THE USE OF NEAR INFRARED SPECTROSCOPY (NIRS) TO DETERMINE THE VENTILATORY THRESHOLD AND THE RELATION BETWEEN SKELETAL MUSCLE OXYGENATION AND RPE

by

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This study: 1) compared three (i.e., V-slope, Bhambhani et al., 1997, and Belardinelli et al., 1995) techniques of measuring the ventilatory threshold (VT), 2) examined the relation between OMNI RPE and muscle deoxygenation (MD), and 3) evaluated the impact of gender on MD and RPE. Subjects included 20 males and 13 females, aged 25-29 years. A commercial NIRS sensor (NIRS Micro-Run Man model # MRM-96) was placed over the right vastus lateralis and secured with an elastic wrap. Next, each subject underwent a progressive multistage cycle ergometer test to establish the VT using the V-slope method and NIRS methods. The V-slope (58.62 ± 10.47% VO_{2peak}), Bhambhani et al. 1997 (49.75 ± 20.13% VO_{2peak}), and Belardinelli et al. 1995 (60.87 ± 10.15% VO_{2peak}) methods did not result in different ($F(2,49) = 2.77$, $p > 0.05$) VT values. The following significant linear regression equation was generated ($p = 0.016$): OMNI RPE = 5.97 – (15.20)MD ($R = -0.20$, $R^2 = 0.04$, and SE = 2.76). The two-way ANOVA (gender x power output) conducted on OMNI RPE revealed a significant main effect for gender ($F(1,193) = 19.53$, $p < 0.05$). Males had lower RPEs (6.32 ± .17) than females (7.16 ± .28). A significant main effect for power output was also found ($F(9, 193) = 56.21$, $p < 0.05$). In addition, a significant gender x power output interaction was found ($F(7, 193) = 2.11$, $p < 0.05$). With respect to MD, the two-way ANOVA revealed a significant main effect for gender ($F(1, 133) = 10.61$, $p < 0.05$). Females had less MD (0.012 ± .007) than males (0.002 ± .005). The results of this study

indicate that the three methods of determining the VT were not different. MD values decreased with increasing ratings of perceived exertion. RPE differed between genders, with males having lower RPE's than females ($p < 0.05$). Also, a significant gender x power output interaction was found ($p < 0.05$). Finally, women experienced less skeletal MD than men during a progressively incremented cycle ergometer protocol.

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1.0 INTRODUCTION

The ventilatory threshold (VT) has been used to prescribe exercise intensity, predict endurance performance, compartmentalize aerobic/anaerobic energy metabolism, and diagnose clinical status in adults that vary in aerobic fitness and training status (Caiozzo, Davis, Ellis, Azus, Vandagriff, Prietto, and McMaster, 1982; Davis, 1985; Demello, Cureton, Boineau, and Singh, 1987; Kanaley and Boileau, 1988; Reybrouck, Weymens, Ghesquiere, Van Gerven, and Stijns, 1982; Robertson, Goss, Boer, Gallagher, Thompkins, Bufalino, Balasekaran, Meckes, Pintar, and Williams, 2001; Wasserman, Whipp, Koyar, and Beaver, 1973; Weltman, Snead, Stein, Seip, Schurrer, Rutt, Weltman, 1990). As exercise intensity progressively increases toward maximum, a point is reached where ventilation increases disproportionately compared with oxygen consumption. This is referred to as the ventilatory threshold (Wilmore and Costill, 2004). Typically, when work rate exceeds 55% to 70% of VO_{2max}, oxygen delivery to the muscles can no longer meet the oxygen requirements (Wilmore and Costill, 2004). Skeletal muscles compensate by deriving more energy from glycolysis, resulting in increased lactic acid production and accumulation (Wilmore and Costill, 2004). This lactic acid combines with sodium bicarbonate and forms sodium lactate, water, and carbon dioxide (Wilmore and Costill, 2004). This increase in carbon dioxide stimulates chemoreceptors that signal the inspiratory center to increase ventilation (Wilmore and Costill, 2004). Thus, the ventilatory threshold reflects the respiratory response to increased carbon dioxide levels secondary to the onset of

anaerobiosis (Wilmore and Costill, 2004). Because the VT is useful, practical, and a noninvasive procedure, to identify the physical and physiological markers of this methodological construct would be valuable.

One method of detecting the ventilatory threshold is through near infrared spectroscopy (NIRS). NIRS is based on the principle of differential absorption properties of oxygenated and deoxygenated forms of hemoglobin/myoglobin (Hb/Mb) in the near infrared range (Bhambhani, Buckley, and Susaki, 1997). By monitoring the difference in tissue absorbance, oxygenation levels in the small blood vessels can be evaluated (Bhambhani, et al., 1997; Chance, Dait, Zhang, Hamaoka, and Hagerman, 1992). Studies have shown NIRS to be a valid and reliable non-invasive technique to identify the VT and other muscle oxygenation trends during exercise (Bhambhani, Buckley, and Susaki, 1999). Studies have used NIRS technologies to examine various populations, including healthy subjects and diseased patients (Bhambhani, et al., 1999; McCully and Hamaoka, 2000).

Two studies have shown that NIRS can be used to non-invasively identify the VT during cycle ergometry exercise in healthy subjects (Bhambhani, Buckley, and Susaki, 1997; Belardinelli, Barstow, Porszasz, and Wasserman, 1995). However, these two studies used different NIRS criteria to identify the VT. Belardinelli et al. (1995) identified the VT as the intensity at which there was an "accelerated fall in tissue oxygen saturation" as shown by the NIRS signal during incremental exercise. Bhambhani et al. (1997) found that the point at which the NIRS signal crossed the starting baseline value was strongly correlated with the ventilatory threshold determined from expired gases (the traditional V-slope method). Although both the Belardinelli et al. (1995) and Bhambhani et al. (1997) technologies have been shown to be strongly correlated with both the VO_2 ($r = 0.95$, $P < 0.0001$ and $r = 0.90$ in men; $r = 0.89$ in

women, $P < 0.01$), respectively, and work rate ($r = 0.94$, $P < 0.0001$ and $r = 0.88$ in men; $r = 0.86$ in women, $P < 0.01$), respectively, the two techniques are based on different criteria. However, no previous investigation has compared the two NIRS methods to the gold standard of detecting the VT through gas exchange methods.

Another non-invasive procedure for identifying the VT involves the use of ratings of perceived exertion (RPE). RPE can be defined as "the feelings of effort, strain, discomfort, and fatigue that a person experiences during exercise" (Robertson and Noble, 1997). With the use of a numerical, and/or a numerical and pictorial scale, one can subjectively rate feelings of exertion. A vast number of studies have examined the perceptual responses of adults and children during progressively incremented exercise. A fairly uniform finding of these investigations is that RPE's of 11 to 14 (on the Borg 6-20 scale) span the VT. This relation between the VT and RPE has shown to be stable for female and male adults (Robertson, et al., 2001).

A substantial number of investigations using adults have demonstrated that the RPE corresponding to either the lactate inflection point or VT was not affected by gender, training status, exercise modality, or training specificity (Boutcher, Seip, Hotzler, Pierce, Snead, and Weltman, 1989; Demello, Cureton, Boineau, and Singh, 1987; Hetzler, Seip, Boutcher, Pierce, Snead, and Weltman, 1991; Hill, Cureton, Grisham, and Collins, 1987; Hurley, Hagberg, Allen, et al., 1984; Mahon, Gay, and Stolen, 1998; Purvis, and Cureton, 1981; Robertson, et. al., 2001; Seip, Snead, Pierce, Stein, and Weltman, 1991). Regarding gender, some investigations have reported differences in the VT determined using NIRS technology. It is unclear based upon previous results whether the gender difference in muscle oxygenation is in fact real or whether it is an artifact of measurement technique (Bhambhani, et al., 1999).

Therefore, if the VT can be detected using both NIRS and RPE methods, it follows that there may be a relation between RPE and skeletal muscle oxygenation determined using NIRS. However, no previous investigation using NIRS technology has examined the relation between skeletal muscle oxygenation and RPE during progressively incremented cycle ergometer exercise in adults. Therefore, this study will compare the criterion measure of VT, the V-slope method, to the NIRS techniques (i.e., Belardinelli and Bhambhani) of establishing the VT. Also, this study will explore the impact of gender on the relation of RPE and skeletal muscle oxygenation variables across the physiological and perceptual continuum. This study will provide further evidence on the practical utility of using NIRS technology to detect the ventilatory breakpoint. In addition, the relation between skeletal muscle oxygenation and ratings of perceived exertion will be examined.

1.1 STATEMENT OF THE PROBLEM

The purposes of the investigation are:

1. To compare the NIRS methods of Belardinelli et al. 1995, Bhambhani et al. 1997, and the V-slope method in detecting the VT.
2. To determine the relation between RPE and muscle deoxygenation during progressively incremented cycle ergometer exercise.
3. To determine if there is a gender difference in the muscle deoxygenation during progressively incremented cycle ergometer exercise.
4. To determine if there is a gender difference in the RPE responses during progressively incremented cycle ergometer exercise.

1.2 SCOPE OF THE STUDY

1. The scope of the study was limited to young men and women who were currently participating in a NIH funded project, *Epidemiology of Physical Activity: Teenage to Adult Years*.

1.3 SIGNIFICANCE OF THE STUDY

The study is designed to advance the existing knowledge-base concerning the use of NIRS technology to determine the ventilatory threshold. In addition, the relation of skeletal muscle deoxygenation to ratings of perceived exertion will be examined. The findings of this investigation will increase our knowledge of the relation of ratings of perceived exertion to the ventilatory threshold, as well as further validate the use of NIRS as a means of detecting the ventilatory threshold. The findings will contribute to our understanding of the impact of gender on skeletal muscle oxygenation during cycle ergometer exercise.

2.0 REVIEW OF LITERATURE

The purpose of this chapter is to examine the body of literature on near-infrared spectroscopy (NIRS), ratings of perceived exertion (RPE), and ventilatory threshold. The concept of NIRS and what it indicates about oxygen saturation in skeletal muscle will be discussed. Also, three different methods of detecting the ventilatory breakpoint during exercise will be examined. In addition, the relation of ratings of perceived exertion and the ventilatory breakpoint will be discussed.

2.1 SKELETAL MUSCLE OXYGENATION

Oxygen saturation in skeletal muscle plays a critical role in aerobic energy metabolism. The importance of oxygen delivery and the potential impact of an insufficient oxygen supply have been widely studied (McCully and Hamaoka, 2000). It would be difficult to argue against the precept that almost any experiment that involves exercising muscle would be well served by measuring oxygen delivery and oxidative metabolism (McCully and Hamaoka, 2000).

Quantifying oxygen delivery to exercising skeletal muscle is methodologically difficult (McCully and Hamaoka, 2000). However, two noninvasive methods (i.e. Doppler imaging and plethysmography) are commonly used (McCully and Hamaoka, 2000). These techniques provide global measures of blood flow, with Doppler imaging corresponding to the volume of flow in a large artery, and plethysmography measuring changes in limb volume corresponding to total arterial inflow (McCully and Hamaoka, 2000). Although useful, these methods do not

provide a measure of oxygen delivery and utilization in skeletal muscle. Magnetic resonance spectroscopy (MRS) of high-energy phosphorus compounds can provide an accurate picture of skeletal muscle energy metabolism (Hamaoka, McCully, Katsummura, Shimomitsu, and Chance, 2000), but this method cannot differentiate between limitations caused by oxygen delivery and utilization (McCully and Hamaoka, 2000). MRS of muscle myoglobin has the potential to measure intracellular oxygen saturation (McCully and Hamaoka, 2000), yet this methodological capability is rarely used because of its high cost and organizational problems (Binzoni, Colier, Hiltbrand, Hoofd, and Cerretelli, 1999).

In contrast, near-infrared spectroscopy (NIRS) has a number of significant advantages. It can be portable, and relatively inexpensive in its simplest forms ($4,000 - $25,000) (McCully and Hamaoka, 2000). Signal detection is based on nonharmful levels of light, which are directed into the subject's muscles through overlying skin (McCully and Hamaoka, 2000).

2.1.1 Near Infrared Spectroscopy

NIRS appears to be the emerging technique for monitoring aerobic metabolism in skeletal muscle (Binzoni, Colier, Hiltbrand, Hoofd, and Cerretelli, 1999; Ferrari, Binzoni, and Quaresime, 1997). Since its introduction in the 1970s (Jobsis, 1977), NIRS has been frequently used to study tissue oxygenation status in humans (Boushel, Langberg, Olesen, Gonzales-Alonzo, Bülow, and Kjær, 2001). The NIRS technique is non-destructive, continuous and operates in real time, thus lending itself to *in vivo* monitoring of tissue oxygenation (Boushel and Piantadosi, 2000). Near-infrared spectroscopy has been used primarily as a research tool to assess dynamic changes in the status of tissue oxyhemoglobin ($tHbO_2$), deoxy-hemoglobin (tHb), total blood volume (tBV), and the oxidation state of the copper moiety (CuA) of mitochondrial cytochrome c oxidase (cytochrome a,a_3) in brain and muscle (Boushel and Piantadosi, 2000).

Recently, using tracers that absorb near-infrared light, it has also become possible to quantitate blood flow through an illuminated region of living tissue (Boushel and Piantadosi, 2000).

The use of NIRS to monitor skeletal muscle oxygenation is based on the principle that near-infrared light absorption characteristics of hemoglobin (Hb) and myoglobin (Mb) depend on their O_2 saturation (Grassi, Pogliaghi, Rampichini, Quaresima, Ferrari, Marconi, and Cerretelli, 2003). However, NIRS does not have the capability to differentiate between Hb and Mb. The NIRS signal in human tissue is derived predominantly from the absorption of light by Hb in small arterioles, capillaries and venules (Boushel et al., 2001; Ferrari, Binzoni, and Quaresima, 1997). The vascular specificity of the signal is due to the differential light absorption between the large and small blood vessels (Boushel, et al., 2001; Chance, Dait, Zhang, Hamaoka, and Hagerman, 1992), which is described by Beer's law, whereby photons successfully migrate through tissue regions with minimal absorption (Boushel, Langberg, Olessen, Nowak, Simonsen, Bülow, and Kjær, 2000). Thus, in the microcirculation, light absorption is small, allowing for multiple complete passages of photons along their pathway and therefore detection of chromophore absorption changes (Boushel et al., 2000).

Several types of NIRS spectrophotometry devices have been developed that vary in sophistication, ease of application, algorithms used and number of wavelengths employed (Ferrari et al., 1997). This section will describe some of the essential aspects of continuous wavelength NIRS, which is the most commonly used and least expensive form of NIRS. However, this technique does not allow quantitative measures of absolute concentrations of the chromophores, but instead provides concentration changes deviating from a baseline value (i.e. rest) during variations of O_2 availability and utilization (Boushel et al., 2001). Also, this section will focus only on issues related to the oxygen saturation measurements and not on blood volume

measurements. With continuous wavelength NIRS, the sensor placed on the skin picks up a continuous stream of light from the light source, as opposed to time-resolved or phase-modulated forms of NIRS in which periodic samples are taken at the site (McCully and Hamaoka, 2000; Sevick, Chance, Leigh, Nioka, and Maris, 1991). Light in the range of 700 – 900 nm is used because a) these wavelengths show good penetration of biological tissues, b) the heme groups of Hb and Mb are among the primary absorbing compounds, and c) the absorption of light by the heme groups is altered by oxygen (McCully and Hamaoka, 2000). The NIRS sensor picks up light after it has traveled through the tissue (McCully and Hamaoka, 2000). In the simplest case, this light is from two different wavelengths, commonly 760 and 850 nm (McCully and Hamaoka, 2000). At 760 nm, deoxygenated heme has a higher absorbency, and at 850 nm, oxygenated heme has a higher absorbency (McCully and Hamaoka, 2000). The difference signal (signal at 760 minus the signal at 850) will be sensitive to changes in heme-oxygen saturation and the sum signal to changes in overall heme concentration (McCully and Hamaoka, 2000). Most continuous NIRS devices use white light with specific filters on the detectors (Bhambhani, Buckley, and Susaki, 1997; Hamaoka et al., 2000; McCully and Hamaoka, 2000; McCully and Natelson, 1999), although some employ unfiltered light detectors, and specific wavelength light sources (Ferrari, Binzoni, and Quaresime, 1997; McCully and Hamaoka, 2000). Reflected light is used to study skeletal muscles in humans as the muscles are too thick to allow light to be transmitted directly from the light source to the detector (McCully and Hamaoka, 2000). When obtaining a reflected light signal from skeletal muscle, the source and detector are usually separated by 3 cm (McCully and Hamaoka, 2000). This results in the light traveling in a shallow arc with a penetration depth of approximately one half the separation distance, or 1.5 cm, into the

tissue (McCully and Hamaoka, 2000). Light is scattered in all directions, but is only picked up by the detectors in measurable amounts from the shallow arc (McCully and Hamaoka, 2000).

There has been some controversy as to the influence of subcutaneous adipose tissue on NIR light propagation in leg muscle and on the sensitivity of NIRS instruments. These limitations have recently been investigated (Homma, Fukunaga, and Kagaya, 1996). The authors demonstrated that the near infrared light penetrates shallow regions of muscle (~2 – 4 cm^3 under the skin and subcutaneous fat) even when the adipose tissue thickness is up to 1.5 cm (Grassi, Quaresima, Marconi, Ferrari, and Cerretelli, 1999). In contrast, another study exhibited a negative correlation between adipose tissue thickness (ATT) and muscle O_2 consumption determined using NIRS ($r = -0.70$, $P \leq 0.01$) (van Beekvelt, Borghuis, van Engelen, Wevers, and Colier, 2001). This indicates a decrease in NIRS measured muscle O_2 consumption with increasing adipose tissue thickness. These findings suggest that ATT has a substantial confounding influence on *in vivo* NIRS measurements.

In the clinical setting, NIRS has been used extensively to monitor cerebral oxygenation and blood flow, as well as circulatory and metabolic responses such as abnormalities in tissue oxygenation, delayed recovery after exercise, and muscle oxidative metabolism abnormalities in specific patient populations (Boushel, et al., 2001). In addition, NIRS has been applied to assess O_2 availability and metabolic responses to exercise such as dynamic knee extension (Boushel et al., 2001), cycling (Belardinelli et al., 1995; Bhambhani et al., 1997; Bhambhani et al., 1999; Costes, Prieur, Féasson, Geyssant, Barthélémy, and Denis, 2001; Matsui, Tamura, Hirakawa, Kobayashi, Takekoshi, and Murakami, 1995), weight lifting (Tamaki, Uchiyama, Tamura, and Nakano, 1994), and rowing (Chance et al., 1992).

Oxygen uptake (VO_2) kinetics have been linked to the rate of muscle bioenergetics and the balance between O_2 supply and demand (Barstow, Lamarra, and Whipp, 1990; Chuang, Ting, Toshihiro, Sun, Chiu, Hansen, and Wasserman, 2002; Sietsema, Daly, and Wasserman, 1989; Wasserman, Van Kessel, and Burton, 1967; Whipp and Wasserman, 1972). Although the rate of oxidative phosphorylation determines the exercise O_2 requirement (Barstow, Buchthal, Zanconato, and Cooper, 1994), the kinetics of change in arterial-venous O_2 content difference and cardiac output can affect VO_2 kinetics, and thereby the bioenergetic components contributing to the O_2 deficit (oxygen stores, phosphocreatine, and anaerobic glycolysis) (Chuang et al., 2002). In order to examine the continuous changes in muscle oxygenation, NIRS has been used to evaluate intracellular oxygenation and mitochondrial function at submaximal and maximal oxygen consumption *in vivo* (Boushel and Piantadosi, 2000; Deblasi, Fantini, Franceschini, Ferrari, and Gratton, 1995; Nioka, et al., 1998). Skeletal muscles deoxygenate to varying degrees during exercise in accordance with work intensity and level of training (Boushel and Piantadosi, 2000; Hansen, Thomas, Harris, Parsons, and Victor, 1996; Nioka, *et al*., 1998). The NIRS signal obtained during exercise is considered to reflect the balance between O_2 use and delivery (Hamaoka, Iwane, Shimomitsu et al, 1996), as demonstrated by its gradual decrease of oxygen during incremental exercise (Belardinelli, et al., 1995; Wilson, Mancini, McCully, Ferraro, Lanoce, and Chance, 1989), and by the decrease of oxygen proportional to the intensity of exercise during bouts of constant work rate of exercise (Belardinelli, Barstow, Porszasz, and Wasserman, 1995b; Costes, et al., 2001).

A large number of studies have used the NIRS method to study oxygen saturation *in vivo*. NIRS-measured oxygen saturation of skeletal muscle has been shown to decline during incremental aerobic exercise (Bhambhani, et al., 1997; Hamoaka, et al., 2000; McCully and

Hamaoka, 2000; Wilson, et al., 1989). This decline has been correlated to decreases in directly measured venous oxygen saturation (McCully and Hamaoka, 2000; Wilson, et al., 1989). Bhambhani et al. (1997) showed that at low levels of exercise, the difference signal increased, indicating increased skeletal muscle oxygenation, and at higher work levels, the signal decreased progressively, indicating deoxygenation.

2.2 VENTILATORY THRESHOLD

The anaerobic threshold, which is also referred to as the lactate threshold (LT), is defined as the lowest oxygen uptake during incremental exercise at which blood lactate increases significantly above resting levels (Wasserman, 1986). The mechanism of lactate accumulation during exercise has been the subject of controversy for several years and as yet is unresolved (Bhambhani, et al., 1997; Brooks, 1985; Davis, 1985). One common theory for the accumulation of lactate during exercise is based on tissue hypoxia (Davis, 1985; Wasserman, 1986). It is theorized that when there is an imbalance between the oxygen supply to an exercising muscle and its demand to sustain aerobic energy production then the balance of energy required for muscle contraction is derived *via* anaerobic metabolism (Bhambhani, et al., 1997). Under these conditions lactic acid is produced within the muscle cell, which dissociates into lactate and free hydrogen ions at the physiological pH (Bhambhani, et al., 1997). The lactate ions diffuse out of the muscle cell into the blood and are buffered by bicarbonate (Bhambhani, et al., 1997). This results in an increase in "non-metabolic" carbon dioxide (CO_2) production which is in excess of the CO_2 produced by aerobic metabolism (Bhambhani, et al., 1997). Hence, the LT can be indirectly identified non-invasively by monitoring changes in the rate of CO_2 production relative to the rate of O_2 production during incremental exercise (Bhambhani et al., 1997). This non-

invasive method of identifying the LT, which is ventilation independent, is referred to as the V-slope method by Beaver et al. (1986) (Bhambhani, et al., 1997).

Numerous studies have examined the ventilatory threshold (VT) from gas exchange data using the V-slope method (Beaver, Wasserman, and Whipp, 1986; Belardinelli et al., 1995; Bhambhani et al., 1997). This threshold, as seen in Figure 1, was identified as the VO_2 above which the rate of VCO_2 production increased more rapidly than the rate of VO_2 production as determined by the slope (ΔVCO_2, ΔVO_2) being greater than 1.0 (Belardinelli et al., 1995). Calculation of the VT using the V-slope method is typically performed by computer software available with open circuit spirometry systems.

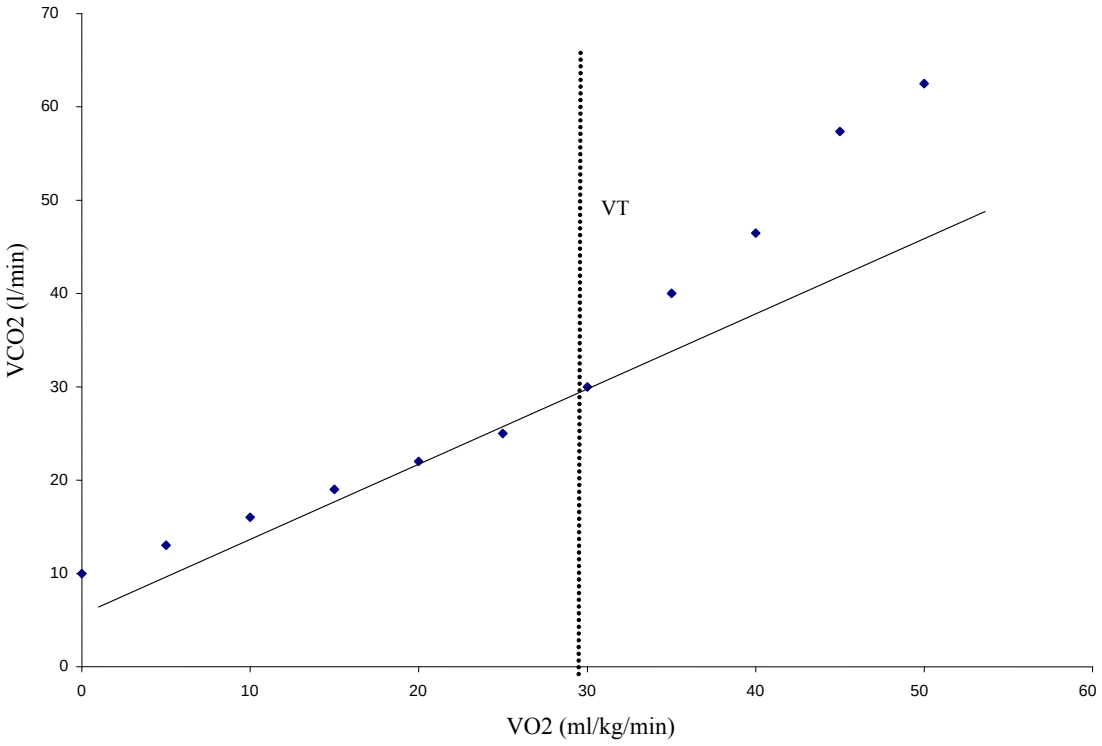

Figure 1. Changes in CO2 production as a function of VO2 during incremental exercise. (Modified from Belardinelli et al., 1995).

2.2.1 Ventilatory Threshold and NIRS

There is a strong correlation between the VT during incremental cycle exercise and the exaggerated reduction in muscle oxygenation measured by NIRS (Bhambhani, 2004). Studies that have documented the NIRS trend during incremental rowing (Chance, et al., 1992) and cycle (Belardinelli, et al., 1995; Matsui et al., 1995) exercise have shown a characteristic four phase response (Figure 2). At the onset of exercise with zero load, there is an immediate, rapid increase in absorbency from the resting baseline values (phase I), implying an increase in muscle oxygenation relative to the baseline level. With increasing power output, phase II occurs in which there is a steady decline in tissue absorbency that continues beyond the resting baseline value, suggesting a decrease in muscle oxygenation relative to the baseline value. Absorbency readings tend to level off with increasing power output until VO_{2max} is attained (phase III). During recovery (phase IV), there is a very rapid increase in the absorbency that extends above the maximum values obtained at baseline in phase I. This response, which has been attributed to hyperemia (Chance, et al., 1992), is indicative of a passive recovery, and tends to level off after the first 2-3 minutes of the recovery phase (Bhambhani, et al., 1997).

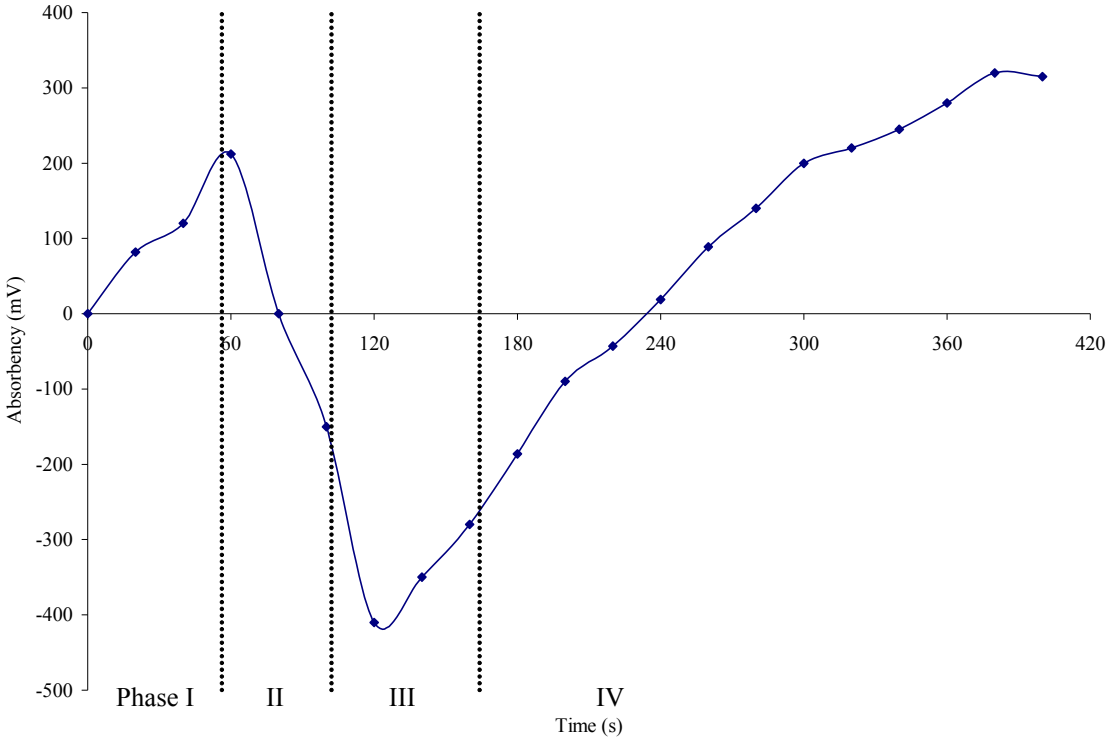

Figure 2. NIRS trend during exercise: Four phase response.

Bhambhani et al. (1997) (Figure 3) and Belardinelli et al. (1995) (Figure 4) both show that NIRS can be used to identify the VT non-invasively during progressively incremented cycle exercise in healthy men and women with different levels of fitness. However, the two studies used different criteria to identify the VT. Belardinelli et al. (1995) identified the VT as the intensity at which there was a rapid desaturation of the tissue as indicated by the NIRS signal during incremental exercise. In the Bhambhani et al. investigation (1997), VT was identified as the exercise intensity at which the NIRS signal crossed the baseline value recorded immediately prior to the onset of exercise. This point was selected because it represents a transition between an increased and decreased level of skeletal muscle oxygenation during incremental exercise

relative to the resting baseline value (Bhambhani et al., 1997). While there is some degree of subjectivity associated with the criteria used by Belardinelli et al. (1995) to identify the ventilatory threshold, the latter method (i.e., Bhambhani et al., 1997) is very objective since there is only one point at which the NIRS signal crosses the baseline value during progressive exercise.

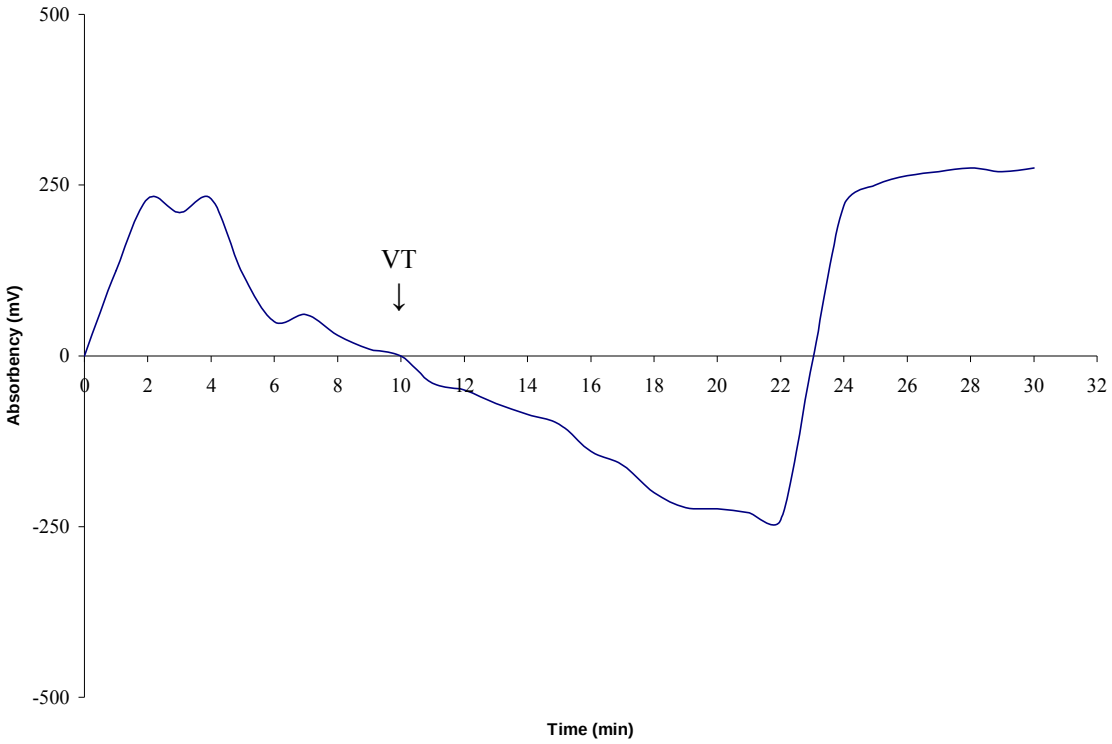

Figure 3. Detection of the ventilatory threshold from tissue absorbency measurements using NIRS. (Modified from Bhambhani et al., 1997).

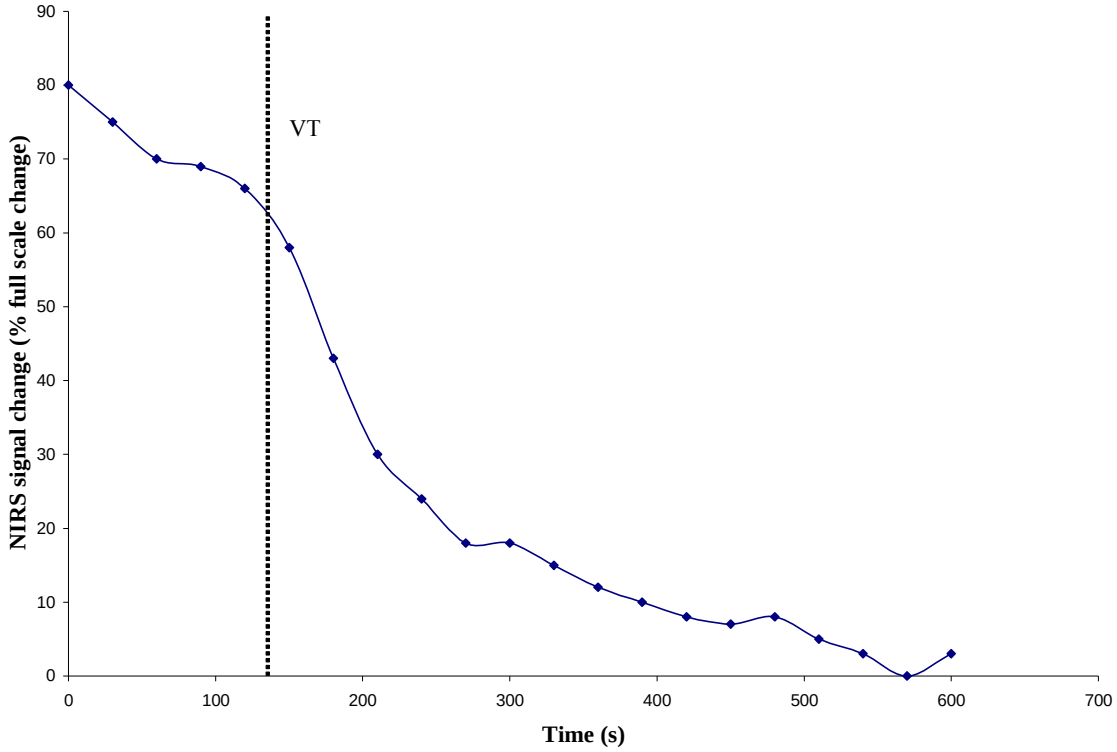

Figure 4. Response of NIRS signal during exercise with the ventilatory threshold (VT) depicted. (Modified from Belardinelli et al., 1995).

Bhambhani et al. (1997) reported that Pearson correlations between the V-slope and Bhambhani methods of detecting the ventilatory breakpoint were 0.90 and 0.88 for the relative oxygen uptake and power output, respectively for men. Similar correlations (0.86 – 0.89) were noted for the female subjects. No significant ($P > 0.05$) gender differences were observed in muscle oxygenation values at the VT, 32% in men and 38% in women (Bhambhani et al., 1997). These results validate the use of NIRS as an alternative noninvasive method for detecting the VT during progressive cycle exercise in healthy men and women. When examining the individual NIRS trends of the 40 subjects Bhambhani et al. (1997) report a rapid decline in tissue absorbency saturation as described by Belardinelli et al. (1995) was only observed in only 50% of the cases. In the remaining subjects, considerable difficulty was experienced in identifying

the reference point (Bhambhani et al., 1997). However, the validity coefficients reported by Belardinelli et al. (1995) for the VO_2 and power output values were higher ($r = 0.94$ and 0.95), respectively, ($P < 0.0001$) than those observed in the study performed by Bhambhani et al. (1997), $r = 0.84$ and $r = 0.88$, respectively, in men and $r = 0.87$ and $r = 0.86$, respectively, in women ($P < 0.01$).

2.3 GENDER AND MUSCLE DEOXYGENATION

There have been some studies that have reported gender differences in the VT determined using NIRS technology. Bhambhani et al. (1997) indicated that muscle oxygenation (%Mox) at the lactic acidosis threshold (LAT) was slightly higher in women compared with men (38% vs. 32%). However, this difference was not significant because of the large standard deviation associated with %Mox. In another study by Bhambhani et al. (1999), %Mox at four separate relative workloads was not significantly different between genders. However, when the skeletal muscle oxygenation values for each workload were pooled for each gender, the mean was significantly higher in women compared with men (35.8% vs. 21.4%) despite the significantly lower absolute VO_2 observed in the female subjects (Bhambhani, et al., 1999). A higher %Mox during exercise indicates that the muscle deoxygenates to a lesser degree, resulting in a reduced amount of oxygen being released by Hb/Mb at that metabolic rate (Bhambhani, et al., 1999). It is unclear, however, based upon the results of previous investigations whether the gender difference in %Mox is a true physiological phenomenon or whether it is an artifact of the measurement technique (Bhambhani, et al., 1999).

2.4 RATINGS OF PERCEIVED EXERTION

During recent decades exercise physiologists have become more interested in different aspects of the human body and how it functions during various modes of exercise. This interest includes how people feel, what aches and pains they have, and how they rate feelings of exertion during exercise. In this context, most exercise scientists and practitioners in the health sciences agree that it is important to understand subjective symptoms of exertion and how they relate to objective physiologic findings (Borg, 1982).

In order to quantify feelings of exertion during exercise, ratings of perceived exertion can be measured. The perception of physical exertion can be defined as the subjective assessment of effort, strain, discomfort and/or fatigue that is experienced during exercise (Noble and Robertson, 1996; Robertson, 2001). Swedish psychologist Gunnar Borg, the father of the concept of perceived exertion, studied the way in which individuals subjectively adapt to physical exercise (Noble and Robertson, 1996). Borg developed the first scale (i.e. Borg Scale) that could be used to rate perceptual intensity during exercise. The typical overall (undifferentiated) rating of perceived exertion (RPE) appears to represent an individual's integration of various physiological sensations that have different subjective weightings (Pandolf, 1982).

Two categories of physiological factors have been suggested as major determinants of RPE during physical exercise (Noble and Robertson, 1996; Pandolf, 1982). The two categories include central and peripheral physiological mediators. Central signals originate in cardio-respiratory adjustments to an increased aerobic metabolic demand (Robertson, Gillespie,

McCarthy, and Rose, 1979). Peripheral physiological mediators are primarily regionalized to exercising muscles in the limbs, trunk, and upper torso (including the shoulder and neck) (Noble and Robertson, 1996).

Central mediators of perceived exertion include:

1. Ventilatory drive (VE) (Mihevic, Gilner, and Horvath, 1981; Noble and Robertson, 1996; Pandolf, 1983; Pandolf, Billings, Drolet, Pimental, and Sawka, 1984; Robertson, 1982)
2. Carbon dioxide excretion (VCO_2) (Mihevic et al., 1981; Noble and Robertson, 1996; Pandolf, 1983; Pandolf et al., 1984)
3. Oxygen consumption (VO_2) (Mihevic et al., 1981; Noble and Robertson, 1996; Pandolf, 1983; Pandolf et al., 1984; Robertson, 1982)
4. Heart rate (HR) (Mihevic et al., 1981; Noble and Robertson, 1996; Pandolf, 1983; Pandolf et al., 1984; Robertson, 1982)
5. Blood pressure (Jones, 1984; Mihevic et al., 1981; Noble and Robertson, 1996; Pandolf, 1983; Pandolf et al., 1984; Robertson, 1982).

Physiological processes that are believed to mediate the intensity of peripheral exertional perceptions are:

1. Metabolic acidosis (pH and lactic acid)
2. Fast- and slow-twitch contractile properties of skeletal muscle fiber
3. Muscle blood flow
4. Blood-borne energy substances (i.e., glucose, free fatty acids, glycerol) (Noble and Robertson, 1996).

The study of human perception during exercise and sport has grown tremendously during the past 30 years. Much of this growth can be attributed to the experimental and practical attractiveness of Borg's 15-category scale (Noble and Robertson, 1996). The 15-category RPE scale increases linearly with the exercise intensity for work on a cycle ergometer, because oxygen consumption and heart rate increase linearly with increasing work load (Borg, 1982).

A newly developed RPE scale known as the OMNI scale, employs pictures of an individual exercising at different exertion levels (Robertson, 2004). The term OMNI is short for omnibus, which in this context means that the perceived exertion scale is applicable for a wide range of clients and physical activity settings (Robertson, 2004). The pictures are combined with short verbal descriptions and arranged along a 0 – 10 numerical scale that depicts gradually increasing exercise intensity such as that encountered when going up a hill (Robertson, 2004). Different sets of pictures, including gender, can be selected to match the exercise mode to be performed. Regardless of the exercise mode depicted on the OMNI scale, the verbal cues remain the same.

The OMNI scale has several distinct advantages over other perceived exertion scales that make it easier for health-fitness and clinical exercise practitioners to use (Robertson, 2004). Foremost, by having the interchangeable picture cues allows the scale to be used for exercise assessment of and program prescription for clients of various ages, fitness levels, clinical statuses, and physical activity preferences (Robertson, 2004).

2.5 VENTILATORY THRESHOLD AND RPE

A number of previous investigations reported that the RPE corresponding to the VT (RPE-Vpt) and/or lactate inflection point ranged from a rating of 11 to 14 on Borg's 15-point scale

(Boutcher, et al., 1989; Demello, et al., 1987; Haskvitz, Seip, Weltman, Rogol, and Weltman, 1992; Hetzler, et al. 1991; Hill, et al., 1987; Hurley, et al., 1984; Mahon, et al., 1998; Purvis and Cureton, 1981; Robertson, et. al., 2001; Seip, et al., 1991). In these investigations, the RPE response range that spanned the VT was stable for males and females who varied in aerobic fitness and endurance training status and was generally independent of exercise mode (Robertson, et al., 2001). In previous investigations involving pediatric samples, RPE-Overall corresponding to the VT ranged from 11 to 14 in female and male children 8 to 12 years if age (Robertson, et al., 2001). Robertson et al. (2001) also reported that a comparatively stable RPE at the Vpt and/or lactate inflection point can be characterized as a response normalized perceptual measurement. This identifies either a stable RPE or RPE range that (a) corresponds to an exercise intensity that produces a prescribed physiological and/or psychological outcome and (b) is common to a specified and defined individual and/or group (Robertson, 2001; Robertson et al., 2001).

2.6 GRADED EXERCISE TESTING

Graded exercise testing (GXT) involves the gradual, progressive, and systematic administration of an exercise stimulus to assess exertional tolerance in clinical, research, and sport settings (Noble and Robertson, 1996). Ratings of perceived exertion are frequently used as an adjunct to standard physiological and clinical responses during a GXT (Noble and Robertson, 1996). It has been established that certain physiological responses mediate the intensity of exertional perceptions. Therefore, perceptual responses during a GXT provide much of the same

information as do physiological responses when determining functional aerobic power, submaximal endurance performance, and tolerance for occupationally related activity (Noble and Robertson, 1996).

The two most common GXT modes include the treadmill and cycle ergometer. The two most commonly used cycle ergometer protocols are the Sjostrand multistage test and the Åstrand-Rhyming single stage test (Noble and Robertson, 1996). A Modified Åstrand test will be used on all current participants.

3.0 METHODS

The purpose of this section is to discuss the methodology of the study. The participants, instrumentation, protocol, and methods of data analysis will be presented.

3.1. SUBJECTS

The participants selected for this study were 20 males and 13 females, aged 25-29 years. The participants were randomly recruited from those currently participating in a NIH funded project, *Epidemiology of Physical Activity: Teenage to Adult Years* (Principal Investigator: Dr. Deborah Aaron). Subject descriptive characteristics are presented in Table 1. Individuals selected for the investigation were sent a letter explaining the purpose of the study and informing them that a research assistant would contact them and invite them to participate in the study. Each subject received a payment of $30.00 for his or her participation. None of the participants reported a history of heart disease, angina, hypertension, heart attack, stroke, asthma or diabetes.

Table 1. Subject characteristics.

	Age (yr)	Height (cm)	Weight (kg)	Body Fat (%)	Vastus Lateralis Skinfold (mm)	VO_{2peak} (ml/kg/ min)
Female (n = 13)	26.2 ± 0.7	160.9 ± 5.6	67.9 ± 14.7	29.0 ± 8.7	32.4 ± 14.0	29.9 ± 8.7
Male (n = 20)	26.0 ± 1.1	177.6 ± 8.1 *	92.6 ± 18.1 *	21.1 ± 7.5 *	20.5 ± 8.3 * (n = 18)	33.8 ±6.3

VO_{2peak} – Peak oxygen uptake
Data are mean ± standard deviation (SD)
*Significant $p < .05$

3.2 EQUIPMENT

Progressive multistage cycle ergometer tests to establish VO_{2peak} were administered on a Monark model # 818 stationary cycle ergometer. A Polar heart rate monitor was utilized to assess heart rates from 45 to 60 s during each minute of exercise. The fraction of expired O_2 (%) and CO_2 (%) were measured every 15 s with an oxygen analyzer, an oxygen sensor model, and a SensorMedics medical carbon dioxide gas analyzer, respectively. Respiratory flow (V_E) (L/min) was measured with a Parvomedics pressure transducer/heater control that was connected to a Hans Rudolph pneumotachograph. The True Max 2400 computer program was interfaced with the electronic gas analyses and flow meter and calculated oxygen uptake and carbon dioxide production and ventilation (STPD) every 15 seconds during exercise. A near-infrared spectroscopy (NIRS) sensor (NIRS Micro-Run Man model # MRM-96) was placed over the vastus-lateralis muscle of the right leg and secured by an Ace bandage. This provided a continuous measure of the skeletal muscle oxygen-hemoglobin saturation during the exercise test.

3.3 EXPERIMENTAL TRIAL

Prior to the experimental trial, the participants were given instructions, advising them to not eat within 12 hours of the test, but to remain properly hydrated, and to wear the appropriate exercise attire. Upon arriving at the lab on the testing day, each participant was required to sign an informed consent approved by the University of Pittsburgh Institutional Review Board (approval # 020221) and complete a medical questionnaire, prior to his or her involvement in the investigation. Next, each participant's height and weight measurements were recorded. Height (mm), without shoes, was measured twice using a wall-mounted Harpendon stadiometer and the average used for analysis. If the two measurements differed by > 4 mm, two additional measurements were taken and the average of the two closest trial values was used in the analyses. Body weight (kg) was measured using a clinic balance beam scale with the participant wearing light clothing and no shoes. Weight was measured to the nearest 0.2 kg. Height and weight was used to calculate body mass index (kg/m^2). Sitting resting blood pressure and resting heart rate were then obtained with a standard sphygmomanometer, stethoscope, and a Polar heart rate monitor.

Anthropometric measurements to assess body size and composition, and fat distribution were performed. Skinfold thickness (mm) was measured with a Lange skinfold caliper at three sites (triceps, suprailiac, and thigh for the females, and chest abdomen and thigh for the males) according to a standard protocol. Three measurements were taken at each site and the average used to calculate percent body fat. Skinfold measurements were also taken of the left vastus lateralis where the NIRS electrode was placed.

A Modified Åstrand graded cycle ergometer exercise test (GXT) was used to assess VO_{2peak}. The subject exercised at a set cadence (50 rpm). A warm-up consisting of cycling with

zero resistance for 2 minutes preceded the progressive protocol. The initial power output was 150 kgm*min^{-1} for two minutes. Every two minutes the power output increased by 150 kgm*min^{-1}. Exercise continued until the subject terminated the test due to exhaustion. Blood pressure (systolic/diastolic) and heart rate (bpm) were monitored throughout the test at the end of each stage. The subjects were cognitively anchored to the Borg 15-category and the OMNI RPE (Appendix B) scales prior to exercise. Subjects estimated their overall body RPE after the first minute of each stage using the Borg 15-category scale, and at the end of the second minute of each stage with the OMNI scale. The OMNI scale was also used to obtain RPE immediately post exercise. The subject had a headset placed on his/her head to secure a mouthpiece. The mouthpiece included a one-way valve that enabled ambient air to enter the body and expired air to be routed into the metabolic cart for analysis. The subject also wore a nose plug throughout the exercise test. Ventilatory parameters (VO_2, VCO_2, and V_E) were measured from the expired air. VO_{2peak} was determined as the highest oxygen uptake value recorded.

The ventilatory threshold (VT) was defined as the VO_2 corresponding to the abrupt increase in ventilation during the graded cycle ergometer exercise test. The VT was identified using the respiratory-metabolic gas exchange measurements obtained every 15 s. The ventilatory equivalents for oxygen (V_E: VO_2) and carbon dioxide (V_E: VCO_2) were plotted as a function of VO_2. The VT was established as the VO_2 at which V_E: VO_2 increased without a concomitant increase in V_E: VCO_2 (Mahon, 1992; Mahon, 1998; Washington, 1988). The VT was detectable from individual plots for each subject and was expressed in both absolute (L/min) and relative (%VO_{2peak}) units. The NIRS unit was used to determine the VT in two ways: the Belardinelli et al. (1995) method and the Bhambhani et al. (1997) method. The Belardinelli et al. (1995) method, as stated previously, defines the VT as the exercise intensity at which there was

an “accelerated fall in tissue oxygen saturation” as shown by the NIRS signal, while the Bhambhani et al. (1997) method identifies the VT as the point at which the NIRS signal crosses the baseline oxygenation value. The Belardinelli et al. (1995) method of determining the VT was determined by two evaluators assessing the VT independently.

A near-infrared spectroscopy Micro-Run Man model #MRM-96 sensor (4 in. x 2 in.) was secured with a tensor bandage without occluding the blood flow, over the vastus-lateralis muscle of the right leg approximately 10-12 mm from the knee, parallel to the major axis of the thigh in order to measure oxygen saturation during the exercise test. Before each exercise test, the NIRS sensor was calibrated according to the manufacturer’s protocol and specifications. This was done while the subject was seated on the cycle ergometer with the leg in a relaxed position at the lowest point of the pedal. In this study, the NIRS unit was interfaced with a computer with the data viewed on-line during each test. Measurements were undertaken continuously throughout exercise and recovery until the subject’s heart rate reached 100 bpm.

Participants were screened prior to administering the VO_{2peak} test based on the American College of Sports Medicine (ACSM) Guidelines for Exercise Testing and Prescription (2000). For individuals 23-27 years of age with no symptoms of CVD, physician supervision of a GXT was not required, however, all tests were administered by experienced technicians and emergency equipment/procedures were in place.

3.4 DATA ANALYSIS

Independent sample t-tests were used to compare the descriptive characteristics (i.e. age, height, weight, % body fat, vastus lateralis skinfold (mm), and VO_{2peak}) in both men and women. A one-way ANOVA was used to analyze the three methods of determining the VT (i.e.,

Belardinelli et al. 1995, Bhambhani et al. 1997, and V-slope). A linear regression analysis was conducted to determine the relation between OMNI RPE and skeletal muscle oxygenation. Two-way ANOVAs (gender x power output) were conducted to examine RPE and NIRS determined muscle deoxygenation. Significant interactions and main effects were examined using the Tukey post-hoc procedure.

4.0 RESULTS

The purposes of this investigation were to: 1) compare the NIRS methods of Belardinelli et al. 1995, Bhambhani et al. 1997, and the V-slope method in determining the VT; 2) determine the relation between RPE-AM and muscle deoxygenation during progressively incremented cycle ergometer exercise; 3) determine if there is a gender difference in muscle deoxygenation during progressively incremented cycle ergometer exercise; and 4) determine if there is a gender difference in the RPE responses during progressively incremented cycle ergometer exercise. The following statistical treatments of the data were employed: independent sample t-tests were used to compare the descriptive characteristics (i.e. age, height, weight, % body fat, vastus lateralis skinfold (mm), and VO_{2peak}) in both male and female subjects, a one-way ANOVA was used to analyze the three methods of determining the VT (i.e., Belardinelli et al. 1995, Bhambhani et al. 1997, and V-slope), a linear regression analysis was used to determine the relation between OMNI RPE and skeletal muscle deoxygenation, and two-way ANOVAs (gender x power output) was used to examine RPE and NIRS determined muscle deoxygenation. Significant interactions and main effects were examined using the Tukey post-hoc procedure.

4.1 SUBJECTS

Twenty males and 13 females participated in the study. Subject demographic data are reported as mean ± standard deviation (see Table 1). There were no significant differences between genders with respect to age or VO_{2peak} (ml/kg/min). The height of the men (177.6 ± 8.1

cm) was different ($p < 0.05$) from the women (160.9 ± 5.6 cm). Weight of the men (92.6 ± 18.1 kg) was different ($p < 0.05$) from the women (67.9 ± 14.7 kg). Body fat, as well as the vastus lateralis skinfold thicknesses were different ($p < 0.05$) between males and females (21.1 ± 7.5 vs. 29.0 ± 8.7, and 20.5 ± 8.3 mm vs. 32.4 ± 14.0 mm, respectively).

4.2 VENTILATORY THRESHOLD METHOD COMPARISON

A one-way ANOVA was conducted in order to compare the three different methods of determining the VT (Belardinelli et al. 1995, Bhambhani et al. 1997, and the V-slope method). The ventilatory threshold was determined using the V-slope method in 21 subjects (9 females and 12 males). The VT was identified in 17 subjects (7 females and 10 males) using the Bhambhani et al. 1997 method and 14 subjects (8 females and 6 males) using the Belardinelli et al. 1995 method. The unequal cell size will be discussed in the next chapter.

All data reported in this section are mean ± standard deviation. The V-slope (58.62 ± 10.47% VO_{2peak}), Bhambhani et al. 1997 (49.75 ± 20.13% VO_{2peak}), and Belardinelli et al. 1995 (60.87 ± 10.15% VO_{2peak}) methods did not result in different ($F(2,49) = 2.77$, $p > 0.05$) VT values. The ANOVA summary for these data is presented in Table 2.

Table 2. ANOVA summary table for three methods of determining VT during graded cycle ergometer exercise in male and female subjects.

	Df	*F*	*p*
Between Groups	2	2.77	0.072
Within Groups	49		
Total	51		

In order to determine whether there were systematic differences between the two NIRS techniques and the criterion measure of the VT (i.e., V-slope), Bland-Altman figures were

generated (Figures 5 and 6). The Bland-Altman plots display the individual subject differences between the criterion (V-slope) method, and the Bhambhani et al. 1997 and Belardinelli et al. 1997 methods of determining the VT. Both Bland-Altman plots display the mean difference (solid line) and the 95% confidence interval (±2 SD; dashed lines). Additionally, the range displayed on the y-axis represents the maximum possible discrepancies. The mean difference between the V-slope method and the Bhambhani et al. 1997 method was 10.0 (range = -27.4, 47.4). The mean difference between the V-slope method and the Belardinelli et al. 1995 method was -2.9 (range = -26.9, 21.1).

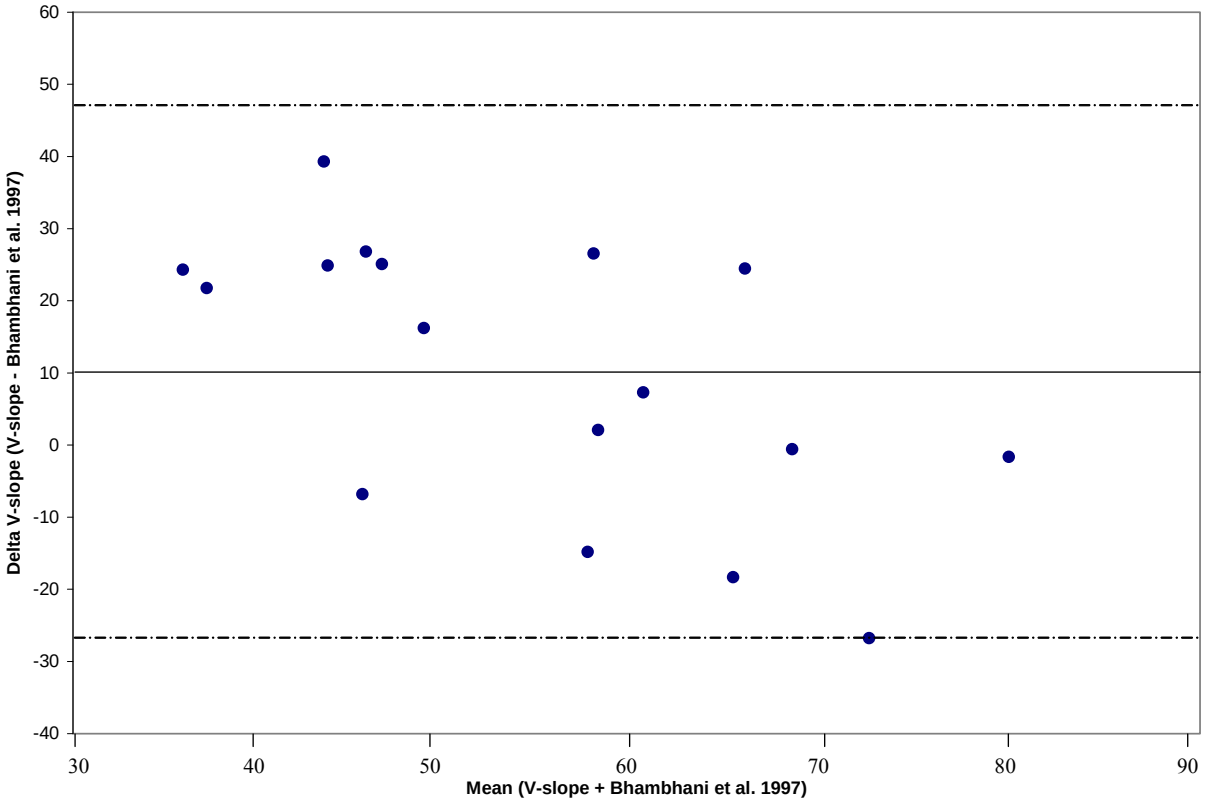

Figure 5. Bland-Altman plot of the individual subject difference between the V-slope and Bhambhani et al. 1997 methods of obtaining the VT.

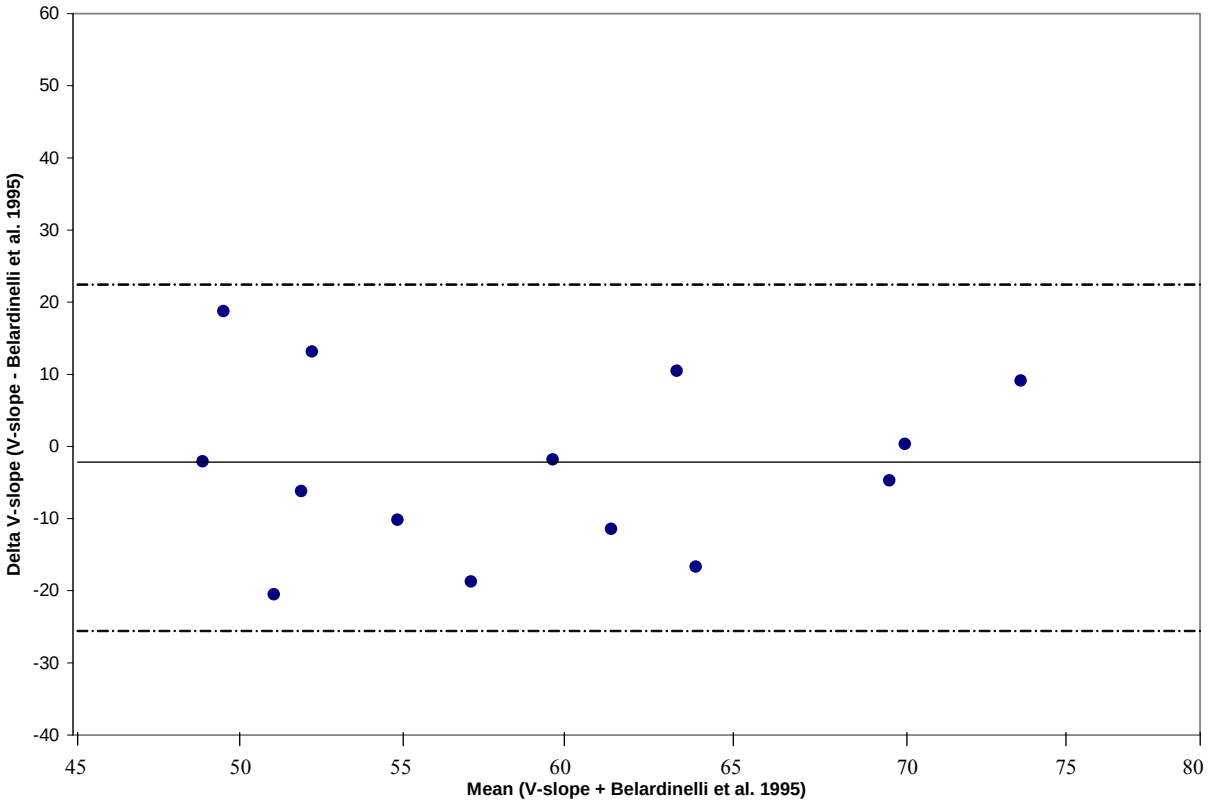

Figure 6. Bland-Altman plot of the individual subject difference between the V-slope and Belardinelli et al. 1995 methods of obtaining the VT.

4.3 RPE AND MUSCLE DEOXYGENATION

A linear regression analysis was conducted using RPE and skeletal muscle deoxygenation at each exercise stage (Figure 7). 32 subjects (12 women and 20 men) from the data set were used in this analysis. One female subject was not included in this analysis due to uninterpretable NIRS data, most likely due to improper placement and/or calibration of the NIRS device. The following significant regression equation was generated ($p = 0.016$): RPE = 5.97 – (15.20)deoxygenation with an R of -0.20, an R^2 of 0.04, and a SE of 2.76. This relationship between RPE and deoxygenation is presented in Figure 7.

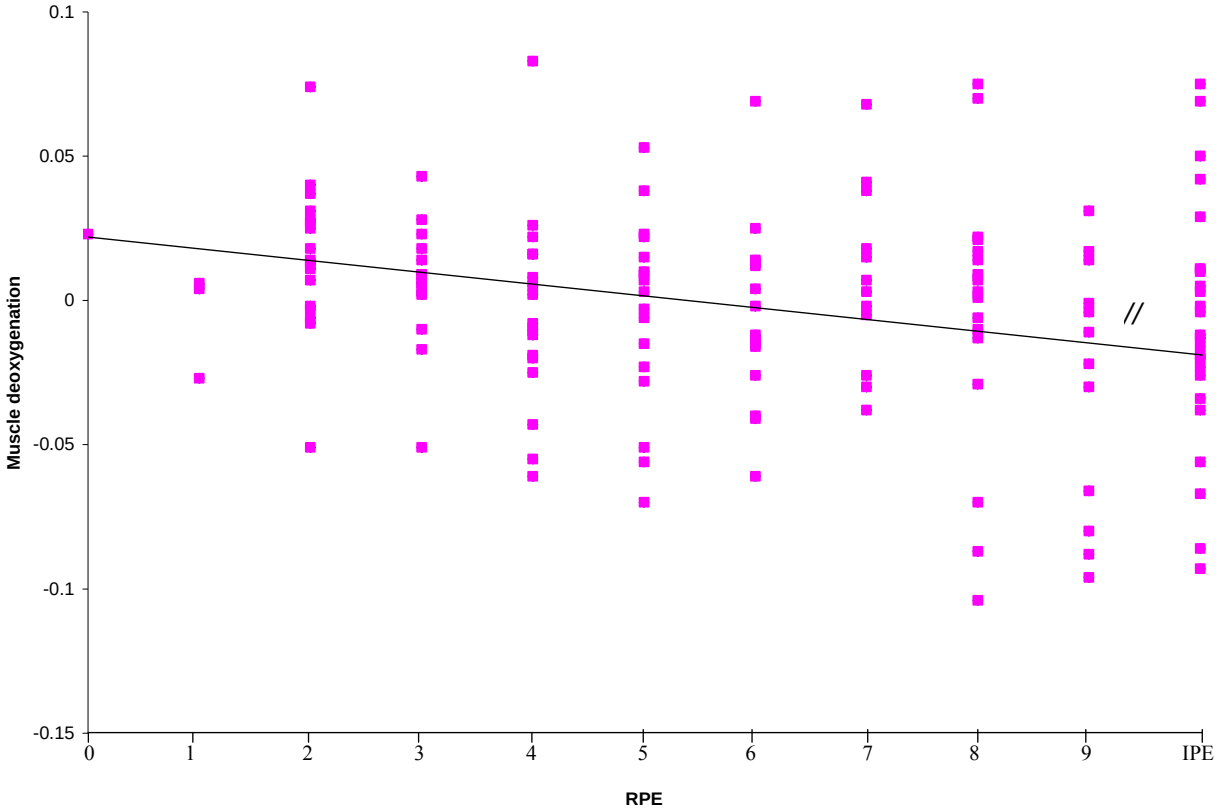

Figure 7. Relationship between muscle deoxygenation and RPE. IPE = Value immediate post-exercise.

4.4 GENDER, RPE, AND MUSCLE DEOXYGENATION

4.4.1 Gender and RPE

A two-way ANOVA (gender x power output) was conducted to examine RPE. The ANOVA summary for these data is presented in Table 3. The RPE responses to the progressively incremented cycle ergometer exercise tests are presented in Figure 8. Data from 33 subjects (13 females and 20 males) were used for this analysis. No females made it past stage 7, whereas some males (n = 2) made it into stage 9. A significant main effect for gender was found ($F(1,193) = 19.53$, $p < 0.05$). Males had lower RPEs (6.32 ± .17) than females (7.16 ± .28). A significant main effect for power output was also found ($F(9, 193) = 56.21$, $p < 0.05$). The

results from Tukey post-hoc analysis conducted on the main effect for power can be found in Appendix C. In addition, a significant gender x power output interaction was found ($F(7, 193) = 2.11$, $p < 0.05$). Independent t-tests were run to determine which power outputs were significantly different in RPE responses.

Table 3. ANOVA summary table for RPE responses during graded cycle ergometer exercise in male and female subjects.

Source	*Df*	*F*	*P*
Gender	1	19.53	.000 *
Power Output	9	56.21	.000 *
Interaction (Gender x Power Output)	7	2.11	.044 *

*Significant p< 0.05

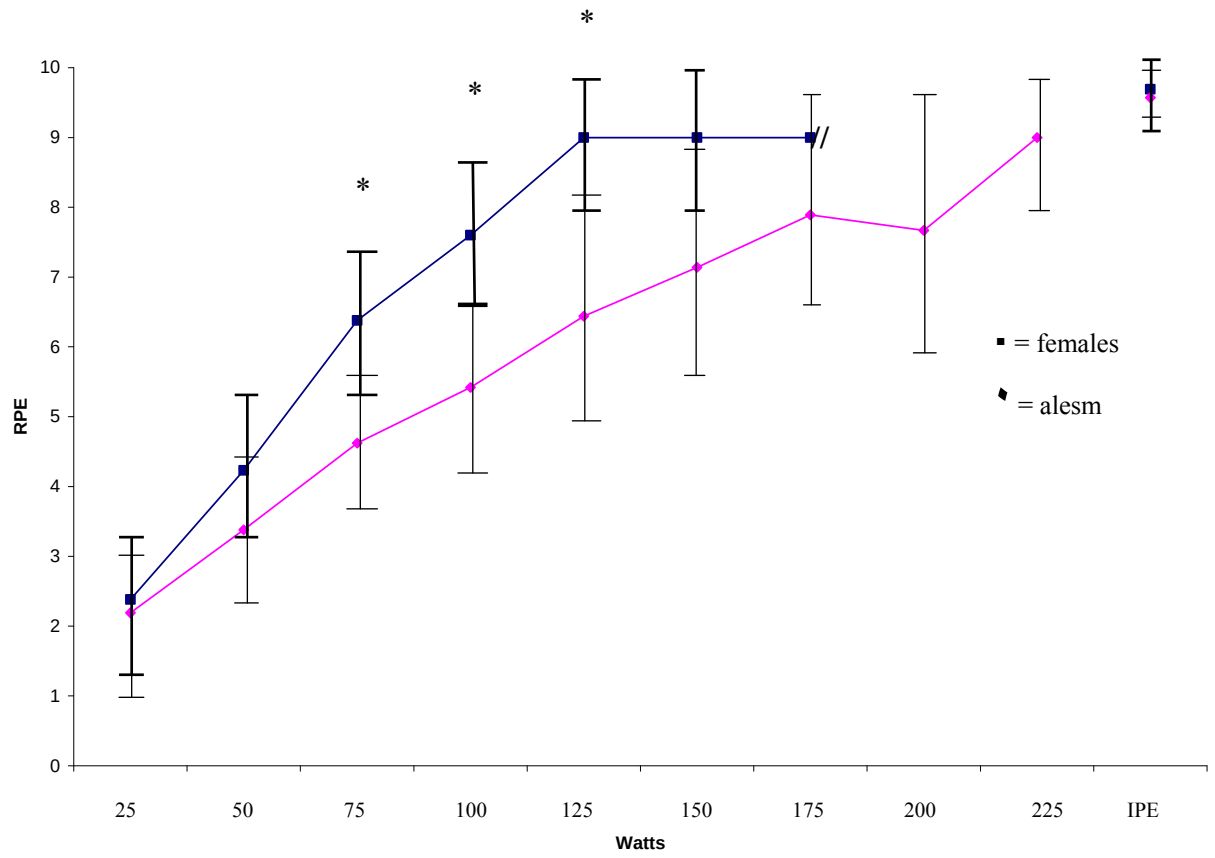

Figure 8. RPE responses during graded cycle ergometer exercise in male and female subjects (p>.05). IPE = Value at immediate post-exercise. * Significant $p < 0.05$

4.4.2 Gender and NIRS Determined Muscle Deoxygenation

A two-way ANOVA (gender x power output) was conducted to examine NIRS determined muscle deoxygenation. Muscle deoxygenation responses to the progressively incremented cycle ergometer tests are presented in Figure 9. The ANOVA summary for these data is presented in Table 4. Data from 23 subjects (9 females and 14 males) were used for this analysis. Some data were not usable do to different reasons that will be presented in the next chapter. Also, no females exercised beyond stage 7. A significant main effect for gender was found ($F(1, 133) = 10.61$, $p < 0.05$). Females had less deoxygenation occur ($0.012 \pm .007$) than males ($0.002 \pm .005$). The main effect for power output was not significant ($F(9, 133) = 1.60$, $p > 0.05$). Finally, the interaction effect (gender x power output) was also not significant ($F(7, 133) = .115$, $p > 0.05$).

Table 4. ANOVA summary table for NIRS determined muscle oxygenation responses during graded cycle ergometer exercise in male and female subjects.

Source	*Df*	*F*	*p*
Gender	1	10.61	0.001 *
Power Output	9	1.60	0.122
Interaction (Gender x Power Output)	7	.115	0.997

*Significant $p < 0.05$

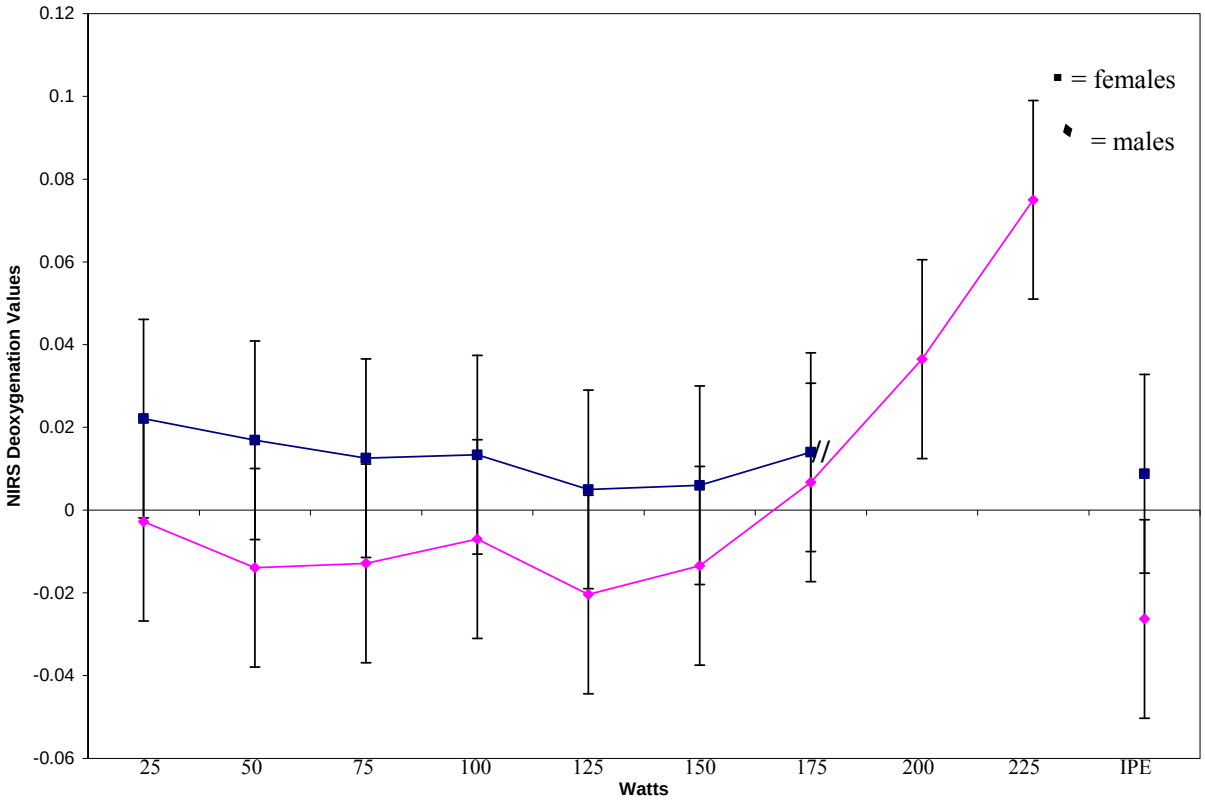

Figure 9. NIRS deoxygenation responses during graded cycle ergometer exercise in male and female subjects ($p > 0.05$). IPE = Value at immediate post-exercise.

5.0 DISCUSSION

The purpose of this section is to discuss the results of this study and to examine the practical applications of the findings. This investigation employed the ventilatory threshold using V-slope as the criterion measure to determine whether NIRS provided a valid assessment of the VT by using the Bhambhani et al. (1997) and Belardinelli et al. (1995) methods. The major findings from this study are:

1. The 3 methods of determining the VT (V-slope, Bhambhani et al. (1997), and Belardinelli et al. (1995) methods) were not different.
2. Muscle oxygenation values decreased with increasing ratings of perceived exertion.
3. Ratings of perceived exertion differed between genders. Males had lower RPE's than females ($p < 0.05$). Also, a significant gender x power output interaction was found ($p < 0.05$).
4. Women experienced less skeletal muscle deoxygenation than men during a progressively incremented cycle ergometer protocol.

5.0.1 Three Method Comparison of the Ventilatory Threshold

Previous studies have validated the use of NIRS as a noninvasive method for detection of the VT during cycle ergometer exercise in adult men and women (Belardinelli et al., 1995; Bhambhani et al., 1997). The NIRS methodology enables the comparison of changes in tissue oxygenation with simultaneous measurements of gas exchange. NIRS allows examination of the pattern of

change in skeletal muscle oxygenation beginning with exercise intensities below the lactic acidosis threshold and continuing until maximum intensity is achieved (Belardinelli et al., 1995).

The results of the present study support the findings of Belardinelli et al. (1995) and Bhambhani et al. (1997) that NIRS can be used to identify the VT non-invasively during cycle exercise in healthy subjects. In the current study, only 21 of the 33 original subjects had usable NIRS oxygenation data available for analysis. Of the 21 subjects, 17 (81%) were able to be included using the Bhambhani et al. (1997) method. The oxygenation values of the four subjects that were not included in the present analyses had NIRS responses that did not cross baseline. Using the Belardinelli et al. (1995) method, only 14 (67%) subjects fulfilled the criteria. As stated previously, two investigators independently assessed the VT, and an average was taken. Seven cases did not meet the criteria set by Belardinelli and colleagues (1995), in that it was indeterminate where the "accelerated fall in tissue O2 saturation" as indicated by the NIRS signal actually occurred. In a previous study by Bhambhani et al. (1997), this "rapid decline" in tissue oxygenation was clearly discernable in approximately 50% of the cases, which is somewhat lower than noted presently (67%). Overall, this loss of data could be due to a number of factors, including possible improper NIRS calibration, incorrect placement of the NIRS sensor over the vastus lateralis, differences in training of investigators, failure to synchronize the timing of the metabolic cart and NIRS device, or faulty criteria. Presently, the NIRS methods resulted in only 81% (Bhambhani) and 67% (Belardinelli) of the data meeting the criteria established for the determination of the VT, therefore the NIRS methods may not be as practical as the traditional V-Slope method of determining the VT.

Bhambhani et al. (1997) compared the mean values for the VT identified by the V-slope method and their method and found no significant differences. These observations were

consistent with those found by Belardinelli et al. (1995). In the current study, the VT obtained from NIRS data occurred earlier (i.e., lower workload) than the V-slope method in 65% of the cases when using the Bhambhani et al. (1997) method, and in 35% of the cases when using the Belardinelli et al. (1995) method. The current results from the Bhambhani method are supported by recent findings (Bhambhani et al., 1997). The discrepancy between NIRS methods is most likely a result of differences in the NIRS technique used to identify the VT. From a physiological standpoint, it seems logical that the VT identified by NIRS should occur earlier than that detected from gas exchange data because the NIRS method evaluates the trends in muscle oxygenation directly at the muscle site, whereas the V-slope method is dependent upon alterations in gas exchange that are associated with the buffering of lactic acid by bicarbonate in the blood (Beaver et al., 1986; Bhambhani et al., 1997). These gas exchange responses are usually delayed because of the time required for the lactate to diffuse out of the muscle cell, enter the blood stream, and react with the bicarbonate to result in non-metabolic CO_2 production (Bhambhani et al., 1997; Wasserman, 1986).

The Bland-Altman plots (Chapter 4, Figures 5 and 6) display the individual subject differences between the V-slope (criterion) method and the Bhambhani et al. (1997) and Belardinelli et al. (1995) methods. As stated before, there were no significant differences between the methods. In addition, these figures do not indicate any systematic differences between the criterion and the Bhambhani or Belardinelli techniques. However, the V-slope and Bhambhani et al. (1997) relationship shows a larger variability. This is supported by the comparatively large standard deviation associated with the Bhambhani et al. (1997) method ($49.75 \pm 20.13\%$ VO_{2peak}). The NIRS techniques used presently rely on very different criteria

which could contribute to the differences in the variability. Also, as stated previously, validity coefficients for the Belardinelli et al. (1995) method have been noted to be higher than the Bhambhani et al. (1997) method.

Dupain (2002) examined the use of NIRS to identify the VT in male and female children. Dupain (2002) reported that no significant differences were found between the V-slope and NIRS methods of determining the VT. The results of these analyses suggest the physiological basis underpinning the abrupt increase in the rate of deoxygenation corresponding to the VT may be due to the Bohr effect (Dupain, 2002). The Bohr effect is a process that reflects alterations in the molecular structure of hemoglobin owing to increases in acidity, temperature, and carbon dioxide concentration (Dupain, 2002). These alterations cause an increase in the release of oxygen from hemoglobin. The abrupt oxy-hemoglobin reduction may provide a physiological mechanism that explains the agreement between the NIRS methods and the V-slope method of determining the VT. It has been proposed that the accumulation of lactate during exercise is at least in part a response to tissue hypoxia (Wasserman, 1986).

5.0.2 Muscle Deoxygenation and RPE

The tissue deoxygenation response during graded cycle ergometer exercise is illustrated in Figure 7 (see Chapter 4). The current investigation found that muscle oxygenation values decreased with increasing RPE. Research has shown that with increasing power output during cycle ergometer exercise, muscle oxygenation values decrease (Belardinelli et al., 1995; Bhambhani et al., 1997; Matsui et al., 1995). The theoretical basis of RPE is supported by a similar rationale; it is expected that as the intensity of exercise increases, corresponding and

interdependent changes occur in both the perceptual and physiological processes (Robertson, 2004). However, previous studies have not examined the relation between RPE and skeletal muscle oxygenation.

The relationship between skeletal muscle deoxygenation and RPE noted presently (r = -.20) does not support a strong sensory link between RPE and skeletal muscle deoxygenation. In contrast other recognized mediators of perceived exertion (i.e., oxygen consumption, muscle blood flow, metabolic acidosis, muscle glycogen, etc.) have been shown to be more strongly related to ratings of perceived exertion. The physiological explanation for the current NIRS findings is that as exercise intensity increases, there is a concurrent increase in aerobic metabolism. With this need for oxygen comes a corresponding increase in oxygen consumption and muscle blood flow. In addition, lactic acid production increases dramatically above the LT. It has been established that skeletal muscles deoxygenate to varying degrees during exercise in accordance with work intensity and level of training (Boushel and Piantadosi, 2000; Hansen, Thomas, Harris, Parsons, and Victor, 1996; Nioka, *et al*., 1998). The results from the current study show similar results, that is, with increasing work intensity, a corresponding decrease in skeletal muscle oxygenation occurs. Desaturation measured with NIRS in normal subjects is due to the decrease in capillary and venous HbO_2 and tissue MbO_2 saturation (Belardinelli et al., 1995). The decrease in skeletal muscle oxygenation during progressively incremented cycle ergometer exercise may serve as a weak mediator of perceived exertion by acting either individually or collectively with other mediators to alter the tension-producing properties of skeletal muscle.

It is possible that the change in muscle fiber recruitment during the progressively incremented exercise test results from the increased reliance on glycolysis. This increase in fast-

twitch muscle fiber activation at higher exercise intensities may have influenced the RPE/NIRS relation noted presently and could have resulted in higher muscle oxygenation due to the low myoglobin and mitochondria size and number in these fibers. In addition, variability in fiber type of the vastus lateralis could have influenced the skeletal muscle deoxygentation pattern.

5.0.3 Gender, RPE, and Muscle Deoxygenation

The male subjects exhibited a lower RPE than the female subjects, indicating that the men did not perceive the level of exertion to be as high as the women. Also, the males and females had different RPE responses at 50, 75, and 100 Watts (Figure 8). These findings are inconsistent with previous research. In general, when females and males perform aerobic exercise at the same % VO_{2max}, their RPEs do not differ (Robertson, 2004). However, when subjects perform at the same absolute exercise intensity, the RPE will be higher for the individual who has the lower aerobic fitness. In general, females tend to have a lower VO_{2max} than males (Robertson, 2004). However, in this investigation, there were no differences in fitness levels between genders, as shown by the similar VO_{2peak} (Table 1). Therefore, any given submaximal power output should represent a similar relative workload for females and as such should result in correspondingly similar RPEs. However, the difference in RPE between genders was only 0.84 RPE units. Although statistically significant, this finding has limited the practical importance.

In the current study, significant differences were observed in muscle deoxygenation at the VT between genders. Females had less deoxygenation occur (0.012 ± .007) than males (0.002 ± .005). It has been reported that approximately 75% of the tissue deoxygenation observed during exercise is due to the desaturation of Hb, whereas the balance is due to the desaturation of Mb (Bhambhani et al., 1999; Chance et al., 1992; Mancini, Bolinger, Li, Kendrick, Chance, and Wilson, 1994).

Similar findings were reported from past studies. Bhambhani et al. (1997) reported that percent muscle oxygenation at the lactate threshold was slightly higher in women compared with men (38% vs. 32%), but these values were not significantly different between genders because of the large standard deviation associated with the measurement.

In another article by Bhambhani et al. (1999), nineteen men and 14 women performed an incremental cycle exercise test to identify the lactate threshold (LAT) VO_{2max}, and an intermittent constant work rate test at an oxygen uptake corresponding to 40% LAT, 80% LAT, 25% LAT-VO_{2max}, and 50% LAT-VO_{2max}. There was no significant difference in percent muscle oxygenation between genders (Bhambhani et al., 1999). The most likely reason for this finding was the large variability in the percent muscle oxygenation values at each of the four intensities (Bhambhani et al., 1999). However, when the four constant work rates were pooled for each gender, the mean percent muscle oxygenation was significantly higher ($p < .05$) in women compared with men (35.8% vs. 21.4%), suggesting less deoxygenation at the same relative exercise intensity (Bhambhani et al., 1999). It was unclear whether this observed gender difference reflects a true physiological phenomenon or whether it is an artifact of the NIRS technique (Bhambhani et al., 1999).

Previous studies have examined the influence of gender on the VT in children. Reybrouck et al. (1982) determined the VT in healthy children during graded exercise and found no difference between genders. Dupain (2002) reported no significant differences in VT using the V-slope and Belardinelli et al. (1995) methods between 40 male and female children between the ages of 8-12 years. The similarity of VT between prepubescent girls and boys may be due to the fact that VO_{2peak} is relatively similar between genders when physical activity and training status are equal (Dupain, 2002).

It has been reported that the NIRS signal penetrates approximately 2.5-3 cm through the subcutaneous tissue (Belardinelli et al., 1995; Bhambhani et al., 1999; Chance et al., 1992). In the present study, the mean skinfold thickness over the vastus lateralis was significantly different between males and females ($p < .05$), (20.5 ± 8.3 mm and 32.44 ± 14.0 mm, respectively). In Bhambhani et al. (1999), the skinfold thickness was not measured, but it was speculated that because the thickness of the fat layer at the measurement site was most likely higher in women compared to men, it was possible that the longer path length in women would have resulted in a weaker NIRS signal, thereby confounding the results of the study. Costes, Jean-Claude, Feasson, Busso, Geyssant, and Denis (1996) reported that variability in the NIRS signal could be due to differences in the thickness of the subcutaneous tissue among subjects, but the precise effect remains unknown. If the attenuation of the NIRS signal was greater in the female subjects due to the thickness of the subcutaneous adipose tissue over the vastus lateralis, this may have contributed to the gender differences in skeletal muscle deoxygenation noted presently.

5.1 CONCLUSIONS

The research foci of the present investigation were:

1. To compare the NIRS methods of Belardinelli et al. 1995, Bhambhani et al. 1997, and the V-slope method in detecting the VT. Based on the findings of the current investigation, the NIRS methods of Belardinelli et al. 1995, Bhambhani et al. 1997, and the V-slope method in detecting the VT did not differ.
2. To determine the relation between RPE and muscle deoxygenation during progressively incremented cycle ergometer exercise. The present investigation revealed an inverse relationship between muscle oxygenation values and RPE.

3. To determine if there is a gender difference in the muscle deoxygenation during progressively incremented cycle ergometer exercise. The present investigation revealed that females had less deoxygenation occur than males.

4. To determine if there is a gender difference in the RPE responses during progressively incremented cycle ergometer exercise. The findings in the current investigation revealed that males had lower RPE's when compared to females. Also, a significant interaction effect was found between RPE and gender.

5.2 RECOMMENDATIONS

The present study compared NIRS and V-slope methods in determining the VT during progressively incremented cycle ergometer tests administered to adults.

1. Future studies could determine whether NIRS technology can be used to identify the VT during other modes of exercise.
2. In the present investigation, the criterion measure for the VT was the V-slope measurement. Future studies could use blood lactate measurements as the criterion variable.
3. Future studies could use larger sample sizes of men and women, including various populations (sedentary, athletes, etc.).

APPENDIX A

INFORMED CONSENT

Approval Date: January 28, 2003
Renewal Date: January 27, 2004
University of Pittsburgh
Institutional Review Board
IRB Number: 020221

CONSENT TO ACT AS A SUBJECT IN A RESEARCH STUDY

TITLE: Factors Contributing to the Change in Physical Activity from Adolescence to Young Adulthood and the Impact on Health Status

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SOURCE OF SUPPORT: School of Education, University of Pittsburgh

Why is this research being done?

Being physically active is an important component of health and has been found to decrease the risk of certain diseases such as heart disease and diabetes. However, many adults do not get the recommended amount of physical activity. In order to help develop better programs to encourage adults to increase their physical activity it is important to understand how and why people's physical activity changes as they get older and how this may affect their health status. The purpose of this research study is to identify factors that contribute to the change in physical activity from adolescence to young adulthood and to examine how this change in physical activity may affect indicators of health such fitness, body composition, and cholesterol, triglyceride, and glucose levels. A total of 40 subjects will be enrolled in this study.

Who is being asked to take part in this research study?

You are being asked to participate in this research study because you are currently participating in a study to examine changes in physical activity from adolescence to young adulthood, have completed a telephone interview of your current health status, and live in the greater Pittsburgh area. You were randomly selected for this additional study based on the physical activity level you reported during adolescence.

What procedures will be performed for research purposes?

Your participation in this research project will last approximately 2 hours and include the following procedures.
You will be asked to fast for approximately 12 hours prior to having your blood drawn. A 11 ml (about 2 teaspoons) sample of blood will be taken to test for cholesterol, triglyceride and glucose concentration. The blood will be drawn by a trained phlebotomist. Following the blood draw, you will be given a light snack.

You will complete a one-on-one interview with a trained research assistant. The interview will include a historical assessment of physical activity at key time periods during your life, i.e., junior high school, senior high school, immediately after high school graduation, etc. At each time period you will be asked to identify factors that may have contributed to any change (either increase or decrease) in your usual level of physical activity. With your permission, the interview will be audio taped. The purpose of the interview is to talk about your physical activity experiences from when you were a child to now as an adult. We are taping these interviews so that we can capture every word that you say. It would not be possible for the research assistant to remember everything that was said or to write everything down. Your name will not be disclosed on any printed or published reports that are produced; ID numbers will be used for all participants in the study. All audio tapes will be confidential and stored in my locked office on the university campus. Tapes will only be reviewed by the investigators and research assistants who will transcribe the tapes. The tape will be destroyed when the project is complete.

Measurements of your height, weight, waist circumference and hip circumference will be taken. The amount of fat in your body will be estimated by the method of bioelectric impedance analysis (BIA). BIA is a common method of assessing body composition. It involves passing a small electric current through your body and measuring the impedance or opposition to the current flow. You will not feel the current. BIA is extremely safe and is currently available for personal use at home. We will also measure the thickness of a fold of skin at three sites on your body (females – thigh, back of upper arm, and hip: males – thigh, chest and abdomen).

Your musculoskeletal fitness will be measured by having you perform several strength and flexibility tests. The tests are commonly performed on school children as part of their regular physical education class. Flexibility will be assessed using a sit-and-reach assessment test for hamstring and low back flexibility. You will remove your shoes and sit on the floor with your feet against the testing box which is placed against a wall. Without bending your knees, you will extend your arms forward across the testing box. The score is the maximum number of inches that you can reach. Grip strength will be measured by the maximal grip effort with your dominant and nondominant hand using a handgrip dynamometer.

Your aerobic fitness level will be measured by having you pedal a stationary bicycle. During the fitness test, we will place a heart rate monitor around your chest and secure it with an elastic strap. A rubber mouthpiece, connected to a headset, will be placed in your mouth to determine the amount of oxygen that you use during exercise. A clip will be attached to your nose to insure that all of the air that you breathe comes in and out through your mouth. Some individuals become anxious when fitted with

the nose clip and mouthpiece. If this occurs to you, please inform the individual performing the test and the test will be stopped. We may have to shave your thigh in order to apply a self-adhesive sensor to measure the amount of oxygen-saturation in the muscle. This is measured through a light sensor. Another sensor will be placed on your forehead to measure cerebral oxygen-saturation. The bicycle test will begin with a 3-minute warm-up. Then every 3 minutes the workload will be increased. You will be encouraged to continue until fatigued. You may stop the test at any time. Following completion of the test, you will cool-down by pedaling slowly for 3-5 minutes. Your heart rate, blood pressure, and the amount of oxygen your body uses will be measured throughout the test. The total duration of the test will not exceed 20 minutes.

What are the possible risks, side effects, and discomforts of this research study?

Abnormal responses, such as excessive rises in blood pressure, mental confusion, shortness of breath, chest pain, heart attack, and death, to maximal exercise tests in young health adults are rare, occurring in less than 1% of people (less than 1 out of 100 people tested). However, some common risks, occurring in 1% to 25% of people (1 to 25 out of 100 people tested), of maximal exercise testing include; heavy breathing, dizziness, muscle fatigue, headache, and overall fatigue. To minimize risks associated with maximal exercise testing, you will be asked to complete a medical history questionnaire. If you have any problems that may be worsened by maximal exercise for example, arthritis, a recent injury, heart disease, hypertension, or diabetes you will be excluded from participation. If an abnormal response occurs during exercise, the test will be immediately stopped and you will be given proper medical attention. Emergency equipment will be on site for all testing procedures. In addition, staff personnel are certified in CPR and First Aid by the American Red Cross.

According to the U.S. Department of Health and Human Services, there have been no reported adverse events induced by BIA. The small current magnitudes involved, less than 1mA, are less that the threshold of pain. However, if you have an implanted device, such as a cardiac defibrillator, you should avoid using BIA due to possible electrical interfaces.

Trained phlebotomists will perform the blood draw. There is a rare risk of infection with the insertion of the needle. It is possible that you may have some minor bruising and/or soreness after blood collection. However, this will be no more than you would encounter during blood donation.

Although rare, there is a potential risk of hamstring or back muscle strain from the flexibility tests, and that subjects may experience muscle or joint pain from the grip strength testing procedures. There are no risks associated with the performance of the musculoskeletal fitness testing, body measurements, or the interview.

What are the possible benefits from taking part in this study?

There are no direct benefits to you from participating in this study. However, you will receive important information regarding your fitness level, body composition, and your cholesterol, triglyceride, and glucose levels.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?

You will be promptly notified if any new information develops during the conduct of this research study that may cause you to change your mind about continuing to participate.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

Neither you, nor your insurance provider, will be charged for the costs of any of the procedures performed for the purpose of this research study.

Will I be paid if I take part in this research study?

You will be paid a total of $30 for completing all parts of this research study. In addition, any parking fees related to your participation in this study will be paid for by the study.

Who will pay if I am injured as a result of taking part in this study?

University of Pittsburgh researchers and their associates who provide services at the UPMC Health System (UPMC HS) recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator or one of the co-investigators listed on the first page of this form.

Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of the UPMC HS. It is possible that the UPMC HS may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the cost of this follow-up unless otherwise specifically stated below. You will not receive any monetary payment for, or associated with, any injury that you suffer in relation to this research.

Who will know about my participation in this research study?

All records related to your involvement in this research study will be stored in a locked file room. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from research records.

Only the researchers listed on the first page of this form and their staff will have access to your research records. Your research records will be destroyed when such is approved by the sponsor of this study or, as per University policy, at 5 years following study completion, whichever should occur last.

Any information about you obtained from this research will be kept as confident (private) as possible. You will not be identified by name in any publication of research results unless you sign a separate form giving your permission (release). In unusual cases, your research records may be released in response to an order from a court of law. It is also possible that the University Research Conduct and Compliance Office may inspect your research records. If the researchers learn that you or someone with whom you are involved is in serious danger or harm, they will need to inform the appropriate agencies as required by Pennsylvania law.

Is my participation in this research study voluntary?

Your participation in this research study is completely voluntary. You do not have to take part in this research study and, should you change your mind, you can withdraw from the study at any time. Your current and future care at a UPMC HS facility and any other benefits for which you qualify will be the same whether you participate in the study or not.

If I agree to take part in this research study, can I be removed from the study without my consent?

It is possible that you may be removed from the research study by the researchers to protect your safety or you are unable or unwilling to complete the research protocol.

VOLUNTARY CONSENT

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (412-578-8570). By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____ _____

Participant's Signature Date

_____ _____

Witness' Signature Date

INVESTIGATOR'S CERTIFICATION

I certify that nature and purpose, the potential benefits and possible risks associated with participation in this research study have been explained to the above individual and that any questions about this information have been answered.

_____ _____

Investigator's Signature Date

APPENDIX B

OMNI RPE SCALE

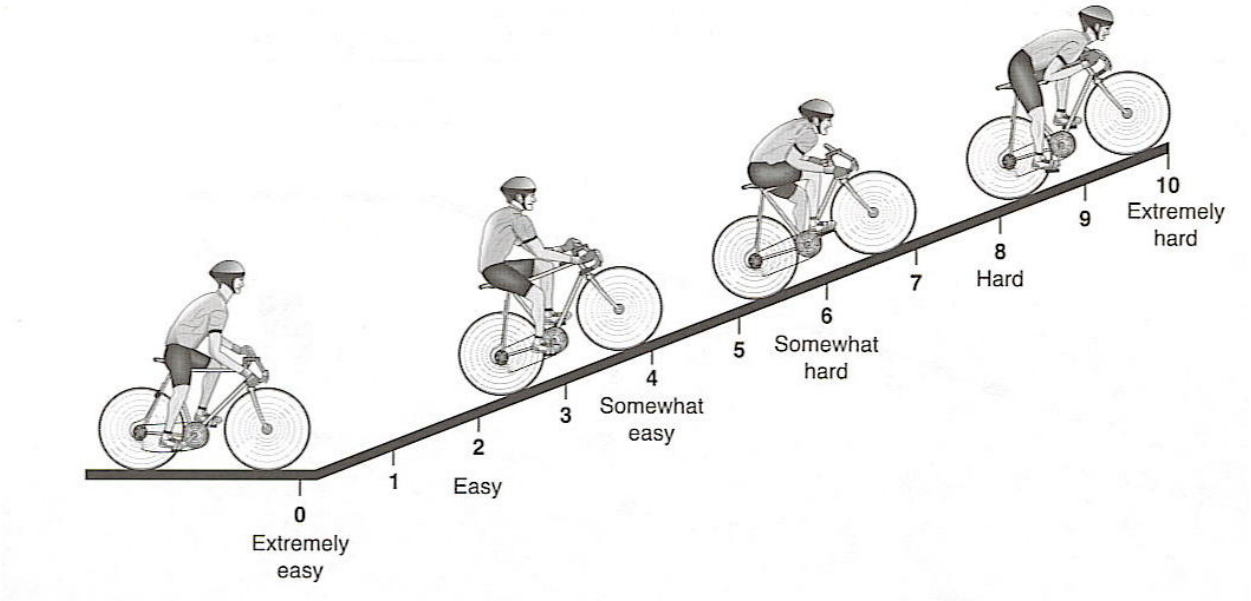

Figure 10. OMNI Scale of Perceived Exertion: Adult, Cycle FormatFrom Perceived Exertion for Practitioners, Robertson, 2004.

APPENDIX C

TUKEY POST-HOC TEST (GENDER X RPE)

A Tukey *post-hoc* analysis conducted on RPE revealed that workload 1 is significantly different from all other workloads (2-9, and 10 (immediately post-exercise)); workload 2 is significantly different from all workloads; workload 3 is significantly different from all workloads, excluding 4 and 8; workload 4 is significantly different from workloads 1, 2, and 10; workload 5 is significantly different from 1-3, and 10; workload 6 is significantly different from 1-3, and 10; workload 7 is significantly different from 1-3; workload 8 is significantly different from workloads 1 and 2; workload 9 is significantly different from 1-3; and workload ten, the RPE taken immediately post-exercise, is significantly different from workloads 1-6.

Table 5. Tukey post-hoc summary of RPE responses during graded cycle ergometer exercise in male and female subjects.

Stage	Stage	Mean Difference	Significance
2	1	1.55*	.000
	3	-1.70*	.000
	4	-2.68*	.000
	5	-3.89*	.000
	6	-4.22*	.000
	7	-4.63*	.000
	8	-3.85*	.000
	9	-5.19*	.000
	10	-5.81*	.000

3	1	3.25*	.000
	2	1.70*	.000
	4	-0.98*	.015
	5	-2.19*	.000
	6	-2.52*	.000
	7	-2.93*	.001
	8	-2.15*	.018
	9	-3.48*	.000
	10	-4.10*	.000
4	1	4.23*	.000
	2	2.68*	.000
	3	0.98*	.015
	5	-1.21*	.018
	6	-1.54*	.017
	7	-1.95*	.022
	8	-1.17	.200
	9	-2.51*	.024
	10	-3.13*	.000
5	1	5.44*	.000
	2	3.89*	.000
	3	2.19*	.000
	4	1.21*	.018
	6	-0.33	.637
	7	-0.74	.408
	8	0.04	.967
	9	-1.29	.256
	10	-1.92*	.000
6	1	5.77*	.000
	2	4.22*	.000
	3	2.52*	.000
	4	1.54*	.017
	5	0.33	.637
	7	-0.41	.676
	8	0.37	.719
	9	-0.96	.424
	10	-1.58*	.012
7	1	6.18*	.000
	2	4.63*	.000
	3	2.93*	.001
	4	1.95*	.022
	5	0.74	.408
	6	0.41	.676
	8	0.78	.507
	9	-0.56	.674
	10	-1.18	.159

8	1	5.40*	.000
	2	3.85*	.000
	3	2.15*	.018
	4	1.17	.200
	5	-0.04	.967
	6	-0.37	.719
	7	-0.78	.507
	9	-1.33	.330
	10	-1.95*	.032
9	1	6.73*	.000
	2	5.19*	.000
	3	3.48*	.002
	4	2.51*	.024
	5	1.29	.256
	6	0.96	.424
	7	0.56	.674
	8	1.33	.330
	10	-0.62	.569
10	1	7.35*	.000
	2	5.81*	.000
	3	4.10*	.000
	4	3.13*	.000
	5	1.92*	.000
	6	1.58*	.012
	7	1.18	.159
	8	1.95*	.032
	9	0.62	.569

*Significant $p < .05$

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