triglyceride is hydrolyzed into glycerol and three molecules of free fatty acids. Both glucose and free fatty acids are taken up by the working muscle to be metabolized generating ATP (Rodahl et al, 1964; Winder, 1985; Langfort et al, 1999; Sutton, 1977).

In healthy individuals the proportion of energy provided by carbohydrate oxidation increases as the level of exercise intensity increases (Kang et al, 1998). At a low intensity of exercise (<50% VO_{2max}) the majority of energy requirements of the working muscle are met largely by oxidation of free fatty acids mobilized from adipose tissue. The contribution of glucose from hepatic production is less at this point (~15%), but still a contributing factor (Andres et al, 1956; Dagenais et al, 1976; Ahlborg & Felig, 1976). At a moderate intensity (50% - 75% VO_{2max}) hepatic glucose production begins to increase and the contribution of glycogen to energy requirements is increased (~50%) (Kang et al, 1998; Kjaer et al, 1991; Kjaer et al, 1993). There is also an increase in the absolute amount of triglyceride-derived energy but its relative contribution is now approximately equal to that of glycogen (~50%) (Ahlborg & Felig, 1982; Ahlborg et al,

1974). At a high intensity (>80% VO_{2max}) glycogen is now the predominant source of energy (~85%) through its hepatic breakdown to glucose, while the relative contribution of the triglycerides is decreased (~15%) (Rodahl et al, 1964: Romijn et al, 1993; Baldwin et al, 1973; Wehren et al, 1971).

Glucose plays a role in all stages of the progress of increasing intensity exercise. For short, immediate exercise requirements, energy is provided through the splitting of high-energy phosphocreatine in conjunction with cellular ATP, but glycolysis is also considered an immediate source of energy for ATP production as carbohydrates (mainly in the form of muscle glycogen) are oxidized to pyruvic acid and to lactic acid when the demand for nicotinamide adenine dinucleotide (NAD^+) is high. In addition to, and most predominately after, the immediate energy requirements during exercise, liver glycogenolysis (glycogen broken down to glucose) is accelerated to meet the demands of the increased glucose utilization rate (Winder, 1985). This begins from the onset of exercise and increases in absolute amount and relative contribution to energy requirements throughout the duration (Romjin et al, 1993). When liver glycogen is depleted (fasting or prolonged exercise), gluconeogenesis becomes a major source of blood glucose. This glucose is created by using amino acids, lactate, pyruvate, and glycerol as carbon sources for synthesizing glucose in the liver (Winder, 1985).

Aerobic metabolic pathways involve the combustion of carbohydrates, fats, and some amino acids in the presence of oxygen to provide a higher capacity for ATP energy (Gastin, 2001). Early research sought to depict the interaction and contribution of these processes along with anaerobic pathways as sequential steps in the response to the demands of intense exercise (Fox et al, 1969). It is accepted now that the energy systems

aren't distinct processes but rather a melding of resources in which each of the pathways is used during almost all exercise activities (Gastin, 2001).

To better define the various energy generation processes during exercise related to measures of anaerobic threshold, Skinner and McLellan (1980) proposed a three phase model. The first phase (phase 1) occurs at less then 40% of maximal oxygen consumption (VO_{2max}) and primarily involves the aerobic system. Phase 1 was associated with linear increases in oxygen consumption (VO_2), carbon dioxide production (VCO_2) and ventilation (V_E). The respiratory exchange ratio (RER), calculated as the amount of VCO_2 relative to VO_2 in cellular respiration (Lamb & Murray, 1998) was considered to be low. The second phase (phase 2) occurs between 40% and 60% VO_{2max} and was associated with continued increases in VO_2, VCO_2 and V_E, with the last two variables increasing nonlinearly. Blood lactate began an initial rise at a concentration of approximately 2mM which was believed to correspond with the onset of anaerobic threshold (Wasserman et al, 1999; Kumagai et al, 1987; Chwalbinska-Moneta et al, 1989). The RER was near 1.0 at phase 2 and was associated with increasing glycolytic production of ATP generation. With further increases of intensity to about 65 to 90% VO_{2max},VO_2 continued to rise linearly along with further increases in V_E and VCO_2 marked by hyperventilation and was termed phase 3. At the onset of phase 3, blood lactate concentration was approximately 4mM and increased rapidly with continued exercise. The RER in phase 3 was greater than 1.0 and indicated a supplementation to ATP generation by anaerobic glycolysis. The onset of this phase with the "break-away" in ventilation is considered by some to correspond with anaerobic threshold (MacDougall, 1977).

2.2 Anaerobic Threshold

Anaerobic threshold is defined as the exercise VO_2 above which anaerobic metabolism supplements aerobic metabolism with increasing lactate production at the site of cellular anaerobiosis (Wasserman et al, 1999). The hypothesis states that:

> (1) the O_2 required by the metabolically active muscles can exceed the O_2 supply to the mitochondria when the work rate is sufficiently high; (2) the imbalance between the O_2 supply and the O_2 requirement (that is, O_2 requirement greater than supply) brings about a net increase in anaerobic oxidation in the cytosol of the cell with pyruvate conversion to lactate; (3) H^+ is buffered in the cell primarily by HCO_3^-; (4) the CO_2 generated from buffering increases CO_2 output, while HCO_3^- exchanges for lactate across the muscle cell membrane according to the new electrochemical gradients; and (5) the buffering and acid-base disturbances produce predictable changes in gas exchange (Wasserman, 1984).

The concept of anaerobic threshold was first published by Wasserman and McIroy (1964) based on the assumption that an oxygen deficiency was the cause for the increased blood lactate concentrations during incremental exercise (Wasserman et al, 1971). In the years since, the concept has been widely examined for its relation to the endurance athlete (MacDougall, 1977; Farrell et al, 1979; Davis et al, 1976; Davis, 1985; Kumagai et al, 1987; Weltman, 1989) and also been a topic of debate over its validity. Davis (1985) published a review stating that anaerobic threshold has an enduring importance with widespread use due to its non-invasive determination and accurate prediction of exercise tolerance. In criticism of this review, Brooks (1985) stated that the concept of anaerobic threshold is a simplistic explanation of indirectly related phenomena flawed as its essential assumption that muscle lactate production results from oxygen-limited ATP production and this notion was not in agreement to findings that increased lactate is

produced as a result of increased work and metabolic rate (Brooks, 1985; Brooks, 1985; Davis, 1985; Davis, 1985). Amongst debate, the concept of anaerobic threshold has remained a pivotal measure within exercise and sport science (Hollmann, 2001; Anderson & Rhodes, 1989; Billat et al, 2003; Gaskill et al, 2001; Svedahl & Macintosh, 2003).

The measure of anaerobic threshold at increasing exercise intensity corresponds to the transition between evenly and unevenly matched aerobic conditions (Antonutto & Di Prampero, 1995). Noticeable in the phase description of substrate metabolism during exercise is the discrepancy in defining the point of anaerobic threshold. The criteria used to identify the metabolic shift gives rise to this discrepancy. Several researchers define the initial blood lactate increase associated with phase 2 as anaerobic threshold (Wasserman et al, 1999; Kumagai et al, 1987; Chwalbinska-Moneta et al, 1989). MacDougall (1978) and Skinner & McLellan (1980) define phase 3 as anaerobic threshold characterized by the abrupt increase of blood lactate to 4mM or greater and the "break-away" in ventilation. Skinner and McLellan (1980) argue that using the second phase is inaccurate as it is still primarily associated with aerobic metabolism. Alternatively it can be difficult to accurately determine anaerobic threshold using criteria of the third phase (MacDougall, 1978) which is susceptible to overestimation of the AT workload (Skinner & McLellan, 1980).

2.3 Lactate Threshold

Lactate threshold (LT) is considered the "gold standard" in anaerobic threshold determination as several studies have examined the reliable associations between lactate response and the various energy pathways (Wasserman et al, 1999; Skinner & McLellan, 1980; Neary et al, 1985; Antonutto & Di Prampero, 1995; Weltman et al, 1990; Weltman

et al, 1989; Wasserman et al, 1991; Stainsby et al, 1991). Antonutto and Di Prampero (1995) describe the lactate response to graded exercise in three broad ranges. The first is during low intensity in which blood lactate does not increase substantially above resting levels. The second phase occurs at moderate intensities where blood lactate initially increases, while abrupt increases are noted in the third phase at intensities close to or above VO_{2max}. The relationship of lactate to anaerobic threshold arises out of the imbalance between the supply and requirement of energy from different metabolic pathways during and beyond AT. The rate of anaerobic glycolysis increases as the ability to generate ATP through aerobic metabolism reaches its maximum. This results in the increased rate of anaerobic conversion of pyruvate to lactate at AT and thus an increase in blood lactate concentration which has been associated with the shift to anaerobic glycolysis (Wasserman, 1984; Wasserman, et al, 1999; Weltman, 1989; Antonutto & Di Prampero, 1995).

The determination of LT has been defined in many ways since its observation in the early 1900's. Some have set LT at absolute values of 2 or 4 $mmol \cdot l^{-1}$ of blood lactate concentration (Hughson & Green, 1982; Kindermann et al, 1979; Sjodin et al, 1981; Heck et al, 1985). Others defined the threshold of lactate as the initial increase in blood lactate concentration (Davies et al, 1976; Wasserman et al, 1973) or at the intensity of an abrupt increase in lactate concentration (Aunola and Rusko, 1984; Brooks et al, 1985). The definitions based on absolute values do not account for the variability between individuals, and thus the definitions based on changes in lactate concentration during the exercise bout may be better suited for anaerobic threshold determination (Anderson and Rhodes, 1989). Using Wasserman et al (1999) definition of LT as:

> The exercise VO2 above which a net increase in lactate production results in a sustained increase in central blood lactate concentration (Wasserman et al, 1999),

the threshold of lactate can be generally described as the point where the rate of lactate production increases greater than lactate removal.

2.4 Ventilatory Threshold

An alternative non-invasive method to predicting anaerobic threshold is ventilatory threshold. This method measures CO_2, which is generated in association with lactate during exercise (Beaver et al, 1986; Svedahl & Macintosh, 2003). A common protocol, termed the V-slope method, draws on the coinciding lactate increase and the carbon dioxide/oxygen relationship during incremental exercise (Beaver et al, 1986; Wasserman et al, 1999). The relationship is characterized by the graphical slope of the relationship between VO_2 and VCO_2. There is a double linear composition of the slope with the first portion having a slope less than one and the second portion having a slope greater than one. The point of the change in slope determined visually or mathematically by the examiner is determined to be anaerobic threshold (Beaver et al, 1986). The ventilatory equivalent method uses the point of anaerobic threshold as defined by an increase in the V_E/VO_2 relationship without a concurrent increase in the V_E/VCO_2 relationship. These two methods are both based on the interrelationships of lactic acid, oxygen consumption and anaerobic metabolism. At anaerobic threshold total energy requirement exceeds the rate that aerobic metabolism can supply and there is increased production of lactic acid. As lactate accumulates in the blood so does CO_2 production because of bicarbonate buffering of the hydrogen ion dissociated in the muscle from lactic acid (Beaver et al, 1986; Wasserman et al, 1999; Svedahl and Macintosh, 2003).

As with all areas of anaerobic threshold there is debate on the validity of ventilatory threshold. Neary et al (1985) found that LT and VT do not occur together and that LT does not cause VT. These findings may arise out of the simplicity of associating increased ventilation and CO_2 production to lactic acid, when research has shown there is an assortment of physiological factors influencing the ventilatory changes including respiratory mechanics, temperature effects, skeletal muscle neurogenic stimulation, and chemoreceptor sensitivity, among others (Beaver et al, 1986; Walsh & Bannister, 1988; Svedahl & Macintosh, 2003). It has also been supported that VT is a valuable tool with strong test-retest relationships to work rate as long as test conditions and personnel are kept constant, and finds value in its practicality as it requires no invasive blood sampling (Prud'homme et al, 1984; Yamamoto et al, 1991; Svedahl & Macintosh, 2003).

2.5 Glucose Threshold

In recent years the addition of the glucose threshold to the toolbox of anaerobic threshold determinants has been examined for validity. Northius et al, (1995) was the first to identify the possibility of blood glucose as a predictor of lactate threshold. Before this observation previous research had commented on blood glucose responses during incremental exercise test but there was no acute examination of its relation to AT. Hartley et al (1972) observed that plasma glucose levels increased from rest to maximal work and a decrease in glucose was recorded at moderate intensity which was not commented on by the researchers. Several other researchers noted that blood glucose levels decreased at lower intensities (<70% VO_{2max}) and increased as the intensity approached VO_{2max} (Rodahl et al, 1964; Pruett, 1970; Sutton, 1977). It is the decrease in blood glucose concentration preceding an increase during incremental exercise tests that

other researchers have identified as the point of glucose threshold (GT) (Northius et al, 1995; Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). The term nadir was used in association to this possible glucose threshold as nadir by definition is the lowest point, and it is the lowest concentration of blood glucose during the incremental test that is identified as the threshold (Northius et al, 1995; Wasserman et al, 1984). Simoes et al (1999) determined that the responses of blood glucose concentrations were similar to those of blood lactate concentration and that the threshold of glucose could be used just as lactate in evaluation of aerobic capacity.

2.6 Glucose Metabolic Pathways

Glucose is a simple sugar, the smallest type of carbohydrate, the primary source of energy for adenosine triphosphate (ATP) generation, and is stored in cells as glycogen (a polymer of glucose). When glucose is abundant (following a carbohydrate meal) glucose absorbed from the intestine is converted into liver glycogen, while in situations of glucose need, glycogen is converted back into glucose (Winder, 1985). The liver plays a primary role in glucose homeostasis through glycogenolysis in which glycogen is broken down into glucose. There are several mechanisms for liver glycogenolysis activation. One mechanism is the series of enzyme phosphorylations initiated by increased cyclic adenosine 3,5 monophosphate (cAMP) (Hems and Whitten, 1980; Winder, 1985). The pathway of this mechanism is initiated by catecholamine or glucagon interaction with site specific receptors on the hepatocyte plasma membrane. This in turn activates the enzyme adenylate cyclase catalyzing the formation of cAMP from ATP. The newly formed cAMP dissociates catalytic subunits of protein kinase from regulator subunits. The active protein kinase catalytic subunits cause the addition of

ATP phosphate into serine amino acid residues of phosphorylase kinase, activating it. Finally the active glycogenolytic enzyme phosphorylase-a is created by phosphorylase kinases phosphorylation of phosphorylase-b. A similar mechanism begins when catecholamines interact with the alpha-adrenergic receptors of the hepatocyte plasma membrane (alpha-adrenergic receptor system). In this sequence the interaction causes a release of mitochondrial, endoplasmic reticulum, and plasma membrane binding site calcium into the cell cytoplasm which stimulates the allosteric activation of phosphorylase kinase which proceeds to glycogenolysis. A third mechanism involves the direct sympathetic innervation of the liver. Action potentials trigger norepinephrines release from sympathetic terminals of the liver. The norepinephrine then binds to alpha-adrenergic receptors of the hepatocyte plasma membrane. This results in calcium ion release which continues on to activate phosphorylase kinase which progresses to glycogenolysis (Winder, 1985).

As energy needs increase glycogenolysis is activated and glycogen is broken down into glucose by hydrolyzation or directly to glucose-6-phosphate by combining with a cytoplasmic inorganic phosphate group. The requirement of this energy is to drive the two primary ATP generating pathways. The first is the aerobic system which occurs in the presence of oxygen and generates the greater yield of ATP, and the second, is the anaerobic system which although generates ATP more quickly, does so at the expense of yield (Gastin, 2001; Skinner & McLellan, 1980; Winder, 1985). The mechanisms of both aerobic and anaerobic metabolism begin with glycolysis. In glycolysis one molecule of glucose is converted by a series of enzymatically catalyzed reactions through glucose-6-phosphate into fructose-1,6-biphosphate which is split into two molecules of pyruvate

(when glycogen is broken down directly to glucose-6-phosphate the glucose to glucose-6-phosphate step is omitted and there is an additional yield of 1 ATP). During this transition there is a net gain of two ATP molecules for each glucose molecule processed. In aerobic metabolism the pyruvate is converted into acetyl coenzyme A (acetyl CoA), with the loss of carbon dioxide and generates 34 more ATP through stages of the citric acid cycle and electron transport chain. In anaerobic metabolism the pyruvate from glycolysis is converted into lactate through a mechanism involving the enzyme lactate dehydrogenase. In anaerobic glycolysis only 2 ATP are generated per molecule of glucose (Winder, 1985; Campbell, 1999).

2.7 Cellular Glucose Uptake

Glucose transport into skeletal muscle is predominately achieved through the use of GLUT-4 glucose transporters (Marette et al, 1999). GLUT-4 transporters stored in the cytoplasm of the muscle cell are translocated to the cellular membrane enabling the cells to take up glucose by facilitated diffusion. Facilitated diffusion is used because the polar glucose molecules are unable to pass easily through the lipid core of the cellular membrane. (Winder, 1985; Terjung, 1979: Kahn, 1992; Jones & Dohm, 1997).

Translocation of GLUT-4 to the cellular membrane is achieved through two distinct processes. The first most predominant mechanism is insulin dependent glucose transport in which insulin combines with its receptor sites on muscle tissue beginning a series of phosphorylation cascades which activate the translocation of the GLUT-4 transporters (Winder, 1985; Terjung, 1979; Kahn, 1992; Jones & Dohm, 1997; Krook et al, 2004). GLUT-4 can also be translocated to the plasma membrane by non-insulin mediation, as it is activated by muscle contraction/exercise (Goodyear, 2000; Greiwe et

al, 2000; Krook et al, 2004; Sigal et al, 2004). The regulator 5'-AMP-activated-kinase (AMPK) is elicited in response to metabolic stress and functions as the metabolic switch that phosphorylates target proteins which activate the translocation of the GLUT-4 transporters independent of insulin (Krook et al, 2004; Sigal et al, 2004). Regardless of whether the translocation is insulin dependent or independent the resulting response is the mobilization of GLUT-4 to the cellular membrane to allow the passage of glucose into the muscle cell (Sigal et al, 2004).

2.8 Exercise Glycolysis Kinetics

In humans, euglycemia is maintained at approximately 5mM (Houston, 1995; Wasserman et al, 1999; Lewis et al, 1999; Wahren et al, 1971). During maximal exercise the rate of glycolysis may show an increase of up to 100 times that of rest although this rate can not be sustained (Gastin, 2001). Specific to liver action it has been shown that the rate of liver glycogenolysis increases ~ 8 fold in response to intense exercise (Winder, 1985; Kreisman et al, 2000). It has generally been accepted that glucose uptake by exercising muscles increases proportionally with work rate (Reichard et al, 1961; MacLean et al, 1999); although it has been hypothesized that whole body glucose turnover was not related in a simple linear manner to metabolic rate (Cooper et al, 1989; Coggan, 1991). After anaerobic threshold glucose production begins to exceed uptake, resulting in hyperglycemia during intense exercise (Kjaer et al, 1986; Kjaer et al, 1991; Galbo et al, 1981). It has been reported that preceding this acute hyperglycaemic state there is a "nadir" or a minimum in blood glucose concentration (Northius, 1995; Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004).

2.9 Hormonal Effectors

Glucagon and insulin are two hormones secreted by the endocrine cells of the pancreas that work in the regulation of glucose metabolic pathways. The clusters of these endocrine cells are referred to as the islets of Langerhans, containing four distinct cell types. The first are the alpha (α) cells which secrete glucagon. Second are the beta (β) cells which produce insulin. Most of the remaining cells are D cells which secrete somatostatin, while a few cells are a fourth rare type which produce a pancreatic polypeptide (Adrian et al, 198; Winder, 1985; Terjung, 1979).

Insulin release from the β cells may be stimulated by elevated plasma glucose (>100 mg/dL) or amino acid concentrations, and increased parasympathetic input. The release of insulin results in decreased plasma glucose concentration as its transport into the cells and metabolic use is increased (Winder, 1985; Terjung, 1979; Adrian et al, 1981; Aarnio et al, 2001; Krook et al, 2004; Sigal et al 2004). Insulin influences glucose transport across the cell membrane by regulation of GLUT-4 (Winder, 1985; Terjung, 1979; Kahn, 1992; Jones & Dohm, 1997; Krook et al, 2004; Sigal et al 2004). When insulin combines with its receptor sites on muscle tissue, GLUT-4 transporters stored in the cytoplasm are translocated to the cellular membrane enabling the cells to take up glucose by facilitated diffusion (Winder, 1985; Terjung, 1979: Kahn, 1992; Jones & Dohm, 1997; Krook et al, 2004; Sigal et al, 2004).

Glucagon release from pancreatic α cells is stimulated by decreased plasma glucose concentrations (<200 mg/dL; with a maximum secretion below 50 mg/dL) or sympathoadrenal influence (Terjung, 1979). Once released, the mechanism of action involves binding to a glucagon receptor on the liver, which through cyclic AMP-

dependent activation of phosphorylase leads to increased production of glucose by glycogenolysis, synthesizing glucose from glycogen, and gluconeogenesis, synthesizing glucose from non-carbohydrate precursors notably amino acids (Winder, 1985;Terjung, 1979).

During strenuous exercise insulin levels decrease with a magnitude closely linked to exercise intensity while glucagon concentration increases (Felig et al, 1972; Wolfe et al, 1986; Cooper et al, 1989; Galbo et al, 1975; Aarnio et al, 2001; Terjung, 1979; Winder, 1985). The immediate effect of the plasma glucagon concentration increase is increased glucose production through liver glycogenolysis along with providing alternative glucose supply through gluconeogenesis. The decrease in plasma insulin concentration facilitates these processes since insulin works antagonistically to glucagon inhibiting glucagon's activation of phosphorylase. Thus a decreased concentration of insulin has a stimulatory effect on glucagon (Wolfe et al, 1986; Terjung, 1979; Winder, 1985). Even though there is a decrease in plasma insulin concentration, there is not necessarily a reduction in cellular glucose uptake as insulin independent glucose uptake mechanisms activated by the working muscle itself ensure continued cellular glucose uptake (Terjung, 1979; Krook et al, 2004; Sigal et al, 2004). Glucagon and insulin are identified as the primary regulators of hepatic glucose production (HGP) (Wasserman et al, 1990).

Cortisol is a third metabolic hormone that has a protective effect against hypoglycemia. Its release from the adrenal cortex can be stimulated by low blood glucose concentrations, bypassing its circadian rhythm of tonic secretion which is stimulated by adrenocorticotropic hormone (ACTH) of the anterior pituitary (Terjung,

1979). Cortisol works to raise blood glucose levels by activating gluconeogenic enzymes of the liver increasing blood glucose levels (Kietzmann et al, 1998).

Cortisol response to exercise is dependent on the intensity of that exercise, as during low and moderate intensity the concentration is observed to remain stable or decline with changes associated with the psychological stress response to a work situation (Terjung, 1979; Davies and Few, 1973; Felig & Wahren, 1979). In contrast, during intense bouts of exercise circulating cortisol is shown to increase as a protective effect against hypoglycemia (Wasserman et al, 1984; Terjung, 1979; Sutton, 1978; Tharp, 1975; Shephard and Sidney, 1975). This may be due to the role of cortisol in longer duration adaptation to stress as opposed to an immediate glycemic debt response (Wasserman et al, 1984). This hormone in addition to glucagon works to raise blood glucose levels (Terjung; 1979). In the absence of cortisol, glucagon alone is unable to stop a hypoglycemic challenge of moderate duration (Wasserman et al, 1984).

2.10 Adrenergic Activity

Wasserman et al (1984) observed that during glucagon suppression with hypoglycemia an excessive release of epinephrine was observed at the nadir of plasma glucose concentration, which indirectly inhibited glucose uptake by the muscle, preventing a further decrease in plasma glucose concentration using an animal model. It is well established that as exercise intensity increases from low to high work rates there is a disproportionately large increase in the sympathoadrenal response. Adrenergic activity (activity of epinephrine and norepinephrine) increases from the onset of exercise along with HGP (Kjaer et al, 1993; Kreisman et al, 2000; Hartley et al, 1972; Sigal et al, 1996; Richter et al, 1982; Arnall et al, 1986; Galbo et al, 1975; Stainsby et al, 1991; Urhausen

et al, 1994). Epinephrine and norepinephrine concentrations can both increase approximately 15 fold and have significant correlations with glucose production during intense exercise (Kreisman et al, 2000; Marliss et al, 2000; Sigal et al, 1996; Sigal et al, 2000; Sigal et al 1994). HGP must increase to maintain euglycemia during exercise. Therefore, the significant role of the catecholamines has been demonstrated in observations where decreased adrenergic activity resulted in a reduction in HGP (Kjaer et al, 1993; Richter et al, 1982; Balikian et al, 2001). Balikian et al, 2001 demonstrated that during an incremental test with β adrenergic blockade, glucose concentration presented a continuous decrease not observed in the tests without the blockade. As a result of this, the inflection point (nadir) of the glucose profile used to determine threshold was not present, which provides evidence for the importance of adrenergic stimulation in glucose threshold determination (Balikian et al, 2001). During exercise, the catecholamines increase glucose production while also decreasing its uptake into the muscle cell (Kreisman et al, 2000; Marliss et al, 1991; Sigal et al, 1996; Hoelzer et al, 1986).

The increased glucose demand during exercise stimulates the release of the catecholamines from the adrenal medulla. After binding to α and β adrenergic receptors of neurons, pancreatic endocrine cells, heart and blood vessels, the catecholamines cause an increase in glucagon and decrease in insulin secretion. This has a whole body action of increasing plasma glucose concentration through increased glycogen breakdown and decreased cellular glucose uptake (Winder, 1985; Sigal et al, 1996; Kriesman et al, 2000; Aarnio et al, 2001; Marliss et al, 1991; Weber & Macdonald, 1993).

When subjects were infused with epinephrine it was found that plasma glucose increased supporting a regulatory role for epinephrine during intense exercise (Kreisman

et al, 2000). Kreisman et al (2000) use this finding and the observation that other studies which dispute an epinephrine causality role have intensities below that which catecholamines become key regulators. There is debate as to whether epinephrine causes increases in HGP (Wasserman et al, 1990; Kjaer et al 1993; Arnall et al, 1986; Sigal et al, 1996). Kjaer et al (1993) showed no attenuation of glucose appearance despite lowering plasma epinephrine. Arnall et al (1986) found that epinephrine infusion stimulated muscle glycogen depletion and blood glucose increase, but had no influence on the liver to break down glycogen any faster using an animal model. Using an islet cell clamp technique to prevent significant changes in glucagon-to-insulin ratios during exercise Sigal et al (1996) found that in comparison to the control group there was a similar glucose production response, thus providing evidence for the relative lack of importance of insulin and glucagon and by inference the importance of the catecholamines.

2.11 Summary

During incremental exercise there is a shift in substrate mobilization towards increasing anaerobic processes (Skinner & McLellan, 1980). The point at or near this shift is termed anaerobic threshold (Wasserman, 1984; MacDougall, 1977). The various energy generation processes are described in a three part model by Skinner and McLellan (1980) in which the first phase was considered to be primarily aerobic and was associated with linear increases in VO_2, VCO_2, and V_E. The second phase was depicted by nonlinear increases in V_E and VCO_2 with an initial rise in blood lactate. The third phase was marked by hyperventilation with sharp increases in blood lactate. Anaerobic threshold was defined as the exercise VO_2 above which anaerobic metabolism

supplements aerobic metabolism with increasing lactate production at the site of cellular anaerobiosis (Wasserman et al, 1999).

In anaerobic systems the observation of greater lactic acid production supports the practice of lactate threshold as the "gold standard" in AT prediction. The threshold of lactate can be described as the point where the rate of lactate production increases more than the rate of lactate removal (Wasserman, 1984; Skinner & McLellan, 1980; Neary et al, 1985; Antonutto & Di Prampero, 1995). A second method to determine anaerobic threshold has been ventilatory threshold. This non-invasive method has been based on excess CO_2 which occurs in correspondence with lactate increase. Determined graphically or mathematically, the ventilatory method considers the relationship of carbon dioxide production and oxygen consumption, with the point where VCO_2 exceeds VO_2 being near anaerobic threshold (Beaver et al, 1986; Hansen et al, 2003). In recent years a new method of threshold determination through examination of blood glucose concentration has been validated. The glucose threshold is identified as the point during an incremental exercise test at which blood glucose concentrations reach their nadir or minimum preceding an increase. This response has been shown to be similar to that of blood lactate concentration (Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004).

Muscle glycolysis increases during exercise to satisfy metabolic demands to maintain euglycemia (Wahren et al, 1971; Lewis et al, 1999; Wasserman et al, 1999). This increase occurs non-linearly to metabolic rate (Cooper et al, 1989). Near anaerobic threshold glucose production by way of glycolysis begins to exceed glucose uptake, resulting in hyperglycemia during intense exercise (Kjaer et al, 1986; Kjaer et al, 1991; Galbo et al, 1981). Preceding this hyperglycemic state there has been observation of a

nadir or minimum in blood glucose (Northius, 1995; Wasserman, 1984, Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). Glucagon, insulin, cortisol and the catecholamines play mediating roles in glucose production and uptake. Glucagon stimulates enzymes to synthesize glucose while insulin initiates a cascade reaction allowing transport of glucose across the muscle cell wall. During exercise, insulin levels decrease with a magnitude closely linked to exercise intensity while glucagon concentration increases (Felig et al, 1972; Terjung, 1979; Winder, 1985). The outcome of this is increased glucose production through liver glycogenolysis which is activated by glucagon. The process is facilitated by the decreased influence of insulin (Terjung, 1979; Winder, 1985; Wolfe et al, 1986). Cortisol response to exercise is dependent on intensity as it is shown to increase at intense bouts of exercise. The cortisol increase works along with glucagon to increase plasma glucose levels (Terjung, 1979; Winder, 1985). The catecholamines also increase during exercise and have been shown to coincide with plasma glucose increases which may be due to a combination of the generation of hepatic glucose from glycogen and an inhibition of cellular glucose uptake (Kreisman et al, 2000; Marliss et al, 1991; Sigal et al, 1996).

The nadir or minimum of blood glucose during incremental exercise has been identified as a possible predictor of anaerobic threshold (Northius, 1995; Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). The intention of this study was to further validate the glucose threshold as a predictor of anaerobic threshold. A second objective was to examine the related glucoregulatory responses including the hormones insulin, glucagon and cortisol concentrations in relation to the glucose concentration during a graded exercise test.

2.12 References

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CHAPTER 3

THE NADIR IN SERUM GLUCOSE AS A PREDICTOR OF ANAEROBIC THRESHOLD

3.1 Introduction

The existence and measurement of anaerobic threshold has been a controversial topic but remains a pivotal measure within exercise and sport science (Hollmann, 2001; Svedahl & Macintosh, 2003). Blood lactate measurements and ventilatory parameters have been the most widely used methods of anaerobic threshold determination and have been examined for reliability and validity (Wasserman et al, 1972; Bhambhani and Singh, 1980; Wasserman, 1984; Prud'homme et al, 1984). A method of threshold identification using blood glucose measurement has been supported as a possible alternative or addition to these established measures (Northius et al, 1995; Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). This glucose response to graded exercise follows the identification of a low point or nadir in blood concentration during exercise and has been shown to evaluate aerobic capacity as well as being related to the lactate and ventilatory thresholds (Simoes et al, 1999).

During graded exercise the blood glucose concentration reaches a low point before beginning a steady increase (Northius et al, 1995). Similar to the identification of a shift in lactate concentration (lactate threshold) during graded exercise, it is this changing in glucose concentration profile (glucose threshold) that is used as a marker of anaerobic threshold (Simoes et al, 1999). Simoes et al (1999) used a discontinuous graded exercise test in which participants had periods of rest between exercise bouts.

Although this protocol elicited a lactate, ventilatory and glucose threshold response, it may not be reflective of the metabolic environment associated with a continuously graded exercise test where accumulating metabolic activities influence anaerobic threshold.

With the applicability of lactate determination of anaerobic threshold for aerobic fitness testing and monitoring of athletes, it is possible that a valid measure of glucose threshold could provide the same benefits. Despite such associative possibilities, the validity of this measure has not been extensively examined. As well, previous research has only speculated on the mechanisms underlying this glucose response (Simoes et al, 2003), which has not been systematically studied.

It has been shown that blood glucagon and cortisol concentrations increase while insulin concentrations decrease during exercise of increasing intensity. Glucagon triggers the production and release of glucose by glycogenolysis in the liver, while cortisol works to raise plasma glucose levels by increasing amino acid availability for gluconeogenesis through the enzymes fructose 1,6-biphosphate and phosphoenolpyruvate carboxykinase (Kietzmann et al, 1998). The decrease in insulin facilitates the glucagon response since insulin has been shown to inhibit glycolytic enzymes (Terjung, 1979; Winder, 1985). The cumulative result of these responses is an increase in plasma glucose concentration to maintain euglycemia during exercise (Wasserman et al, 1999; Wahren et al, 1971). With the close associations of these hormones to circulating levels of glucose it is necessary to examine these hormones in relation to glucose changes during graded exercise and the potential relationships with the glucose threshold.

Therefore, the purpose of this study was to investigate the response of serum glucose and the changes in glucagon, insulin and cortisol concentration to incremental

exercise. The response of glucose was defined by the concentration of glucose in serum observed during exercise of increasing intensity. It was hypothesized that there is a nadir of serum glucose which occurs in a predictable fashion to lactate and ventilatory thresholds. It was further hypothesized that the occurrence of glucose threshold would be mirrored by the increased concentration of glucagon and cortisol, and decreased concentration of insulin to maintain euglycemia amidst the metabolic demands leading up to and beyond anaerobic threshold.

3.2 Participants

Twenty-two healthy active males residing in the city of Edmonton volunteered to participate in this investigation. A healthy, active individual was defined as a person who participated in regular aerobic physical activity a minimum of 3 times a week and was free of conditions that may have impeded their metabolic function or their effort and performance during the graded exercise test. Participants were required to complete and sign the Physical Activity Readiness Questionnaire (PAR-Q) (Appendix D), the Healthy Physical Activity Participation Questionnaire (Canadian Physical Activity, Fitness and Lifestyle Approach, 3rd Edition, 2004) (Appendix C), and an informed consent (Appendix B). Subject characteristics appear in Table 3-1. This investigation was reviewed and approved by the Faculty of Physical Education and Recreation Ethics Board at the University of Alberta.

Physiological Testing

The exercise testing involved three visits to the exercise physiology lab. One session for maximal oxygen consumption (VO_2 peak) testing, one for anaerobic threshold (AT) testing, and a third for a fasted blood sample. The peak VO_2 test was conducted

first to also provide participants with experience to the testing environment. Between 2 and 5 days after the peak VO_2 test the participants returned to the lab in the rested state for the AT test. This test included blood samples taken at rest before exercise and in the last minute of each power output. Between 2 and 5 days after the AT exercise test and the morning after an overnight fast, the participants returned on a different day to provide a fasting blood sample.

Peak VO_2 Test

Each subject arrived at the lab for his first exercise test in the rested state. This required that they refrain from any formal exercise 24 hours before the exercise test. All subjects were also asked to have a light meal and water 2 to 3 hours before the peak VO_2 exercise test.

The peak VO_2 test required graded, incremental exercise to volitional exhaustion on a cycle ergometer (Monark, Sweden) similar to that outlined by the American College of Sports Medicine (ACSM) guidelines for a graded cycle exercise test (pedal rate = 75 rpm, power out starts at 74 w and is increased by 37 w every 2 minutes). During the test, each subject was suited with a headgear and mouthpiece apparatus to collect all the air they expired which was collected and analyzed in a calibrated metabolic measurement system (ParvoMed True Max 2400, Utah). Volitional exhaustion was defined as the point at which the subject could not continue to exercise due to fatigue despite further verbal motivation. Peak VO_2 was defined as the highest VO_2 that was recorded during the exercise test and was associated with a respiratory exchange ratio greater than 1.1, achievement of age-predicted or known maximum heart rate and volitional exhaustion. A 5 minute warm-up and cool-down as well as stretching was included. Heart rate was

recorded every minute using telemetry from Polar Pacer heart rate monitor (Polar USA, Connecticut). This test established peak VO_2 in each individual, and also served to provide testing experience for the subjects prior to the experimental anaerobic threshold test.

Anaerobic Threshold Test

After a minimum of two days and maximum of five days, each subject returned for the second exercise test that was also a graded, incremental protocol but not to a maximum intensity. This test was scheduled between 10:00 am and 1:00 pm. The same preliminary exercise testing requirements were followed as described previously. However, each subject was provided with one can of ENSURE™ which is a meal replacement drink that contains a known amount of carbohydrate, fat and protein as well as other vitamins and minerals (See Appendix G) three hours before the anaerobic threshold test. Each subject was also requested to consume 500 ml of water in small amounts (125 ml every 30 minutes) two hours before the test. This was done to establish a standard starting nutritional state and a baseline blood glucose concentration for each subject.

Upon entering the exercise testing laboratory the participants were seated and had a cathelon placed in a forearm vein. Once secure, a resting blood sample was drawn from the cathelon, after which sterile saline was inserted into the cathelon to prevent clotting at the site. In the last minute of each power output during the graded exercise test, 3ml of blood was drawn to remove any saline solution, followed by the experimental blood sample used for analysis. Once again sterile saline would be injected into the cathelon to prevent blood clotting before the next blood sample to taken at the power output.

The same metabolic system and setup was used as previously described for the peak VO_2 test. The exercise protocol was performed on the same cycle ergometer and subjects began with cycling at 75 pedal rpm and a power output of 74 watts. Subsequently, the power output was increased every 3 minutes by 37 w. The occurrence of ventilatory threshold was determined using the V-Slope method of Wasserman et al. (1999) by observation of the graph of VCO_2 versus VO_2. Once the subject exceeded their ventilatory threshold by 2 power output increments the test was terminated. Heart rate was recorded every minute using telemetry from a Polar Pacer heart rate monitor (Polar USA, Connecticut).

Threshold Determination

Threshold determinations were made for glucose, lactate and ventilatory measures. The power output at which the respective threshold was determined was used as a common scale of measurement between the different threshold variables. For each of the threshold measures two researchers independently (blind) determined the threshold point in a random order based on the following stated guidelines. If the independent determinations of the power output at threshold differed, a third researcher adjudicated the difference. All three examiners had to unanimously agree on the point or the data was rejected.

Glucose Threshold

The threshold of glucose was defined as the lowest point in serum glucose concentration preceding a significant rise. This point was operationalized by the power output at which it was observed and was used as the dependent variable for glucose

threshold (Northius 1995; Simoes et al, 1999; Simoes et al, 2003). The VO_2 corresponding to the glucose threshold was also determined.

Lactate Threshold

The threshold of lactate was defined as the exercise VO_2 above which a net increase in lactate production is observed to result in a sustained increase in deproteinized blood lactate concentration (Wasserman et al, 1999). The graphical point of lactate threshold was determined as the first rise from baseline of plasma lactate concentration. This point was operationalized by the power output at which the lactate threshold was observed, as well as the VO_2 corresponding to this point.

Ventilatory Threshold

Ventilatory threshold was determined using the V-slope method of Wasserman et al (1999). This method involved the analysis of VCO_2 as a function of VO_2 and is found graphically as a division of the scatter plot of data into two slopes, one being less than one and the other being greater than one. The intersection of these two slopes is determined as the ventilation threshold (Beaver et al, 1986). To standardize comparisons this point was also operationalized by the power output and VO_2 at which it was observed.

Blood Collection

A registered nurse took all exercise blood samples from an arm vein. Blood samples were taken prior to exercise and during the last minute of each three minute power outputs during the anaerobic threshold graded exercise test. A 22 gauge cathelon was inserted into a forearm vein and secured with tape and capped. Sterile saline (0.9 % NaCl) solution was injected into the cathelon to prevent clotting at the site. The blood

samples (3 ml each) were drawn with a syringe after a 0.5 ml sample was removed and discarded to remove any saline solution at the site.

On a separate day, a 5 ml fasting rested blood sample was drawn by an individual trained in venipuncture blood sampling. Subjects refrained from food after 9 pm the night before the 8 am blood sample was taken; water was permitted.

Blood Analysis

Immediately after sample collection, 0.25 ml of whole blood was pipetted from the blood collection tube and added to 1 ml of ice cold 8% perchloric acid, vortexed and centrifuged at 3000 xg for 10 minutes before being frozen for later spectrophotometric assay analysis of lactate concentration (Appendix E). A 50 ul micro-hematocrit tube was filled from the blood collection tube and hematocrit was measured using the micro-centrifuge method. The remaining whole blood was allowed to clot (~30 minutes), centrifuged at 3000 xg, and the serum supernatant drawn off and frozen for later analyses of glucose using spectrophotometric assays (Appendix E), and serum free cortisol, glucagon, and insulin using commercially available radioimmunoassay (RIA) kits (Oxoid, Stillwater, MN) (Appendix F).

The commercial radioimmunoassay kit for glucagon stated a protease inhibitor was recommended during specimen collection, which was not done in this study. It would be expected that the glucagon values would be elevated in absence of this step, but the relative relationship of sample concentration changes would not be differentially affected. Comparison of samples treated with a protease inhibitor and those not treated were compared by Oxoid, Stillwater, MN, and it was found that the samples without a

protease inhibitor were equal to 1.07(with the protease inhibitor) + 52 $pg \cdot ml^{-1}$ (Oxoid, Stillwater, MN; Double Antibody Glucagon; PIKGND-3, 2003-11-04).

For the glucose assay samples were run in triplicate, while participant samples for the lactate, cortisol and insulin assays were run in duplicate (Appendix E, F). Only fasted blood samples were run in duplicate for the glucagon assay, as the volume of exercise blood samples was not enough for duplicate analysis (Appendix F). The mean coefficient of variance (± standard deviations) for the glucose, lactate, glucagon, insulin and cortisol assays were 2.4 ± 1.9 $mmol \cdot l^{-1}$, 2.4 ± 1.8 $mmol \cdot l^{-1}$, 6.7 ± 3.9 $pg \cdot ml^{-1}$, 8.2 ± 4.7 $ulU \cdot ^{-1}$, 5.0 ± 3.3 $ug \cdot dl^{-1}$, respectively. Due to extreme outliers (greater than 3 standard deviations from the mean) in the insulin concentrations, of a few participant samples, 3, 2, 1, and 2 were removed from analysis at measurement times of fasted, pre-exercise, 74w, and 184w, respectively.

Statistical Analysis

Statistical analyses were performed using a commercially available statistical software package (STATISTICA, Oklahoma City, Oklahoma). All group data were expressed as means and standard deviations. Extreme outliers determined as greater than three standard deviations from the mean were removed from analysis. Simple linear regression was conducted to determine the relationships between lactate threshold and glucose threshold, ventilatory threshold and glucose threshold, and between lactate and ventilatory thresholds. Two separate one-way analysis of variance (ANOVA) with repeated measures were used to compare the oxygen consumption and power output of the three different threshold techniques. Separate one-way ANOVA's with repeated measures were conducted to determine differences in concentrations across common

measurement times (at rest and each power output during the graded threshold exercise test). Each participant's individual hormonal profile during the graded exercise test was divided into two slopes, prior to and after the glucose threshold to the end of the exercise test. A one-way ANOVA with repeated measures was conducted to compare the slopes of these two segments of hormonal response to graded exercise Significant F ratios were further examined with a Newman Kuels multiple comparison procedure and alpha was set a-priori, at $P < 0.05$ for all analyses.

3.3 Results

Subject Characteristics

The mean age, height and weight of the participants was 24.5 ± 4.0 years, 182.0 ± 9.1 cm, and 84.8 ± 17.4 kg respectively. The participants in this study were all regularly involved in physical activity, participating in 5.4 ± 2.4 intense activity sessions per week (range from 2.0 to 10.0 intense activity sessions per week) (Table 3-1). These participants included athletes from hockey, football, cycling and iron man triathlon sports.

Exercise Test Performance

Table 3-2 provides the mean performance information of the individual power outputs during the anaerobic threshold exercise test. All participants completed the first 4 power outputs (74, 110, 147, & 184 watts) while 21, 18, 13, and 5 participants completed the final 4 graded exercise test power outputs (221, 258, 294 & 331 watts, respectively). The actual calculated power output at each workload was not statistically significantly different from the power output outlined in the testing protocol. Oxygen consumption and carbon dioxide production significantly increased with each increase in power output during the graded exercise test (Figure 3-1). The mean absolute and relative peak oxygen

consumption of the peak VO_2 test was 4.33 ± 0.56 L/min and 52.7 ± 9.4 ml/kg/min respectively (Table 3-1). The mean peak absolute and relative oxygen consumption of the anaerobic threshold test was 3.97 ± 0.56 L/min and 48.0 ± 9.3 ml/kg/min respectively.

VO_2 and VCO_2 increased significantly at each increase in power output during the graded exercise test (Figure 3-1).

Lactate concentration increased significantly during the graded exercise test. The concentration of lactate in deproteinized blood at power outputs above 147 watts was significantly greater than that of power outputs of 147 watts or less (Figure 3-2).

Thresholds

Experimenters agreed on the lactate and ventilation threshold point for all 22 participants while they agreed on the glucose nadir (threshold) point for 20 participants according to the established definition. Figure 3-3 depicts the ventilatory curve, and the lactate and glucose concentrations as a function of power output for a single participant with indications of the threshold determination for each parameter.

The PO and VO_2 at the threshold of glucose and lactate were not significantly different from each other (Table 3-3; Figure 3-4; Figure 3-5). There was also no statistically significant correlation between lactate threshold and glucose threshold measurements as power output ($r=0.41$; $p=0.070$) or oxygen consumption ($r=0.43$; $p=0.058$) (Figure 3-6; Figure 3-7).

The PO and VO_2 at the ventilatory threshold was significantly greater (~ 46 Watts; ~ 1 workload) than the glucose threshold ($p<0.05$) (Table 3-3; Figure 3-4; Figure 3-5). There was a significant correlation between the PO ($r=0.51$; $p=0.022$) and VO_2

(r=0.58; p=0.007) using the ventilatory threshold and glucose threshold measures (Figure 3-8; Figure 3-9).

The power output and oxygen consumption at the ventilatory threshold was significantly greater (~ 32 Watts; ~ 1 workload) than at lactate threshold ($p<0.05$) (Table 3-3; Figure 3-4; Figure 3-5). The correlation between measures was not significant for the PO (r=0.38; p=0.096) at ventilatory threshold and lactate threshold but it was significant for VO_2 using these two measures (r=0.47; p=0.036)(Figure 3-10; Figure 3-11).

Hematocrit measures of blood samples

There was no significant change or difference in hematocrit measures pre-exercise, during the graded exercise or in the fasted sample (Data not shown).

Glucose response to a graded exercise test

Serum glucose concentrations during the fasted state, pre-exercise and at each power output appear in Table 3-4. There was no significant differences between glucose concentrations at any measurement time (Table 3-4; Figure 3-12).

Hormone responses to a graded exercise test

Table 3-4 lists the concentrations of insulin, glucagon and cortisol in the fasted state, pre-exercise and at each power output during the graded exercise test.

Insulin concentrations during the graded exercise test decreased significantly from the pre-exercise state and at 74 watts throughout the protocol to 221, 258, 294 and 331 watts. The insulin concentration at the final two power outputs were significantly lower than those at the power outputs of 110, 147 and 184 watts (Table 3-4; Figure 3-13).

The serum glucagon concentration at 294 and 331 watts, was significantly greater than at 74, 110, 147 and 184 watts (Table 3-4; Figure 3-14).

Fasted serum cortisol concentrations were significantly higher than pre-exercise samples. During the graded exercise test, 331 watts was associated with serum cortisol concentrations greater than pre-exercise and all other power outputs (Table 3-4; Figure 3-15).

Hormone responses in relation to glucose threshold

Figure 3-16 depicts serum hormonal concentrations and serum glucose concentrations at rest, before exercise and during the graded exercise test.

Insulin concentration decreased during the graded exercise test (Figure 3-13; Figure 3-16). The slope of the insulin concentration profile was significantly lower after glucose threshold compared to before (Figure 3-17).

Glucagon concentration increased during the graded exercise test (Figure 3-14; Figure 3-16). The slope of glucagon concentration was significantly greater after versus before the glucose nadir (Figure 3-18).

Cortisol concentration increased in the final power output (331w) of the graded exercise test (Figure 3-15; Figure 3-16). There was no significant difference between the slope of cortisol data points before and after the nadir in serum glucose concentration (Figure 3-19).

3.4 Discussion

The experimental protocol selected for this study was designed to examine the existence of a nadir in serum glucose and if this was the same as, or related to, other indices of the anaerobic threshold. Further examination was included to evaluate whether

the profile of serum glucose was explainable by the changes in glucoregulatory hormones. It was hypothesized that there would be a nadir of serum glucose concentration (glucose threshold) which occurs in a predictable fashion to other indices used to indicate the anaerobic threshold. This study confirmed that a nadir in serum glucose occurs during graded exercise, and that this nadir was observable in the majority of subjects (20/22). There was no significant difference between the power output or VO_2 at the glucose and lactate thresholds, further suggesting that one measure (glucose) corresponds to the other (lactate). Furthermore, glucose threshold was correlated to ventilatory threshold.

It was also hypothesized that the occurrence of the glucose threshold would be mirrored by concentration changes in glucoregulatory hormones such as glucagon, cortisol, and insulin. The results of this study support this hypothesis since there was a significant increase in glucagon concentration and a significant decrease in insulin concentration after glucose threshold occurred. However, there was no significant change in cortisol concentration before and after glucose threshold.

The nadir in serum glucose as a predictor of anaerobic threshold

This study confirmed that a nadir in serum glucose occurs during graded exercise that was observable in the majority of subjects. This was in agreement with other studies evaluating blood glucose responses during exercise indicating anaerobic threshold, despite using different exercise protocols (Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). Similar to the findings of Simoes et al (1999), no differences were observed between power output and VO_2 at glucose threshold and lactate threshold. Furthermore, it was possible to observe a decrease in the concentration of glucose prior to the nadir in

serum glucose. The power output at this nadir was similar to the power output observed at the time of the marked increase in deproteinized blood lactate concentration. Lactate and glucose concentrations concurrently increase from this power output to the end of the exercise test. This is consistent with previous reports (Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). A significant correlation was not observed between power output or VO_2 measured at the glucose threshold and lactate threshold ($r=0.41$; $p=0.070$, $r=0.43$; $p=0.058$ respectively). This conflicts with previous reports which found significant correlations between the two measures (Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004), and may be due to the different subject groups and exercise protocols. Further research comparing exercise protocols and the glucose threshold is required to examine this discrepancy.

There was a significant correlation between the power output and VO_2 measured at the glucose and ventilation threshold, as hypothesized. Findings of Simoes et al (2003), supports the suggestion that various metabolic and ventilatory responses might identify the anaerobic threshold. Further, Simoes et al (2003) determined that ventilatory and glucose threshold measures identified the same exercise intensity as the anaerobic threshold. This was not observed in this study, as the power output and VO_2 of glucose and ventilatory threshold were significantly different. Glucose threshold was found to be approximately one power output lower than the ventilatory threshold using a continuous graded exercise test. This finding is in agreement with those of Wasserman et al, (1999) and Svedahl and Macintosh (2003) that suggest the ventilatory indicators of threshold occur at a higher intensity of exercise than metabolic indices of threshold such as lactate. At the intensity of anaerobic threshold, total energy requirement exceeds the rate that

aerobic metabolism can supply and there is increased production of lactic acid. As lactate accumulates in the blood so does CO_2 production because of bicarbonate buffering of the hydrogen ion dissociated in the muscle from lactic acid (Beaver et al, 1986; Wasserman et al, 1999; Svedahl and Macintosh, 2003). In this study, the VO_2 of ventilation threshold significantly correlated to approximately one power output greater than that which elicited the lactate threshold.

Insulin, glucagon and cortisol response to graded exercise and their relationship to the nadir in serum glucose

There has been speculation that the characteristic response of glucose underlying the glucose threshold was at least partly due to the increased liver glucose output and decreased cellular glucose uptake following the nadir in blood glucose (Simoes et al, 1999; Simoes et al, 2003). These homeostatic alterations are primarily influenced by changes in the glucoregulatory hormone concentrations (Winder, 1985; Sigal et al, 1996; Kriesman et al, 2000; Aarnio et al, 2001; Marliss et al, 1991; Weber & Macdonald, 1993). Concentrations of the catecholamines (epinephrine, norepinephrine), insulin, glucagon and cortisol are known to change during exercise with a magnitude that was closely linked to exercise intensity (Felig et al, 1972; Terjung, 1979; Winder, 1985; Wolfe et al, 1986; Kjaer et al, 1993; Kreisman et al, 2000; Hartley et al, 1972; Sigal et al, 1996; Richter et al, 1982; Arnall et al, 1986; Galbo et al, 1975). During the graded exercise test of the present study the hormones glucagon, insulin and cortisol experienced significant changes between power outputs. The concentration of insulin during the final power outputs of the graded exercise test was significantly lower than during the initial power outputs. The lowered concentration of insulin during later stages of the graded exercise test has been observed previously in the literature, as it has been shown to

decrease with a magnitude and be closely linked to the intensity of exercise (Felig et al, 1972; Terjung, 1979; Winder, 1985; Wolfe et al, 1986). Glucagon concentration significantly increased in the final power outputs as opposed to the initial power outputs during the graded exercise test. Previous research indicates that glucagon concentration increases as the intensity of exercise increases (Felig et al, 1972; Terjung, 1979; Winder, 1985; Wolfe et al, 1986). Cortisol was only significantly higher at the final power output of the graded exercise test, but this was greater than all other power outputs. This late cortisol response to graded exercise in the present study is supported by other research that has found that cortisol concentration remains stable during low and moderate intensity exercise of short duration and only increases at intense bouts of exercise when there is a hypoglycemic challenge of moderate duration (Wasserman et al, 1984; Terjung, 1979; Sutton, 1978; Tharp, 1975).

In this study, the low point in the serum glucose concentration paralleled a concomitant increase in serum glucagon and concomitant decrease in serum insulin concentrations. Previous reports indirectly support these observations as the greatest changes in glucagon, insulin and the catecholamines occur around 75% VO_{2max}, which would be near the intensity for the onset of anaerobic threshold, depending on the fitness level of the subject (Wasserman et al, 1984; Terjung, 1979; Sutton, 1978; Tharp, 1975; Felig et al, 1972; Winder, 1985; Wolfe et al, 1986; Wasserman et al, 1999; Hartley et al, 1972; Weltman et al, 1990). Since this study demonstrated that the nadir in serum glucose occurs at the same or similar exercise intensity as other threshold indices, the changes in hormonal concentrations observed after the nadir in serum glucose may

provide more support to the validity of the glucose threshold to reflect a metabolic response and as a marker of anaerobic threshold.

The association of the nadir in serum glucose and other indices of anaerobic threshold to hormonal response during graded exercise

It has been suggested that the concurrent increase in glucose and lactate concentrations after anaerobic threshold was a result of the association between exercise intensity and the glucoregulatory hormones (Simoes et al, 1999). Increased lactate concentration has been reported to result from the increased rate of glycolytic conversion of pyruvate to lactate at intensities associated with the anaerobic threshold (70-85% peak VO_2) (Weltman, 1989; Wasserman, 1984; Wasserman et al, 1999). Increasing catecholamines, glucagon, cortisol, and decreasing insulin have been reported to increase blood glucose concentration during exercise. The greatest increase has been reported to follow stimulation of lactate production and glycogenolysis at intensities above anaerobic threshold (Winder 1985; Wasserman et al, 1991; Stainsby et al, 1991; Urhausen et al, 1994). Simoes et al (1999) suggest that the decrease in glucose concentration up to the point identified as anaerobic threshold was a result of the greater glucose uptake by skeletal muscle versus hepatic glucose production. This relationship reverses during anaerobic threshold intensities as the influence of the catecholamines, glucagon and insulin increase hepatic glucose production relative to cellular glucose uptake. As a result there was the observed increase in blood glucose concentration concurrent to the lactate concentration increase (Winder et al, 1985; Kriesman et al, 2000, Sigal et al, 1996).

Based on the observations of glucagon and insulin in this study, further support is provided to the relationship between exercise intensity, hormonal response and glucose

threshold. During the graded exercise test there was a decrease in the serum insulin concentration. The decrease in insulin concentration may have contributed to the characteristic glucose profile through metabolic action that increases the hepatic glucose production relative to cellular glucose uptake relationship. Insulin release from the β cells of the pancreas can be inhibited by lowered blood glucose concentrations. The result of decreased insulin is an increased blood glucose concentration accompanied by decreased insulin-dependent cellular glucose uptake and facilitation of glucagon metabolic action (Terjung, 1979; Winder, 1985; Krook et al, 2004; Aarnio et al, 2001; Sigal et al, 2004). Thus, concomitant to the low point in blood glucose when the metabolic response aims for euglycemia, the concentration of insulin decreases. Insulin is known to facilitate the processes of glucagon as insulin is antagonistic to glucagon (Felig et al, 1972; Terjung, 1979; Winder, 1985; Wolfe et al, 1986). As the exercise intensity increases, glucagon release from pancreatic α cells can be stimulated by a decrease in glucose concentrations. An increased glucagon concentration results in increases in glucose production through stimulation of liver glycogenolysis. The observed decline in blood glucose levels at the same workload as lactate threshold may be associated with the release of glucagon that contributes to the subsequent increase in blood glucose concentration observed after the nadir (Terjung, 1979; Winder, 1985).

Although this investigation has provided support for the relationship of insulin and glucagon to the nadir of serum glucose, it can not conclude a specific mechanism of action to achieve the nadir of blood glucose. As such, further research specific to glucose threshold and graded exercise is needed to examine the detailed production and clearance

of glucose in the human body, the contribution of non-carbohydrate energy sources, and the hormones that mediate these processes.

Limitations

One limitation of this study was that due to the range of fitness levels of the participants, there was a corresponding range in the length of the graded exercise test. As a result some participants completed fewer power outputs than others. This could have influenced hormonal measurements through time delays in hormonal action. A second limitation of this study was that due to inadequate sample volumes, only the fasting rested samples of glucagon were analyzed in duplicate. This may reduce reliability of the glucagon values during the graded exercise test, although the coefficient of variance for all resting values was low. Nutritionally, the meal replacement drink provided to each participant may have been a limitation as all subjects were given the same quantity preceding the threshold test regardless of body mass. This may have influenced resting glucose and hormonal measures. A final limitation was that all measures from blood samples drawn from an arm vein were assumed to be valid indicators of the whole body glucose, lactate and hormonal response, although this assumption was common in hormonal and metabolic research using human participants.

Conclusions

The results of this study indicate that there was a nadir in serum glucose which occurs in predictable fashion to other accepted indicators of anaerobic threshold. Although this investigation differed from previous research in that a significant difference between the power output of glucose and ventilatory threshold was observed, this may be explained by a delay in ventilatory threshold as a result of the time delay of bicarbonate

buffering of the hydrogen ion dissociated in the muscle from lactic acid (Beaver et al, 1986; Wasserman et al, 1999; Svedahl and Macintosh, 2003). This investigation also demonstrated that insulin and glucagon have concomitant responses to the nadir in serum glucose, with insulin significantly decreasing and glucagon significantly increasing after this power output during the graded exercise test. Future research should continue to examine the validity and reliability of the glucose threshold, as well as examine in greater detail the mechanisms resulting in the relationship of the nadir in blood glucose to anaerobic threshold.

3.5 References

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Table 3-1. Participant characteristics. Values are means ± SD (minimum - maximum)

Age (years)	Body Mass (kg)	Height (cm)	Activity sessions/week (intense)	VO_2max ($ml \cdot kg^{-1} min^{-1}$)	VO_2max ($l \cdot min^{-1}$)
24.5 ± 4.0 (19-34)	84.8 ± 17.4 (62.0 - 134.0)	182 ± 9.1 (168.0 - 197.0)	5.4 ± 2.4 (2.0 - 10.0)	52.7 ± 9.4 (36.8 - 67.8)	4.33 ± 0.56 (3.11 - 5.22)

Table 3-2. Participant submaximal exercise test responses. Values are means ± SD.

Protocol PO (w)	N	Actual P0 (w)	VO_2 ($l \cdot min^{-1}$)	VCO_2 (l·min-1)	Ve/VO_2	Ve/VCO_2
74	22	74.1 ± 2.3	1.42 ± 0.20	1.23 ± 0.14	25.23 ± 2.31	29.05 ± 2.36
110	22	110.4 ± 4.6	1.73 ± 0.17	1.60 ± 0.14	25.73 ± 2.73	27.82 ± 2.40
147	22	147.9 ± 6.9	2.13 ± 0.16	2.07 ± 0.17	26.50 ± 3.25	27.18 ± 2.42
184	22	187.2 ± 9.9	2.61 ± 0.18	2.64 ± 0.22	27.77 ± 4.17	27.50 ± 3.28
221	21	223.8 ± 10.2	3.05 ± 0.20	3.20 ± 0.26	30.76 ± 7.01	29.24 ± 4.88
258	18	259.4 ± 13.0	3.55 ± 0.21	3.81 ± 0.26	32.83 ± 6.39	30.89 ± 4.95
294	13	290.6 ± 19.4	3.96 ± 0.27	4.32 ± 0.29	35.15 ± 5.67	32.23 ± 4.92
331	5	327.0 ± 20.8	4.39 ± 0.31	4.87 ± 0.64	37.60 ± 4.39	33.60 ± 2.97

Table 3-3. Power output and oxygen consumption at the glucose, lactate and venilatory thresholds. Values are means ± SD.

	Power Output (w)	Oxygen Consumption ($l \cdot min^{-1}$)
Glucose Threshold	164.2 ± 30.9	2.34 ± 0.39
Lactate Threshold	178.1 ± 33.0	2.49 ± 0.46
Ventilatory Threshold	210.1 ± 33.4*	2.90 ± 0.49*

* - significantly different from other threshold determinants

Table 3-4. Serum glucose and hormonal response to graded exercise. Values are means ± SD.

	Fasted	Pre-Exercise	74w	110w	147w	184w	221w	258w	294w	331w
Glucose Assay N	22	22	22	22	22	22	21	18	13	5
Glucose ($mmol \cdot L^{-1}$)	5.39 ± 0.63	4.49 ± 0.51	4.86 ± 0.63	4.98 ± 0.66	4.88 ± 0.60	4.97 ± 0.63	5.10 ± 0.55	5.05 ± 0.50	4.93 ± 0.56	5.41 ± 0.48[a]
Insulin Assay N [1]	19	20	21	22	22	20	21	16	13	5
Insulin ($ulU \cdot ml^{-1}$)	5.68 ± 2.93[b]	5.88 ± 2.75[b]	5.26 ± 2.44	4.47 ± 2.23	4.31 ± 2.28	4.46 ± 2.54	3.23 ± 1.84[d]	3.05 ± 2.84[d]	1.50 ± 0.98[c]	1.66 ± 1.78[c]
Glucagon Assay N [2]	22	20	21	22	22	22	21	18	12	5
Glucagon ($pg \cdot ml^{-1}$)	81.21 ± 18.84	78.98 ± 14.94	75.16 ± 13.91	74.64 ± 13.77	72.71 ± 11.62	74.65 ± 11.75	79.35 ± 14.24	79.63 ± 12.53	86.11 ± 11.31[c]	89.25 ± 14.44[c]
Cortisol Assay N	22	22	22	22	22	22	21	18	13	5
Cortisol ($ug \cdot dl^{-1}$)	18.09 ± 4.53[a]	12.72 ± 4.13	14.15 ± 4.23	14.66 ± 4.18	14.86 ± 4.45	14.80 ± 4.82	14.97 ± 5.47	14.81 ± 5.16	16.37 ± 5.52	21.21 ± 5.97[e]

N = Sample size

[1] Extreme outliers removed from analysis (>3 sd from mean)

[2] Not enough volume for duplicate analysis of exercise samples

[a] - significantly different from pre-exercise

[b] - significantly different from final 4 power outputs

[c] - significantly different from initial 4 power outputs

[d] - significantly different from 74w

[e] - significantly different from all power outputs and pre-exercise

Table 3-5. Comparison of slopes for each hormone determined before and after the glucose threshold. Values are means ± SD.

	Before	After
Insulin	-0.142 ± -0.664	-0.670 ± -0.499*
Glucagon	-1.505 ± -3.850	3.318 ± 2.640*
Cortisol	0.453 ± 0.830	0.245 ± 0.605

* - significantly different from before glucose threshold

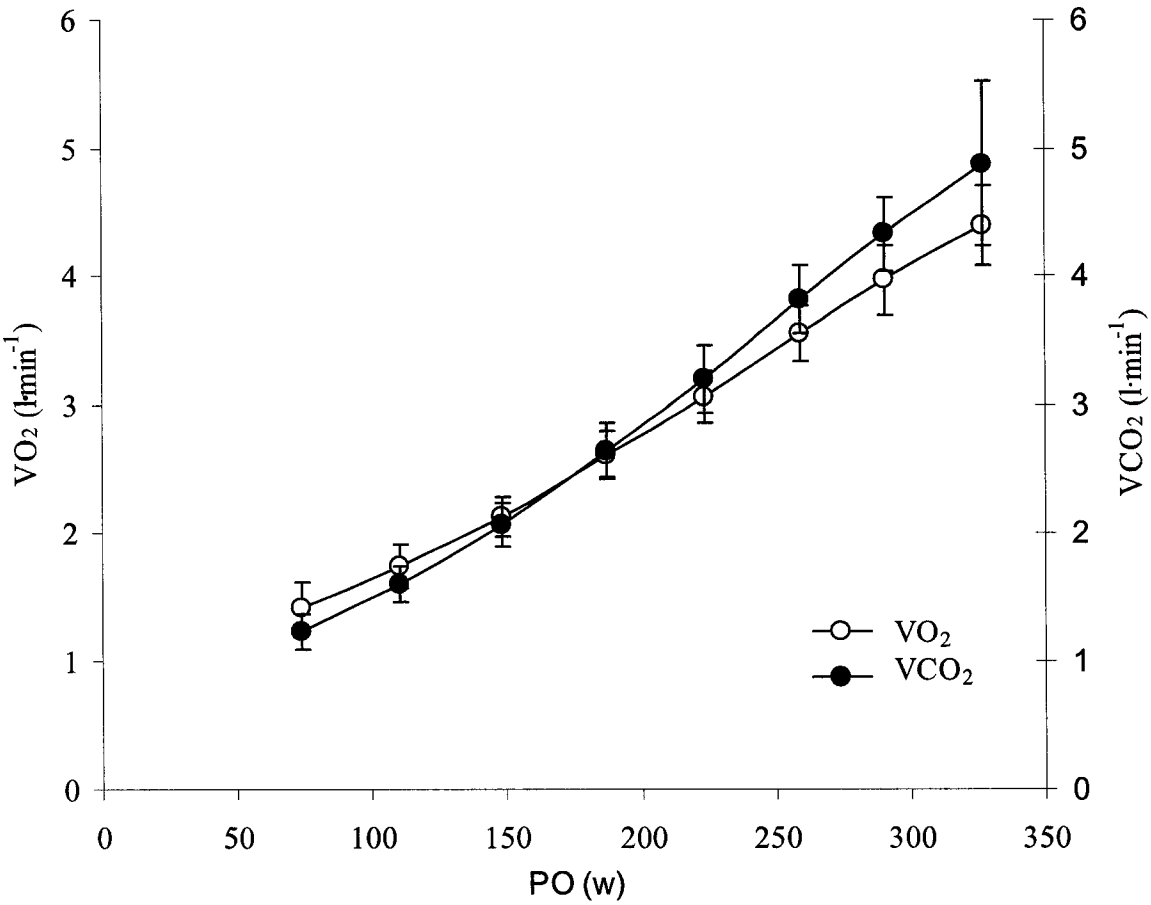

Figure 3-1. Oxygen and carbon dioxide ventilatory responses. (Error bars = SD)

Note: VO_2 and VCO_2 were significantly elevated at each increased PO

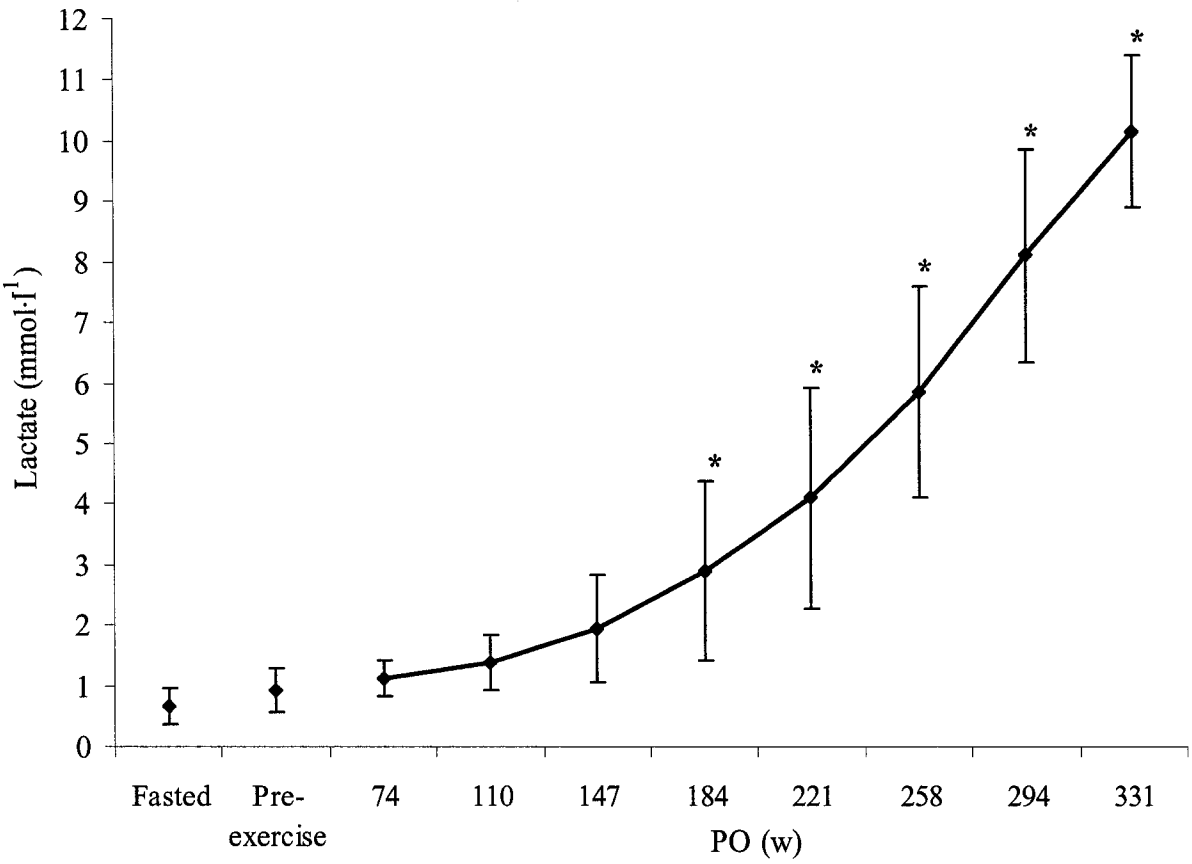

Figure 3-2. Deproteinized blood lactate concentration when fasted, at pre-execise and during graded exercise (Error bars = SD)

* - significantly different from all other lactate samples

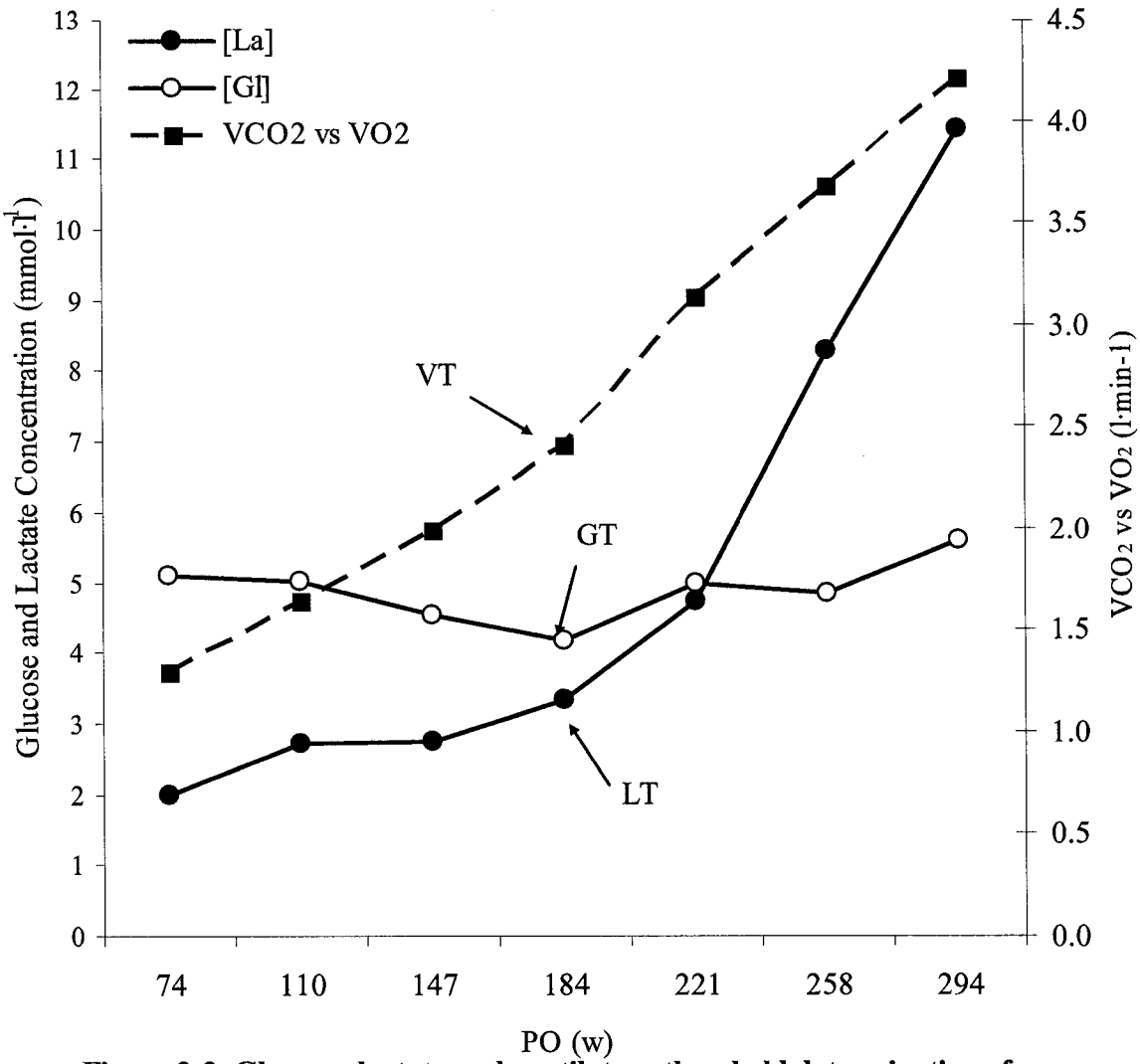

Figure 3-3. Glucose, lactate and ventilatory threshold determinations for a single participant.

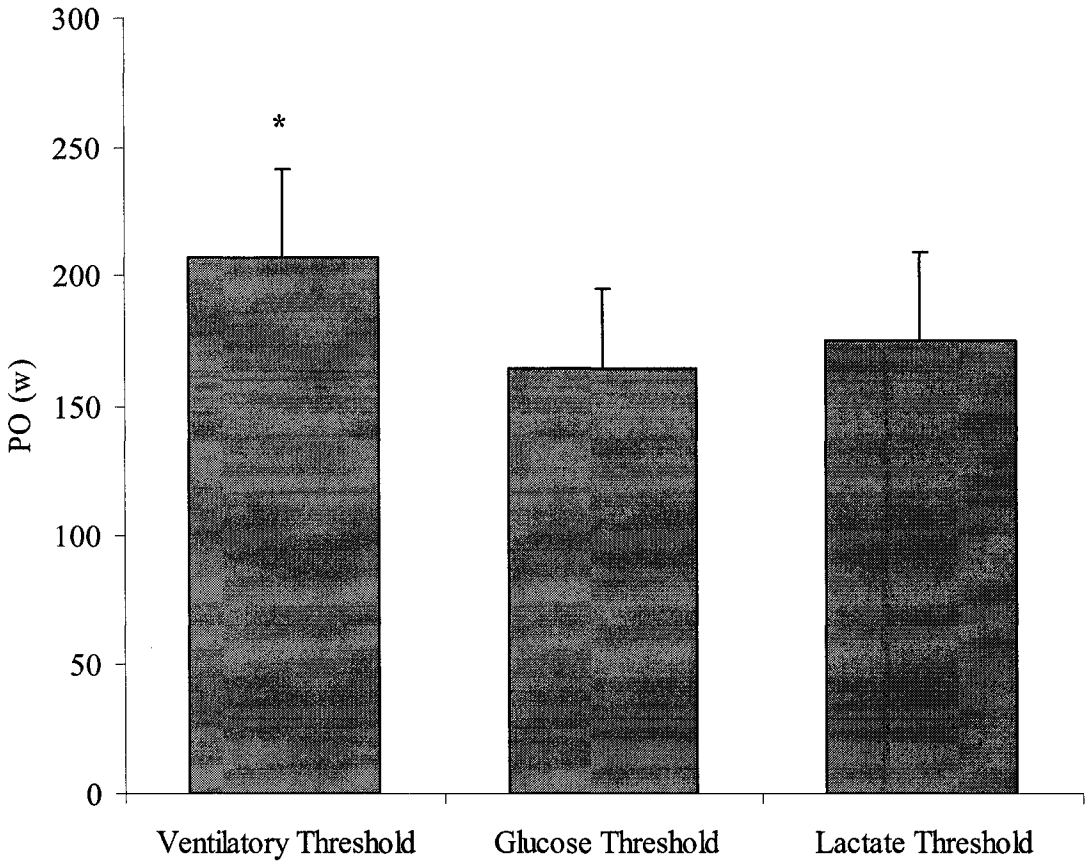

Figure 3-4. Power output at threshold. Values are means ± SD
* - significantly different from other threshold determinants

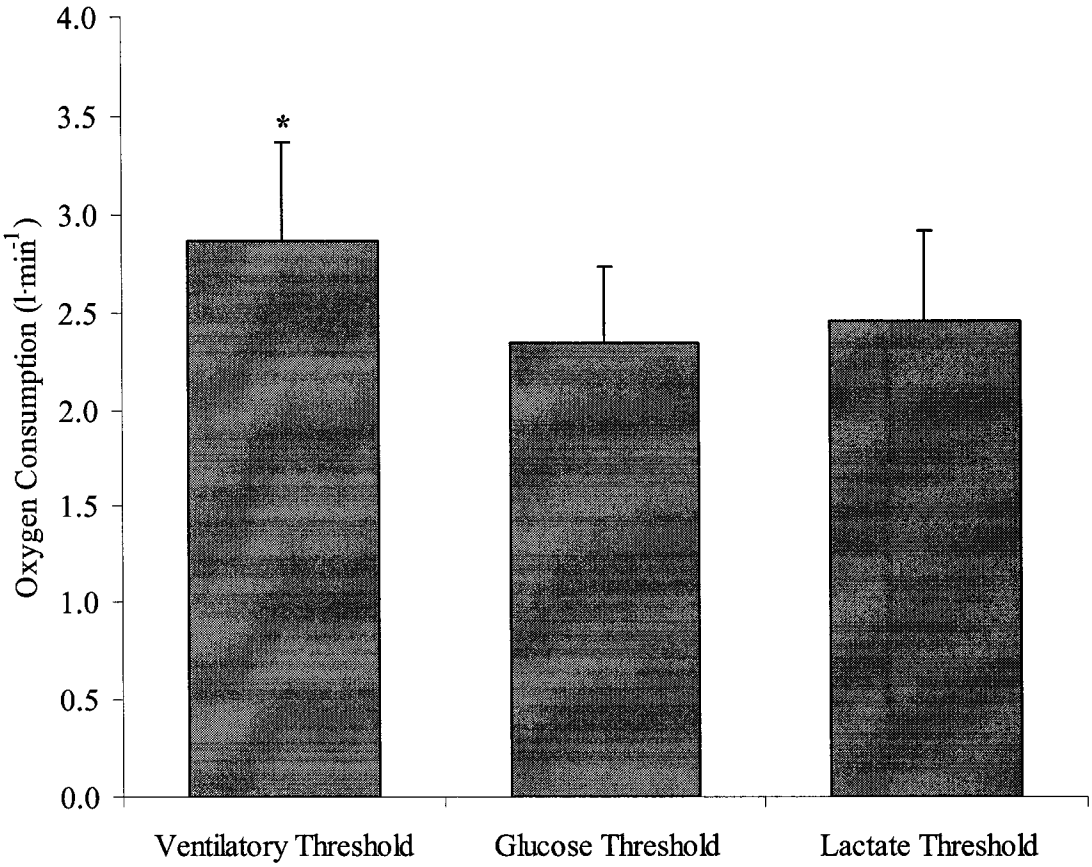

Figure 3-5. Oxygen consumption at threshold.
Values are means ± SD
* - significantly different from other threshold determinants

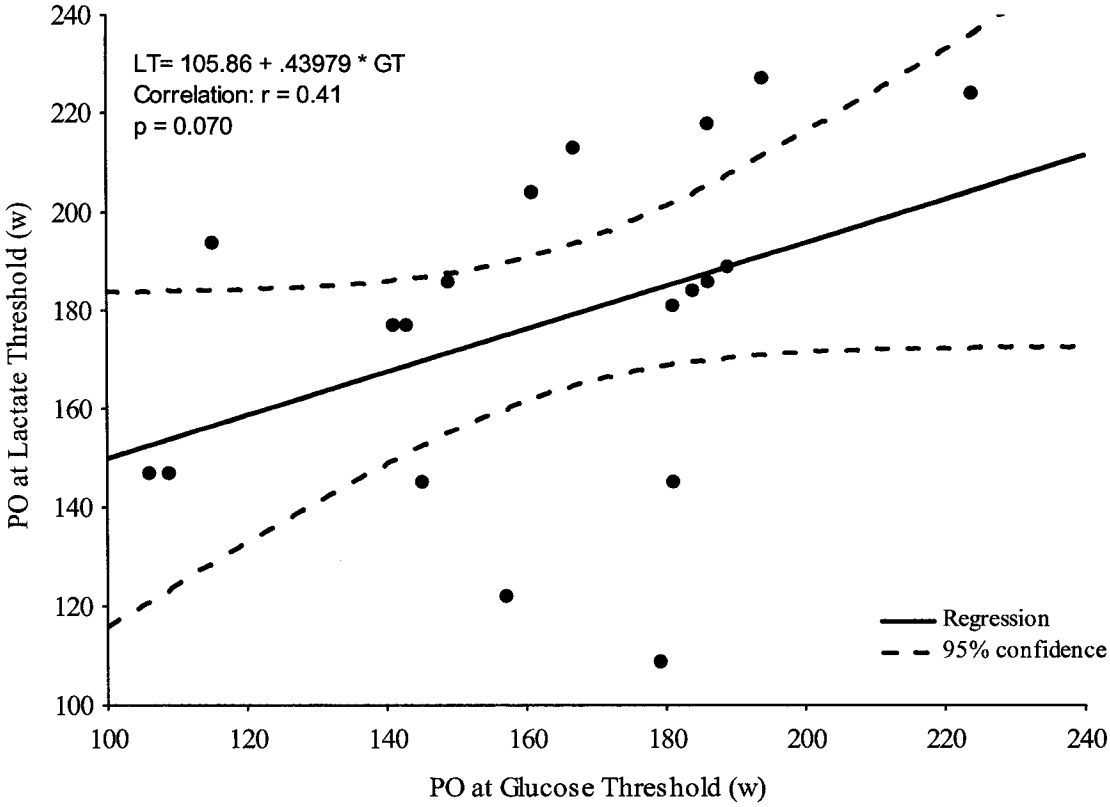

Figure 3-6. Relationship between power output at lactate threshold and glucose threshold

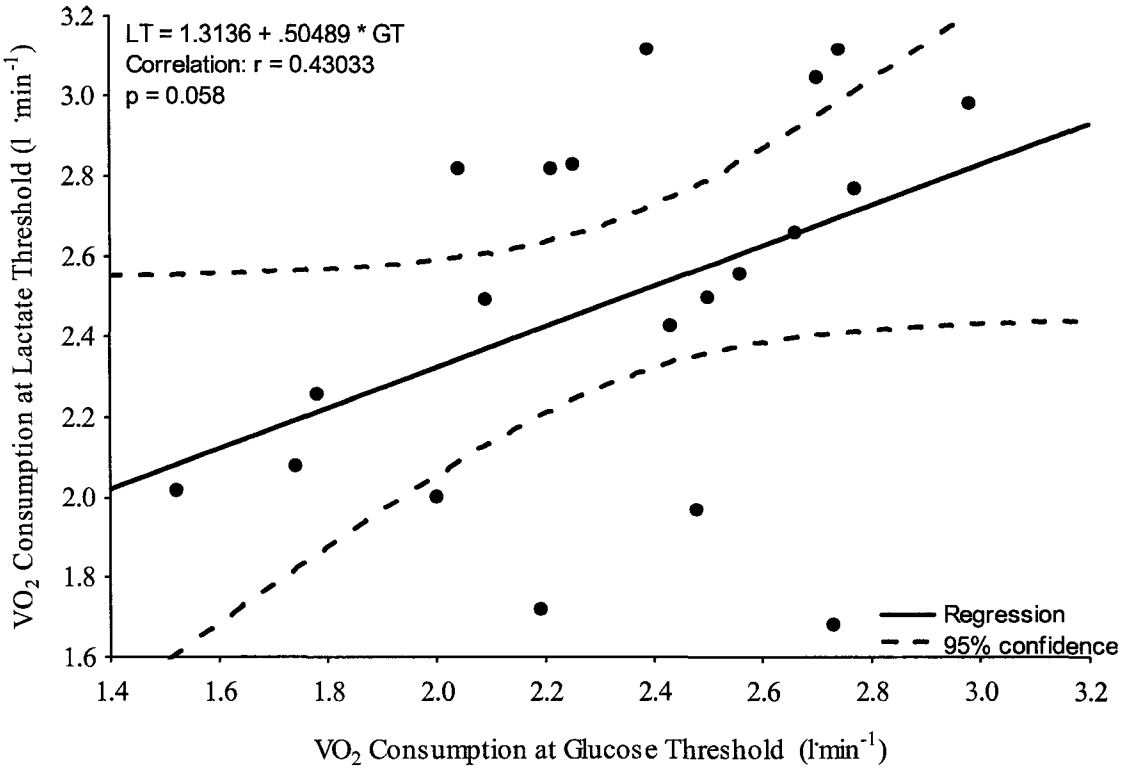

Figure 3-7. Relationship between oxygen consumption at lactate threshold and glucose threshold

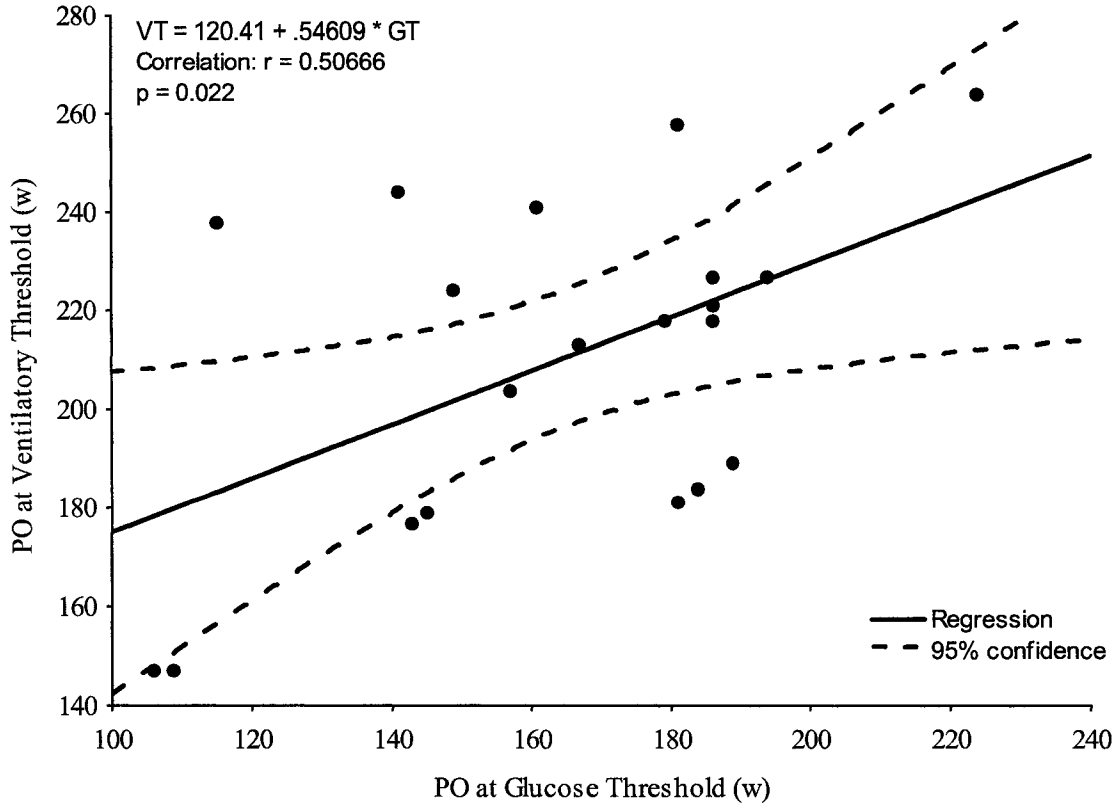

Figure 3-8. Relationship between power output at ventilatory threshold and glucose threshold

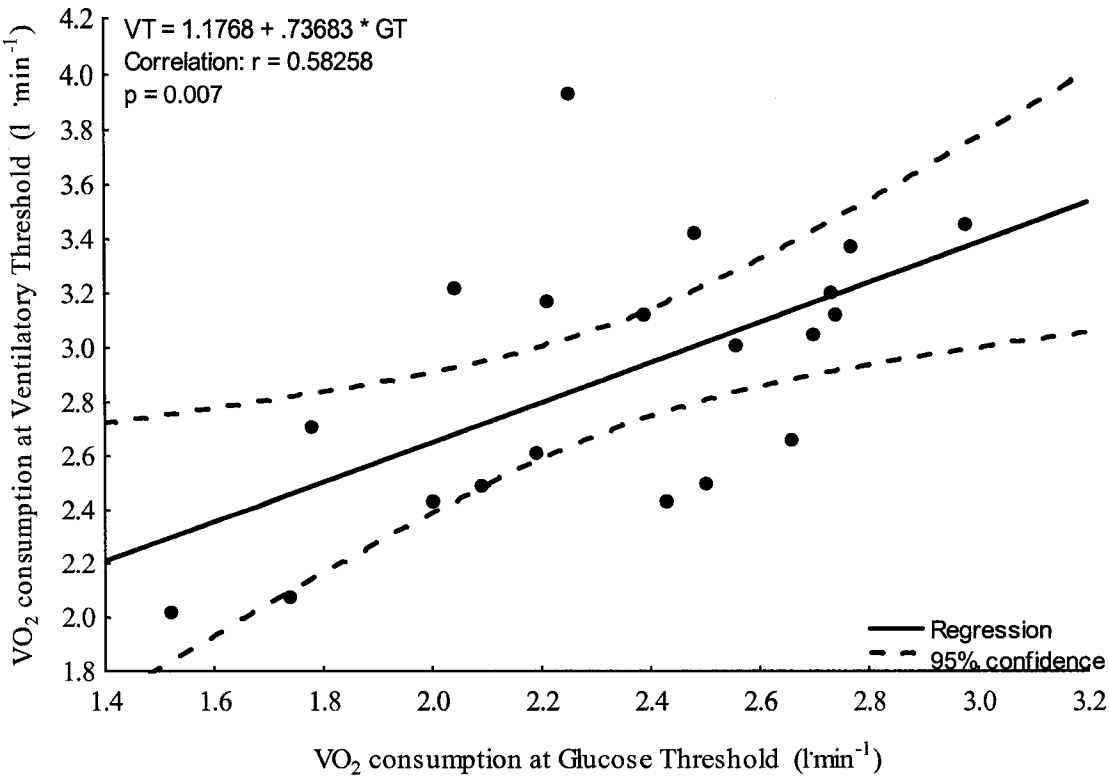

Figure 3-9. Relationship between oxygen consumption at ventilatory threshold and glucose threshold

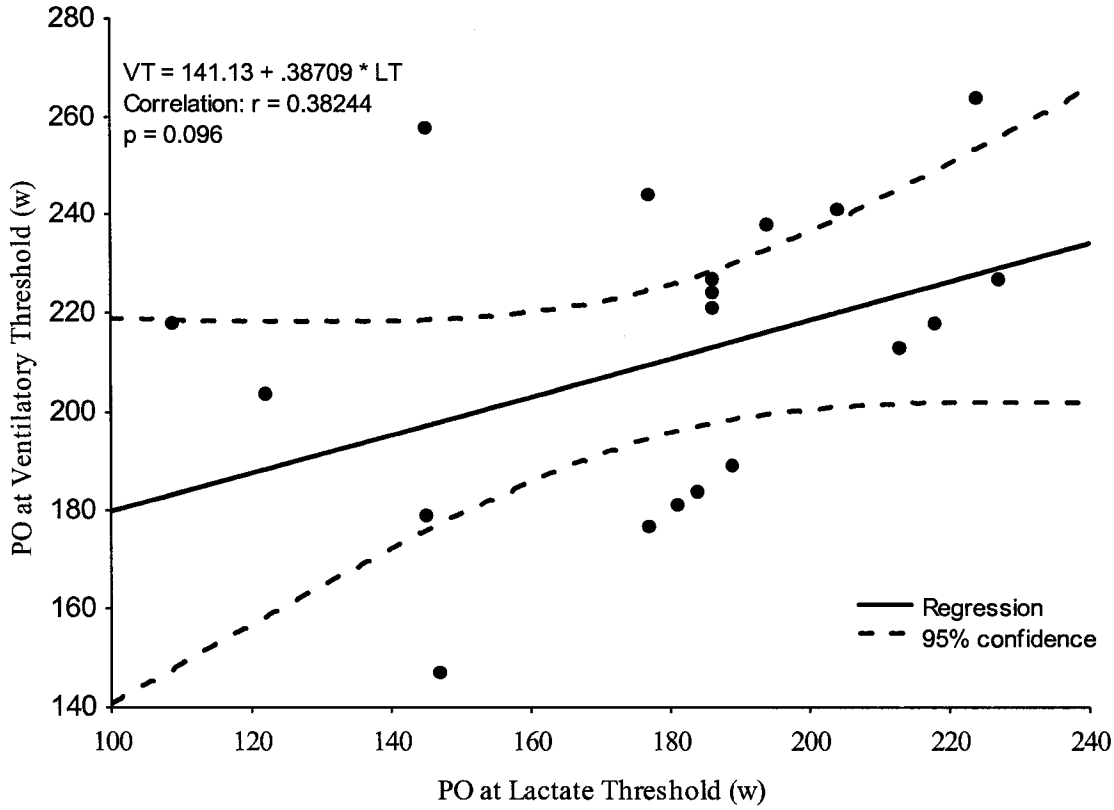

Figure 3-10. Relationship between power output at ventilatory threshold and lactate threshold

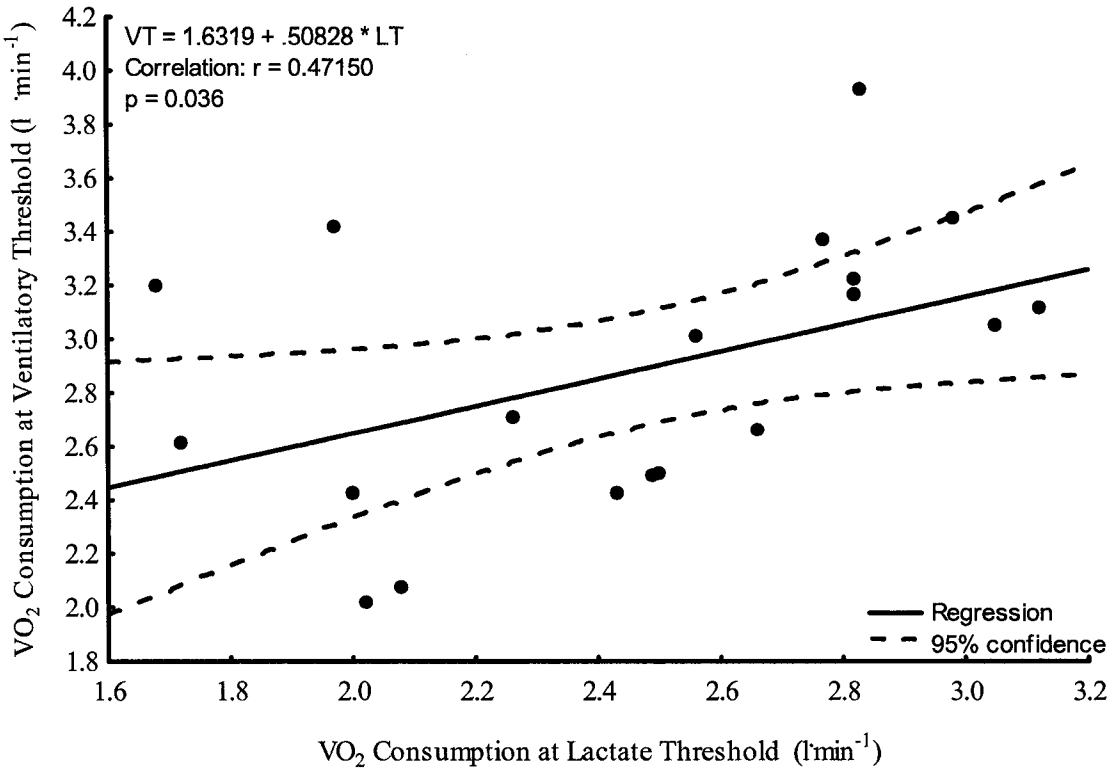

Figure 3-11. Relationship between oxygen consumption at ventilatory threshold and lactate threshold

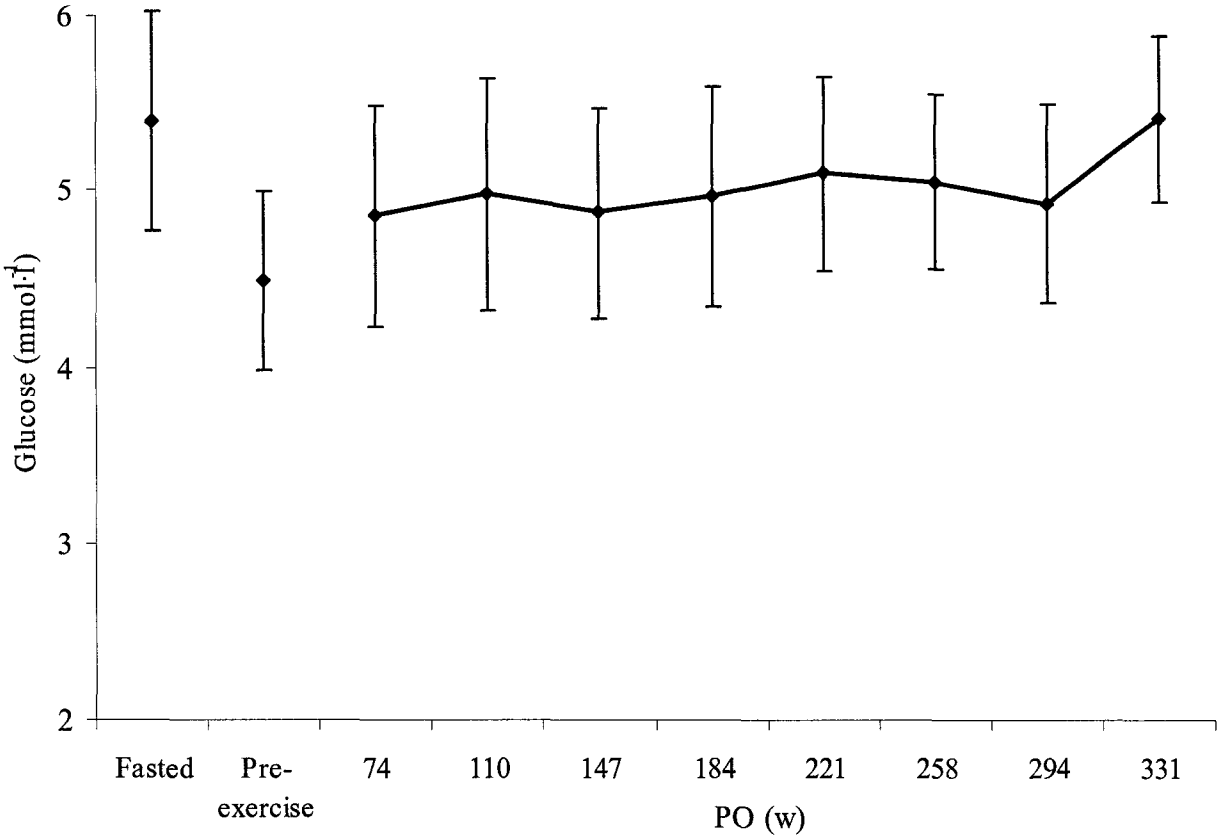

Figure 3-12. Serum glucose concentration when fasted, at pre-execise and during graded exercise (Error bars = SD)

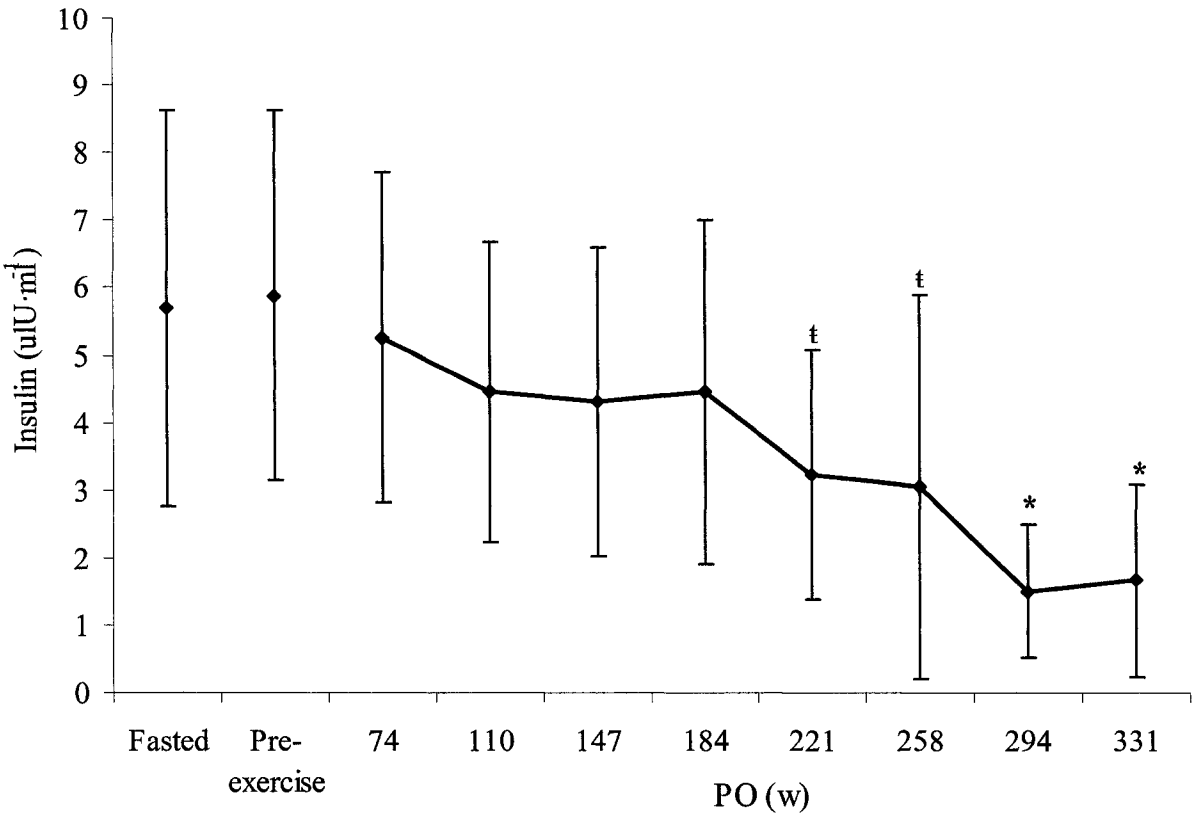

Figure 3-13. Insulin concentration when fasted, at pre-execise and during graded exercise (Error bars = SD)

* - significantly different from fasted, pre-exercise and workloads 1,2,3, & 4

t - significantly different from fasted, pre-exercise and workload 1

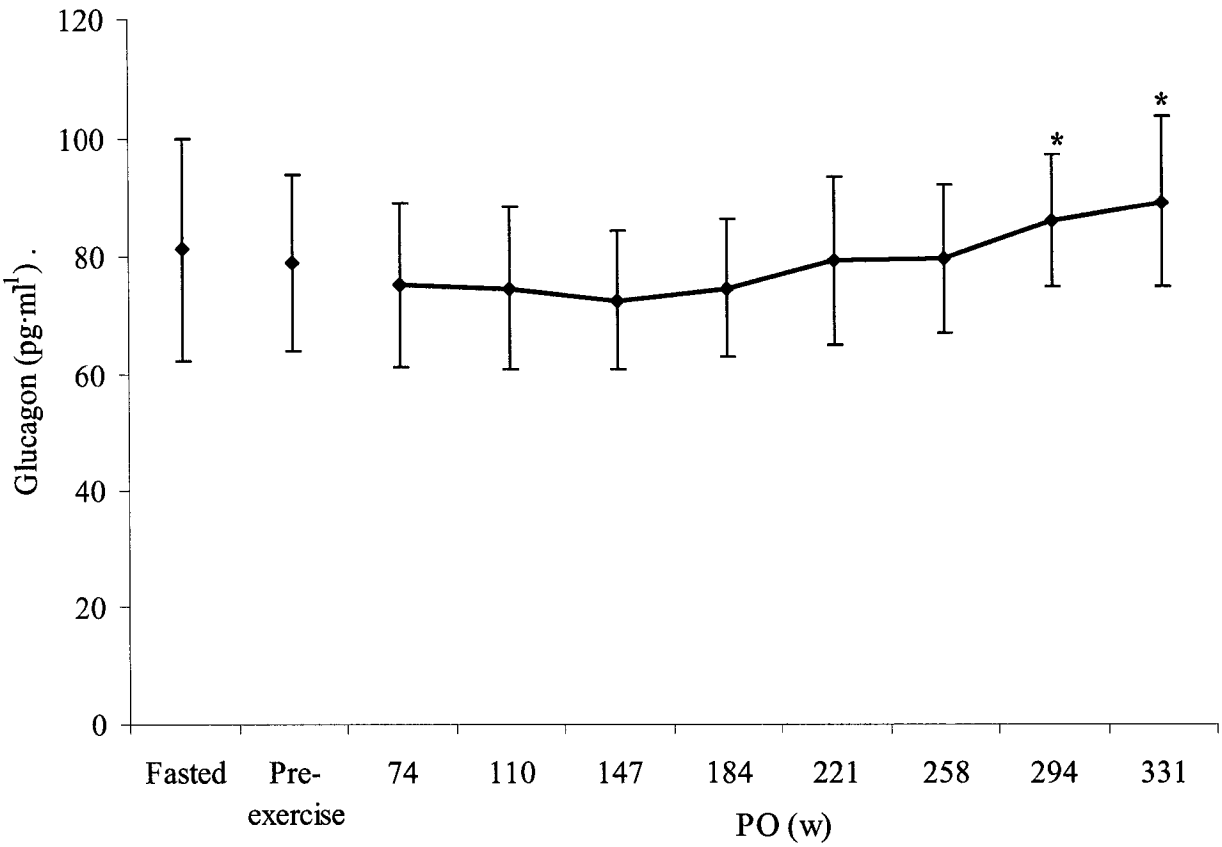

Figure 3-14. Glucagon concentration when fasted, at pre-execise and during graded exercise (Error bars = SD)

* - significantly different from workloads 1,2,3 & 4

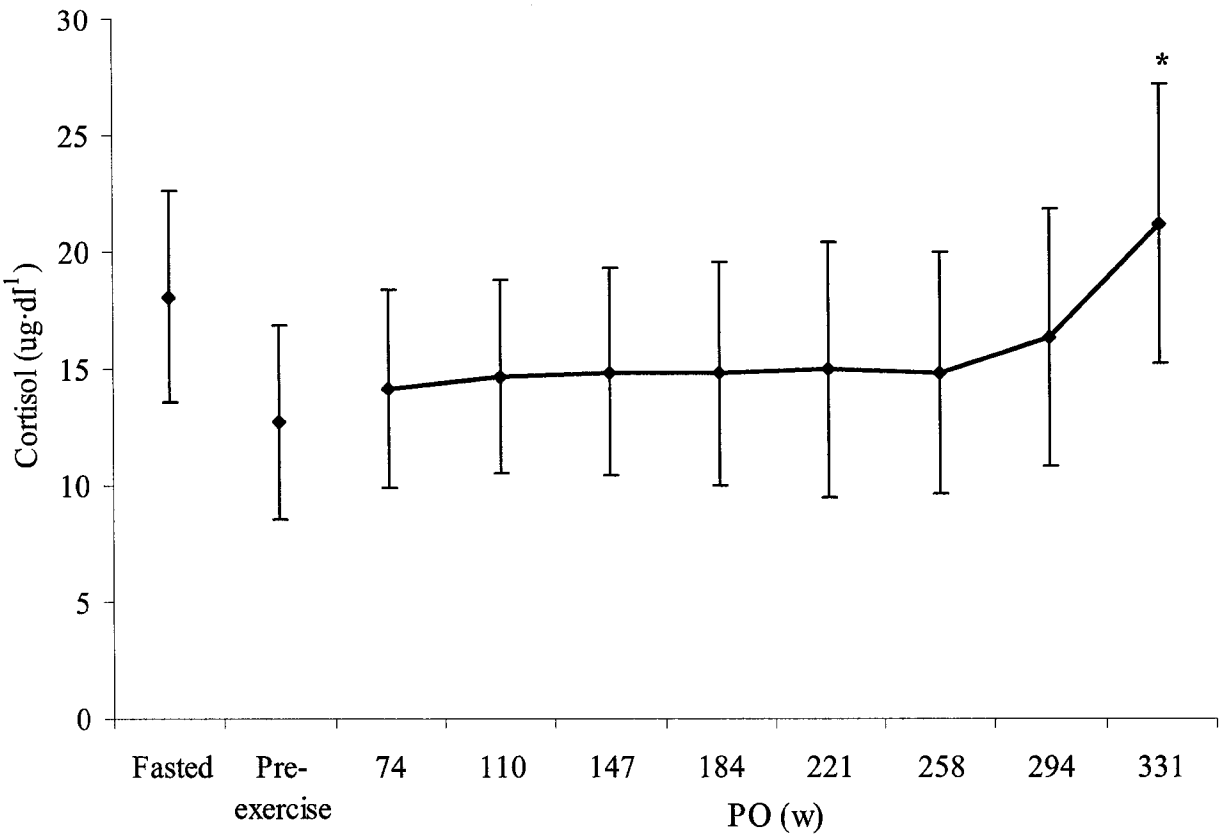

Figure 3-15. Cortisol concentration when fasted, at pre-execise and during graded exercise (Error bars = SD)

* - significantly different from pre-exercise and all workloads

ŧ - significantly different from pre-exercise

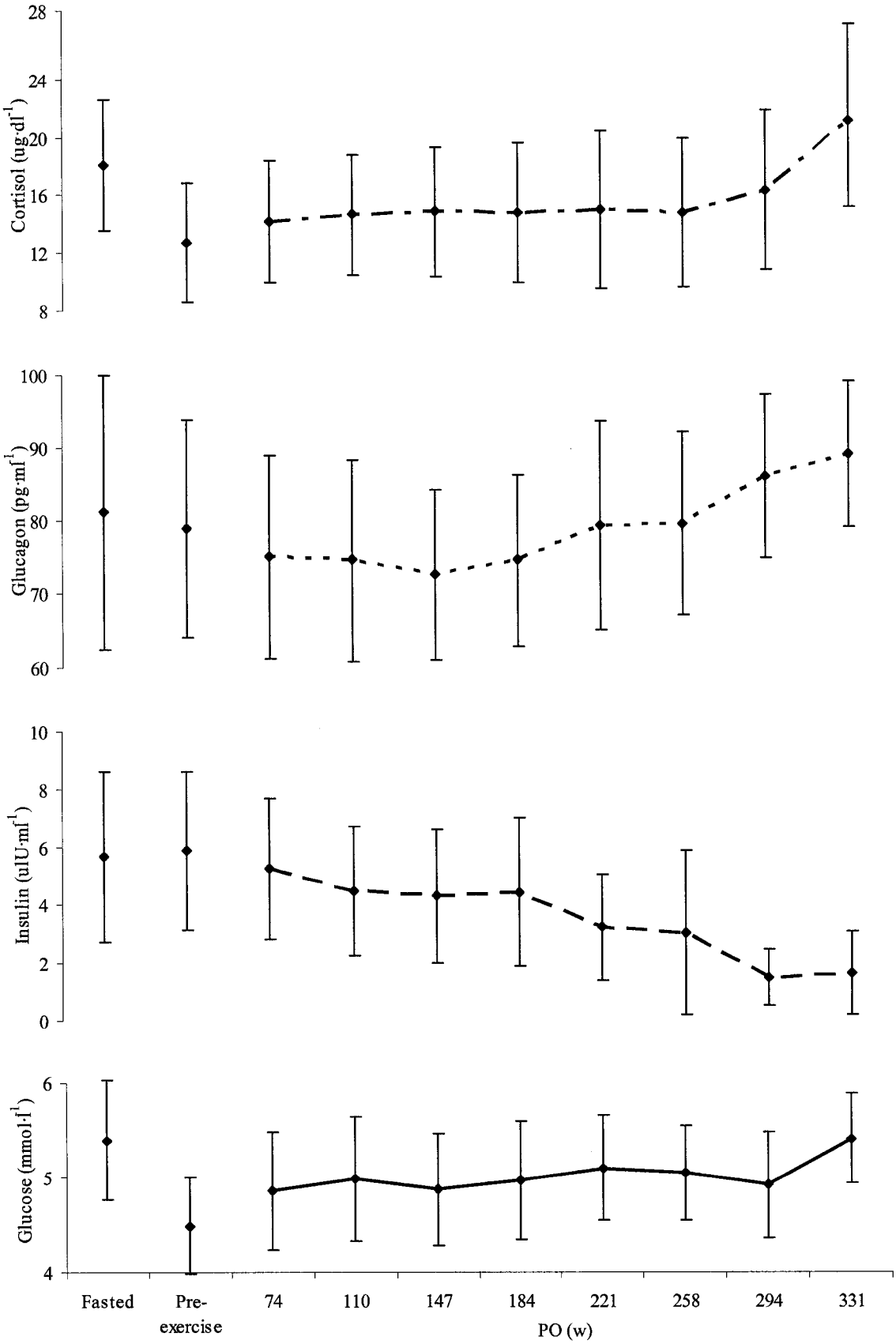

Figure 3-16. Glucose, insulin, glucagon, and cortisol concentration when fasted, at pre-execise and during graded exercise (Error bars = SD)

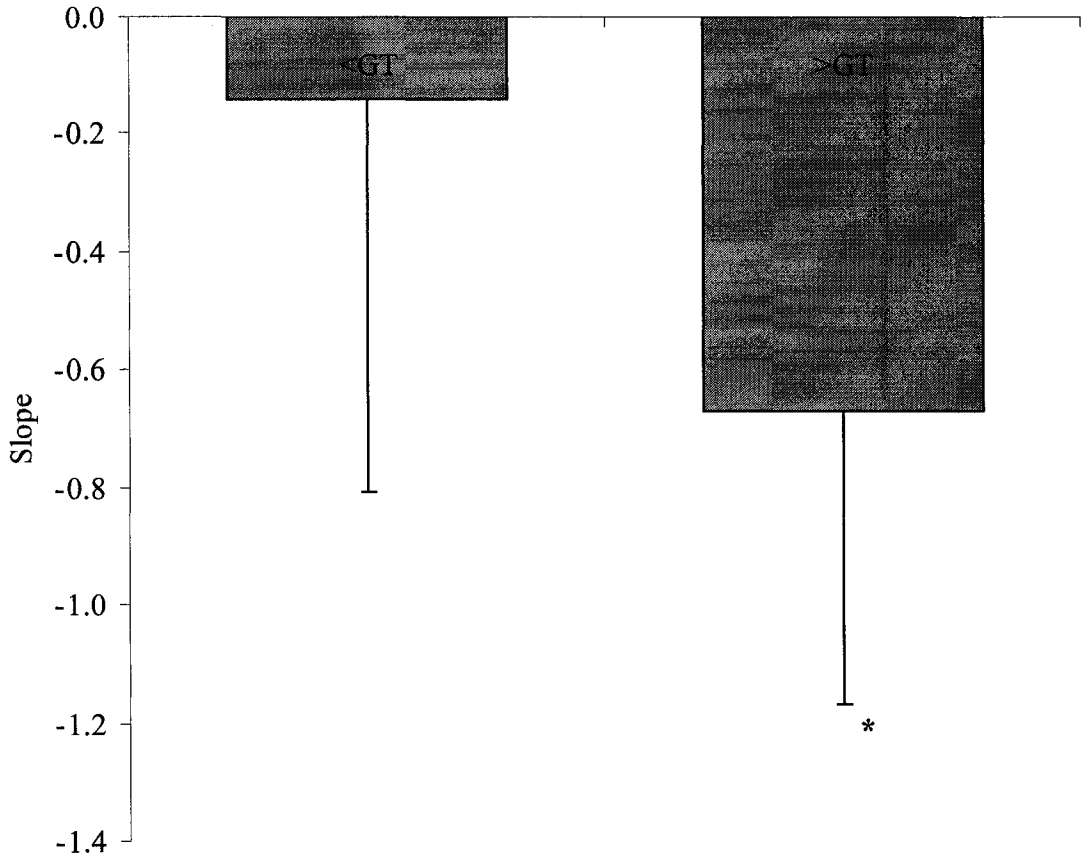

Figure 3-17. Slope comparison before and after glucose threshold in serum insulin concentration (Error bars = SD)

* - significantly different from before glucose threshold

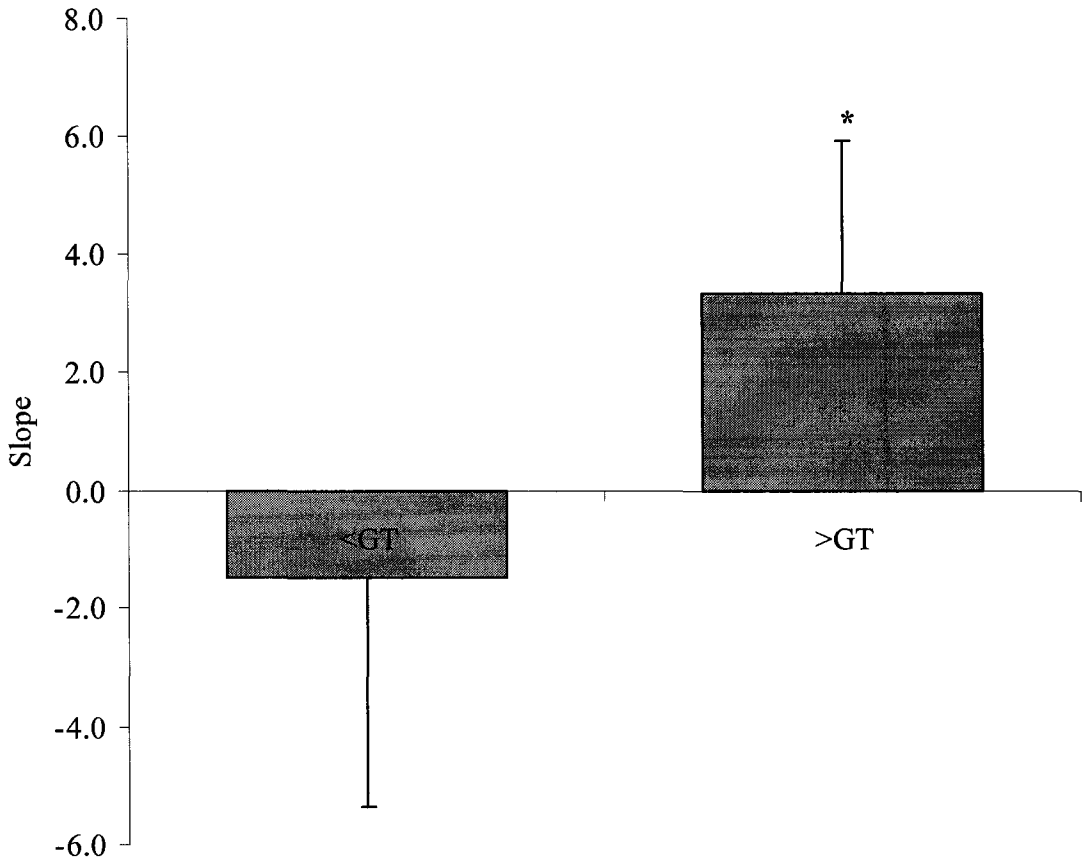

Figure 3-18. Slope comparison before and after glucose threshold in serum glucagon concentration (Error bars = SD)
* - significantly different from before glucose threshold

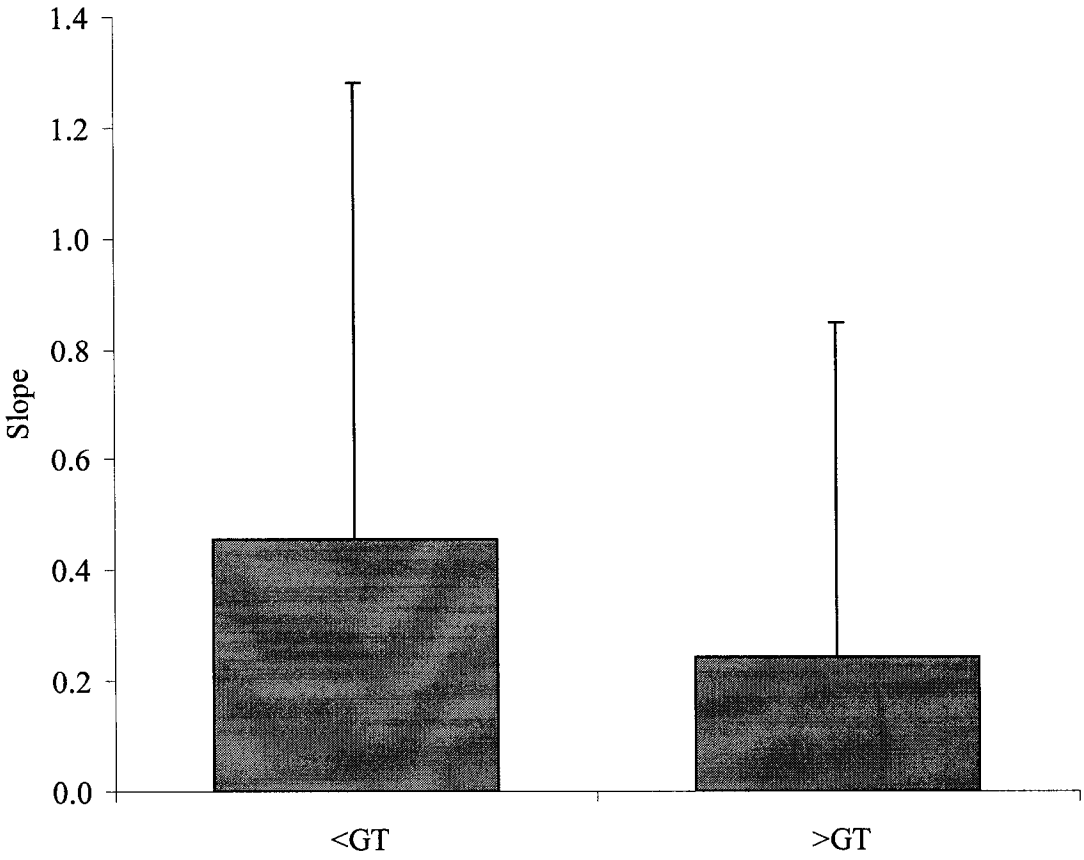

Figure 3-19. Slope comparison before and after glucose threshold in serum cortisol concentration (Error bars = SD)

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

4.1 Discussion

The purpose of this research was to examine the existence of a nadir in serum glucose and if this was the same as or related to other indices of anaerobic threshold. The study also included an evaluation of insulin, glucagon, and cortisol hormone response during the same graded exercise test in relation to the nadir of serum glucose. Previous research has demonstrated that a glucose determination point is identifiable that was adjunct to the lactate and ventilatory determinations of anaerobic threshold (Northius et al, 1995; Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). This point was defined as the nadir in blood glucose concentration during a graded exercise test (Simoes et al, 1999) that could also be described as a glucose threshold. The glucose threshold may be able to provide the same benefits as other anaerobic threshold indices such as AT determination for athletes (Simoes et al, 1999; Simoes et al, 2003; Costill, 1970; MacDougall, 1977; Svedahl and Macintosh, 2003; Sigal et al, 2004). Further, it is possible that glucose threshold determination may also benefit type 2 diabetics by helping determine an exercise intensity that may provide for an optimal blood glucose clearance. The examination of the response of the hormones insulin, glucagon and cortisol in this study provided further support for the nadir in blood glucose.

This study supported the work of previous researchers by determining that there was no significant difference between the power outputs at the thresholds of glucose and lactate. Furthermore, a significant correlation between glucose and ventilatory threshold was observed. In contrast to previous research this study did not find a significant correlation ($r=0.43$; $p=0.058$) between glucose and lactate threshold. Furthermore this

study also found a significant difference of approximately one power output between glucose and ventilatory threshold (Northius et al, 1995; Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). The possible reasons for these discrepancies may be the difference in exercise protocols, but as well it may be possible that the observations between glucose and ventilatory threshold in this study agree with accepted metabolic action around anaerobic threshold. It has been reported that ventilatory threshold is delayed in relation to the metabolic measurements of lactate due to bicarbonate buffering of the hydrogen ion dissociated in the muscle from lactic acid (Beaver et al, 1986; Wasserman et al, 1999; Svedahl and Macintosh, 2003). These observations are in agreement with the observations of this study.

Research has reported several applications for anaerobic threshold, including assessment of cardiovascular health, evaluation of training programs and categorization of the intensity of exercise (Hollmann, 1991; Billat, 1996; Svedahl and Macintosh, 2003). In an athletic population these benefits are used for fitness evaluation and training, as the anaerobic threshold can provide a good estimate of the intensity that can be sustained in endurance exercise. Anaerobic threshold provides a benchmark intensity that can be used for training program design. Training below threshold would be mild, around threshold would be moderate, and above would be intense levels of intensity. These classifications are useful depending on the goal of the training program, and the most appropriate intensity determined from anaerobic threshold can be used to achieve those goals (Billat, 1996; Svedahl and Macintosh, 2003). Because of anaerobic thresholds wide use by the athletic population, one initial glucose threshold benefit results from the observed association between the nadir in blood glucose and anaerobic threshold (Costill, 1970;

MacDougall, 1977; Simoes et al, 1999; Svedahl and Macintosh, 2003). Portable glucose meters cost less than lactate measurement instruments and may be technically more accurate measurement devices. This would be a useful field tool to determine and set exercise training intensities.

It was found that glucagon and cortisol increased significantly while insulin significantly decreased during the graded exercise test. In relation to the glucose threshold, glucagon significantly increased and insulin significantly decreased from the power output of the nadir in serum glucose concentration. These observations support the work of others that found the response of glucagon and insulin to change with a magnitude closely linked to the intensity of exercise, specifically the increase in glucagon and the decrease in insulin (Felig et al, 1972; Terjung, 1979; Winder, 1985; Wolfe et al, 1986).

The joint observations of the threshold of glucose and the associated glucoregulatory hormone response may provide evidence for a secondary benefit related to the type 2 diabetic population. This possibility exists because of the decrease in insulin and increase in serum glucose after the nadir of glucose has been achieved. Type 2 diabetics are usually insulin resistant but may not be resistant to the stimulatory effects of exercise on glucose utilization (Sigal et al, 2004; Kennedy et al, 1999). They often still retain the capacity to translocate GLUT-4 through insulin independent glucose uptake. This recruitment of GLUT-4 coupled with elevated circulating glucose levels can lead to greater glucose utilization (Sigal et al, 2004). Thus, after the intensity of exercise as which glucose threshold has been reached and insulin has decreased, serum glucose concentration increases and perhaps there is an increase in insulin independent glucose

uptake. This would benefit type 2 diabetics since exercising at this level may be associated with a more optimal glucose clearance (Krook et al, 2004; Sigal et al, 2004) than other exercise intensities. Exercise at intensities around anaerobic threshold may be difficult to sustain for sedentary type 2 diabetics but when safely possible, research has supported intensities such as commonly associated with anaerobic threshold to improve glycemic control (Marliss and Vranic, 2002; Sigal et al, 2004). Consideration of all the possible glucoregulation irregularities require attention as many type 2 diabetics may have complications with exercise such as concomitant medications, insulin injection, and ketosis, all of which require attention that supersedes exercise (Sigal et al, 2004). Further research would be required before glucose threshold and type 2 diabetic associations can be accurately determined but the findings of this investigation support the possibility of this application.

Although the findings of this study do not definitively identify a central or peripheral mechanism for the glucose threshold, the observations of the glucose and hormone response does support a change in exercise fuel source as one such proposed mechanism. The drop and subsequent increase in serum glucose concentration and its association to the threshold of lactate may result from an increasing demand for energy provided by carbohydrate oxidation as the level of exercise intensity increases. At low to moderate intensity exercise (up to ~75% VO_{2max}), the relative contribution of free fatty acid oxidation to energy requirements has been shown to be greater than that of carbohydrates. As exercise intensity exceeds a moderate level (~ 75% VO_{2max}) glycogen becomes the predominant fuel source (Kang et al, 1998; Kjaer et al, 1991; Kjaer et al, 1993; Wahren et al, 1971; Baldwin et al, 1973). At this exercise intensity, the circulating

concentration of serum insulin decreases and glucagon increases to preserve blood glucose levels since they were decreasing as most of the energy requirements for exercise were being met through free fatty acid oxidation. It has been suggested the nadir in blood glucose occurs at this point of change to increased glycogen utilization (Simoes et al, 1999). This study supports such a proposed mechanism as it was after the nadir in serum glucose that the glucoregulatory hormones measured changed significantly. Research indicates that anaerobic threshold and thus lactate threshold occurs between 70% and 85% of peak VO_2 (Weltman, 1989; Wasserman, 1984; Wasserman et al, 1999). This is also the intensity level of the increased carbohydrate utilization during exercise and hence the nadir in blood glucose. Further research would be required before such mechanisms can be accurately determined but the findings of this study support this proposed mechanism.

4.2 Conclusion

It can be concluded from this study that the nadir in srum glucose may be a valid measure of anaerobic threshold. Further it was shown that a relationship existed between the nadir in serum glucose and the response of insulin and glucagon hormones during a graded exercise test. The findings of this study overall agree with those of previous research of glucose threshold (Northius et al, 1995; Simoes et al, 1999; Simoes et al, 2003; Ribeiro et al, 2004). Further research is necessary to establish possible mechanisms for the threshold of glucose and to provide further validity and reliability to the nadir in blood glucose as a predictor of anaerobic threshold. Furthermore, research examining the applicability of the glucose threshold to athletic and type 2 diabetic populations may be beneficial.

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cannot continue by stopping the test) to determine your peak aerobic fitness (peak VO2). The actual test usually lasts for about 12 to 15 minutes, with an additional 5 to 10 minutes of warm-up and cool-down exercise before and after. During the test, you will be asked to wear a nose clip and you will be breathing into a mouthpiece attached to a special breathing apparatus so that all the air you breath out is collected into a machine that will determine a variety of things such as your oxygen consumption. Heart rate is monitored continuously with a heart rate monitor that is strapped around your chest. We ask that you do not do any formal exercise (e.g. jogging, cycling, swimming, weight training) the day before any of your exercise tests. As well, we ask that you have a light meal of your choice and drink 500 ml's of water 2 to 3 hours before this exercise test.

After 2 to 5 days, we will set up another time for the second exercise test that does not require you to go to a maximum intensity. This exercise test is the submaximal test (it is known as the threshold test) and is conducted on the same cycle ergometer. We will also perform the same measurements on the air you breath out and measure your heart rate the same way as described previously. This test also gets increasing harder to pedal throughout the test, but the changes in the intensity will be 3 minutes in duration. This test will be stopped by one of the researchers before you are exercising maximally. However, this test will require you to exercise at approximately 80 to 90 % of your maximum ability at the end so it is a demanding test; but it does not go to exhaustion. To make sure that you have an appropriate amount of food and water before this test, we will provide you with a can of ENSURE™, which is a common meal replacement drink that we will require you to drink 3 hours before the threshold test. As well, we will request that you drink 125 ml (about half a cup) of water every 30 minutes for 2 hours before this test (total of 500 ml).

In addition, we will be taking blood samples before, during and after the submaximal threshold test to measure your blood hematocrit, glucose, lactate, and some hormones (glucagon, cortisol, insulin and catecholamines) known to influence blood glucose levels. To do this a registered nurse will put a small tube (cathelon) in a forearm vein with a needle under sterile conditions before you start. This is similar to what is used if you have had an "IV" in a hospital or when you have donated blood at Blood Services. Note that the needle is removed in this procedure and only the soft Teflon tube remains to enable blood samples to be taken. This tube has a cap on top and is taped to your arm during the test. The nurse will put a small amount (about ½ a ml) of sterile saline (water with some "electrolytes") into the tube and during the last minute of each workload the nurse will remove the saline (½ ml) and take a small sample (about 2 ml) from this tube with a syringe. This will happen prior to exercise and during the last minute of each workload on the cycle ergometer. Thus, you will possibly have 6 or 7 small samples of blood taken. After the test, the cap and tube are removed from your arm. The benefit of this method of taking blood is that you do not have to be poked with a needle each time a blood sample is taken.

Note that we will provide you with a schedule of all your required visits to the lab and these will be as flexible as possible to suit your personal schedule.

Risks: The maximal (peak VO2) exercise test requires maximal effort to go to exhaustion and/or perform to each person's maximal capacity. With this type of exercise there may be some health risk. During and after the tests it is possible to experience

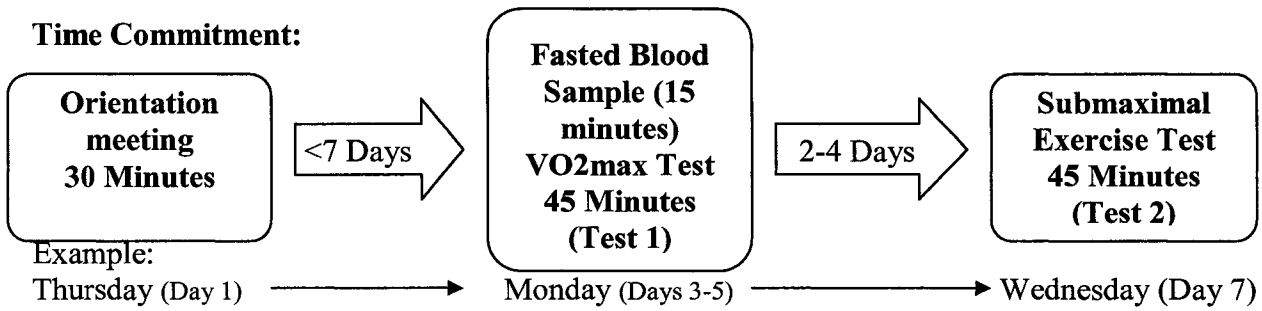
symptoms such as abnormal blood pressure, fainting, lightheadedness, muscle cramps or strain, nausea, and in very rare cases (0.5 per 10,000 in testing facilities such as exercise laboratories, hospitals and physicians offices), heart rhythm disturbances or heart attack for the VO2max Test. While serious health risk to you is highly unlikely, these risks must be acknowledged, and you willingly assume the risks associated with very hard exercise. The submaximal test is difficult but not maximal in nature so it is considered to be less risk.

The blood samples are performed with sterile equipment but there is a small risk of infection at the site if not properly cared for. However, sterile procedures, cleanliness and use of a band-aid greatly minimize this risk. A registered nurse using standard procedures will conduct the blood sample procedures using the cathelon.

Qualified personnel under the supervision of Dr. Gordon Bell will administer the exercise testing. Personnel are trained to handle identifiable risks and emergencies and have certification in CPR. Certifications can be produced upon request. The researchers will continuously watch for adverse symptoms and will stop the test if at any time they are concerned about your safety. You can also stop the test at any time. Please inform the researcher of any of the above- mentioned symptoms experienced during or after the tests.

Benefits: The major benefit of your participation in this study will be to help the researchers understand the nature of the glucose response to graded exercise as a predictor of anaerobic threshold, and the possible mechanisms surrounding it. As a participant you will be provided with a written report of your personal aerobic fitness information and an exercise training prescription. If you are interested in the future outcomes of this study, you may contact one of the researchers for this information

Time Commitment:



Total time is 2 hours and 15 minutes but this does not include travel to and from the lab, changing and showering.

Confidentiality: To ensure confidentiality and anonymity, personal information will be coded and stored in a file cabinet in a locked office to which only the investigators have access. There will be no way to identify individuals in results that are published in an article. Normally, information is retained for a period of five years post publication, after which it may be destroyed. The data will be presented at a research conference and published in a scientific journal.

APPENDIX C – PHYSICAL ACTIVITY PARTICIPATION FORM

Glucose Threshold Study
Physical Activity Participation Form

Name:_____

#1 Frequency

Over a typical seven-day period, how many times do you engage in physical activity that is sufficiently prolonged and intense to cause sweating and a rapid heart rate?

- ☐ Rarely or Never
- ☐ Normally Once or Twice
- ☐ At Least Three Times

if more than three times, how many:_____

#2 Intensity

When you engage in physical activity, do you have the impression that you:

- ☐ Make a Light Effort
- ☐ Make a Moderate Effort
- ☐ Make an Intense Effort

#3 Perceived Fitness

In a general fashion, would you say that your current physical fitness is:

- ☐ Very Poor
- ☐ Poor
- ☐ Average
- ☐ Good
- ☐ Very Good

The above three questions can be used indicate your health benefit rating (other side of sheet).

What was your average frequency per week of exercise?

_____ average frequency/week

What was your average duration per exercise session in minutes from the above?

_____ average duration of exercise session (minutes)

Compare yourself with ACSM standards

The American College of Sports Medicine guidelines suggest exercising at least three times per week, for at least 20 to 30 minutes, at moderate-to-high intensity levels.

Do you exercise:	Yes	No
3 or more times/ week?	___	___
20 or more minutes/session	___	___
At moderate or high heart rate, breathing/session?	___	___

Add up your score from the three questions in the first half of the previous page to determine your health benefit rating:

#1 Frequency	Rarely or Never	Once or Twice	Atleast three times
	0	2	3
#2 Intensity	Light Effort	Moderate Effort	Intense Effort
	0	1	3
#3 Percieved Fitness	Very Poor or Poor	Average	Good or Very Good
	0	3	5

Health Benefit Zone	Total Score
Excellent	9 - 11
Very Good	6 - 8
Good	4 - 5
Fair	1 - 3
Needs Improvement	0

Adapted from the Healthy Physical Activity Participation Questionnaire (Canadian Physical Activity, Fitness and Lifestyle Approach, 3rd Edition, 2004).

APPENDIX E – SPECTROPHOTOMETRIC ASSAYS

Lactate Assay: General Principle

In lactate oxidation, lactate is converted to pyruvate by lactate dehydrogenase (LDH) in the presence of β-nicotinamide adenine dinucleotide (β-NAD+). Every mole of lactate that is converted causes one β-NAD+ to be reduced to β-NADH. β-NADH absorbs light at wavelength of 340 nm, while β-NAD+ does not. If the enzymatic reaction is allowed to run to completion all the lactate will be converted to pyruvate and the number of β-NADH molecules resulting will be equal to the initial number of lactate molecules, thus the concentration of lactate in the initial sample can be determined mathematically using the slope of a standard curve of known concentration of lactate.

Glucose Assay: General Principle

Glucose oxidase oxidizes glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase to form a colored product while oxidized o-dianisidine reacts with sulphuric acid to form a more stable colored product. Measured at 540 nm, the intensity of the pink color is proportional to the original glucose concentration (Sigma-Aldrich, 2004). Glucose concentration can be determined mathematically using the slope of a standard curve of known concentration of glucose.

APPENDIX F – RADIOIMMUNOASSAYS GENERAL PROCEDURE

In radioimmunoassays a fixed concentration of radioactively labelled substance is incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited. When unlabeled antigen is added to this system, there is competition between labelled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free tracer and counting the radioactively labelled substance using a gamma counter. A standard curve is set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated using the shape of the curve.

For each of the measures executed in this examination, the procedure followed similar methodological principles. The only differences were in the materials used and are related to the measured hormone.

Insulin RIA

In the insulin RIA, radioactively labelled (^{125}I) insulin competes for a fixed time (18-24 hours at room temperature) with the sample plasma insulin for sites on the insulin-specific antibody. The antibody is immobilized on the wall of a polypropylene tube and decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled insulin. Using a gamma counter the tube is counted and the resulting number converts into the amount of insulin present in the initial

sample through comparison to a standard curve (Diagnostic Products Corporation, Los Angeles, CA)

Glucagon RIA

The glucagon RIA was a double antibody procedure. The subject glucagon sample is preincubated with anti-glucagon antibody and incubated for 24 hours at 2-8 °C. After this incubation the radioactively labelled (^{125}I) glucagon competes for a fixed time (24 hours at 2-8 °C) with the sample plasma glucagon for sites on the glucagon-specific antibody. After the second incubation, precipitating solution is added to all tubes, which are then vortexed and centrifuged for 15 minutes at 1500xg. Each tube is aspirated leaving the precipitate. Using a gamma counter the tube is counted and the resulting number converts into the amount of glucagon present in the initial sample through comparison to a standard curve (Diagnostic Products Corporation, Los Angeles, CA)

Cortisol RIA

In the cortisol RIA, radioactively labelled (^{125}I) cortisol competes for a fixed time (45 minutes at 37 °C) with the sample plasma cortisol for sites on the cortisol-specific antibody. The antibody is immobilized on the wall of a polypropylene tube and decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled cortisol. Using a gamma counter the tube is counted and the resulting number converts into the amount of cortisol present in the initial sample through comparison to a standard curve (Diagnostic Products Corporation, Los Angeles, CA)

APPENDIX G – ENSURE MEAL REPLACEMENT DRINK NUTRITION FACTS

Nutrient Profile per 8 fl oz: Calories 250; Protein (% Cal) 14.1, Total Fat (% Cal) 22.0, Carbohydrate (% Cal) 63.9

Nutrition Facts

Serving Size 1 can (8 fl oz)

Amount Per Serving

Calories 250	Calories from Fat 50
	% Daily Value*
Total Fat 6g	**9%**
Saturated Fat 0.5g	**3%**
Cholesterol <5mg	**<2%**
Sodium 200mg	**8%**
Potassium 370mg	**11%**
Total Carbohydrate 40g	**13%**
Dietary Fiber 0g	**0%**
Sugars 18g	
Protein 9g	**18%**

Vitamin A	25%	•	Vitamin C	50%
Calcium	30%	•	Iron	25%
Vitamin D	25%	•	Vitamin E	25%
Vitamin K	25%	•	Thiamin	25%
Riboflavin	25%	•	Niacin	25%
Vitamin B6	25%	•	Folate	25%
Vitamin B12	25%	•	Biotin	25%
Pantothenic Acid	25%		Phosphorus	30%
Iodine	25%	•	Magnesium	25%
Zinc	25%	•	Selenium	25%
Copper	25%	•	Manganese	60%
Molybdenum	50%	•	Chromium	25%
Chloride	10%			

*Percent Daily Values based on a 2,000 Calorie diet.

Ensure product handbook (2005). Retrieved July 11, 2005, from http://rpdcon40.ross.com/mn/Ross+MN+Nutritional+Products.nsf/web_Ross.com_XML/36F9395EA34E8A02052569D600581B1F