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HEMODYNAMIC AND METABOLIC RESPONSES DURING SELF-PACED AND RAMP GRADED EXERCISE TESTING TREADMILL PROTOCOLS

Nicholas Mark Beltz
University of New Mexico

Nicholas M. Beltz
Candidate

Health, Exercise and Sports Sciences
Department

This dissertation is approved, and it is acceptable in quality and form for publication:

Approved by the Dissertation Committee:

Len Kravitz, Chairperson

Fabiano Amorim

Ann Gibson

Christine Mermier

Lance Dalleck

Jeffrey Janot

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by

NICHOLAS M. BELTZ
Degrees
B.S. Kinesiology, Human Performance
University of Wisconsin-Eau Claire
2005-2010
M.S. Clinical Exercise Physiology
University of Wisconsin- La Crosse
2011-2012

DISSERTATION

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Hemodynamic and Metabolic Responses During Self-Paced and Ramp Graded Exercise Testing Treadmill Protocols

By

Nicholas M. Beltz

B.S., Kinesiology, Human Performance
University of Wisconsin-Eau Claire, 2010

M.S., Clinical Exercise Physiology
University of Wisconsin- La Crosse, 2012

Ph.D., Physical Education, Sports and Exercise Sciences
University of New Mexico, 2017

ABSTRACT

Purpose: To investigate: 1) if a self-paced (SP) graded exercise testing protocol elicits similar and reliable maximal oxygen uptake (VO_{2max}) responses compared to a ramp (RAMP) treadmill protocol; 2) the impact of SP on cardiac output (Q), stroke volume (SV), and arteriovenous oxygen difference (a-vO_{2diff}); and 3) the metabolic response during SP through blood lactate (BLa^-) accumulation and ventilatory threshold (VT) attainment. **Methods**: Sixteen recreationally trained men (23.7±3.0 yrs) completed two separate treadmill graded exercise testing protocols. SP consisted of five 2-min stages (10 min total) of increasing speed based on the Borg RPE_{6-20} scale. RAMP consisted of increases in speed by 0.16 km/hr every 15 s until volitional exhaustion. All tests were performed at 3% incline. VO_2 was measured via indirect calorimetry, hemodynamic function was measured via thoracic impedance, and BLa^- was measured via portable lactate analyzer. Differences between SP and RAMP protocols were analyzed as group means by using paired samples t-tests (R Core Team (2017))**. Results**: Maximal values for SP and RAMP were similar ($p>0.05$) for VO_{2max} (47.1±3.4 vs. 47.4±3.4 $mL•kg^{-1}•min^{-1}$), heart rate (198±5 vs. 200±6 $beat•min^{-1}$), ventilation (158.8±20.7 vs. 159.3±19.0 $L•min^{-1}$), Q (26.9±5.5 vs. 27.9±4.2 $L•min^{-1}$), SV (145.9±29.2 vs. 149.8±25.3 $mL•beat^{-1}$), a-vO_{2diff} (18.5±3.1 vs. 19.7±3.1 $mL•dL^{-1}$), VT (78.2 ± 7.2 vs. 79.0 ± 7.6% VO_{2max}), and peak BLa^- (11.7±2.3 vs. 11.5±2.4 $mM•L^{-1}$), respectively. **Conclusions**: SP elicits similar physiological responses in comparison to RAMP. These results support SP as a feasible GXT protocol. Electing to employ SP may benefit clinicians and researchers from a time-management perspective.

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SYMBOLS / ABBREVIATIONS

>: greater than

≤: less than or equal to

<: less than

±: plus or minus

~: approximately

a-vO_2: arteriovenous oxygen difference

ANOVA: analysis of variance

AT: anaerobic threshold

ATP: adenosine triphosphate

BLa^-: blood lactate

bpm: beats per minute

cm: centimeters

CO_2: carbon dioxide

CRF: cardiorespiratory fitness

CVD: cardiovascular disease

EDV: end-diastolic volume

ESV: end-systolic volume

GXT: graded exercise testing

hr: hour

HR: heart rate

ICG: impedance cardiography

kg: kilogram

km: kilometer

LV: left ventricle

M: moles

MET: metabolic equivalent to a task

min: minute

mmol: millimolar

mph: miles per hour

O_2: oxygen

Q: cardiac output

RER: respiratory exchange ratio

RPE: rating of perceived exertion

s: seconds

SD: standard deviation

SV: stroke volume

V_{max}: maximal velocity

VCO_2: carbon dioxide expiration

VE: ventilation

VO_2: oxygen consumption

W: watts

wk: week

WR: work rate

yrs: years

CHAPTER 1

Introduction

Since its inception in the pioneering work of Hill, Long, and Lupton (1923), the concept of maximal oxygen consumption (VO_{2max}) has been the most widely examined variable in the field of exercise physiology (Noakes, 2008). Typically, VO_2max is obtained by administering a graded exercise test (GXT) to examine the dynamic relationship between exercise workload and the integrated cardiovascular, pulmonary, musculoskeletal, and neurophysiological systems (Albouaini et al., 2007). Altogether, the relationship depicts the upper limits of systems to collectively deliver oxygenated blood to working skeletal muscle and the ability of skeletal muscle to utilize oxygen for work. Therefore, results obtained from a GXT are the basis for a large spectrum of applications, ranging from clinical exercise tolerance and appropriateness for surgery to research on the efficacy of aerobic training programs in athletic performance.

Historically, GXTs were administered in a discontinuous fashion (Taylor et al., 1955). That is, responses to exercise were evaluated by completing a series of single-stage exercise tests of incremental intensities on separate days. Taylor et al. (1955) were the first to demonstrate the reliability of such testing, noting that modality, fitness status, test duration, and intensity increment were all important considerations when attempting to accurately capture VO_{2max}. In an effort to highlight inconsistencies between continuous GXT protocols, Pollock et al. (1976) evaluated the ability of four popular treadmill protocols (Balke, Bruce, Ellestad, and Modified Astrand) to assess maximal cardiorespiratory responses. Although no differences were found between tests for VO_2max, the unique step-wise challenge applied by each protocol demonstrated the

variability in ventilatory and metabolic responses across protocols. Investigations by Whipp et al. (1981) and Davis et al. (1982) popularized the use of ramp protocols on electronically braked cycle ergometers. Given the workload was delivered in a linear rather than step fashion, observations examined the slope in VO_2-workload relationships and the optimal progression of work rates (Buchfuhrer et al., 1983; Zhang et al., 1991). Since then, there has been a trend in research toward establishing an optimal and standardized GXT protocol suitable for all fitness levels and testing goals.

Despite the widespread importance of measuring VO_{2max}, the methodology in which graded exercise tests are applied and even the conceptualization of VO_{2max} itself has created recent controversy (Mauger and Sculthorpe, 2012; Mauger et al., 2013; Chidnok et al., 2013; Levine, 2008). Hagerman (1984) originally reported confounding evidence, highlighting the limitations of the continuous GXT by demonstrating the ability of individuals to reach a greater VO_{2max} during simulated competition compared to laboratory-based methods. These findings suggested that an individual and his/her ability to self-modulate muscular power output on a momentary basis may serve as the ultimate variable in maximizing physiological responses during exercise. Therefore, strategies in self-pacing similar to those exhibited during athletic competition may be the limiting factor in performance during a GXT.

Noakes (2008) introduced a three-fold model concerning the appropriateness and utility of continuous GXTs to elicit VO_{2max}. First, the test is administered in an "open loop" rather than "closed loop" fashion, meaning that the participant is unaware of when the test will end. Second, the nature of the exercise itself is not representative of natural exercise because it is delivered in predetermined and fixed increments. Lastly, the

subjectivity of the test may limit reliability because the test only concludes upon the participant reaching volitional exhaustion.

These criticisms, coupled with the high inter-subject variability of the primary (VO_2 plateau) and secondary (rating of perceived exertion or 'RPE', blood lactate (BLa^-), respiratory exchange ratio, heart rate) criteria applied to verify the attainment of a 'true' VO_2max have led to the recent application of self-paced GXT protocols coupled with supramaximal verification bouts. Mauger and Sculthorpe (2012) developed a novel "closed loop" GXT design that does not rely on predetermined and fixed intensity increments by a test administrator, but rather allows the participant to self-select intensity throughout the bout via "RPE clamping." The test design consists of five 2-min stages, totaling exactly 10 minutes, at incremental intensities using Borg's Rating of Perceived Exertion (RPE_{6-20}) scale (Borg, 1982). Each 2-min stage is associated with an RPE value and its verbal cue; stage one is clamped at an RPE of 11 ("fairly light"), stage two at 13 ("somewhat hard"), three at 15 ("hard"), four at 17 ("very hard"), and five at 20 ("maximal"). Although the results of their study received considerable criticism surrounding research design issues, their results showed that untrained individuals were able to attain a greater VO_{2max} during a self-paced compared to traditional ramp GXT. In reference to Noakes' criticisms (2008), the self-paced testing delivered exercise in a "closed loop" fashion that simulated the demands of a more 'natural' form of exercise as evidenced by the final kick near the end of the test. Mauger and Sculthorpe (2012) attributed this finding to the self-modulation of power output and subsequent feedback to regulate fatigue, presenting a willingness to endure greater levels of discomfort with an approaching test termination point. Other studies either confirm (Mauger et al, 2013;

Astorino et al., 2015) or question (Chidnok et al., 2013; Hogg et al., 2015; Faulkner et al., 2015) the utility of the self-paced GXT model to elicit a higher VO_2max. However, the underlying mechanism(s) that explain any differences between protocols have yet to be fully elucidated. It has been reported that left ventricular (LV) volumes and twist mechanics limit performance during incremental exercise (Stohr et al., 2011). Furthermore, these limiting characteristics within the LV have a close relationship with cardiac output (Q) and stroke volume (SV) (Stohr et al., 2011). Most recently, Astorino et al. (2015) examined Q responses during self-paced and ramp GXT cycle protocols and found that the greater VO_{2max} during self-paced compared to ramp exercise was attributed to a greater Q. Currently, there remains a need to examine the components that limit Q (SV, filling volumes, contractility) during self-paced compared to traditionally applied GXT. Furthermore, additional examinations should seek to further describe the appropriateness of these protocols to elicit VO_{2max} and the underlying metabolic mechanisms (BLa^-, ventilatory threshold attainment) that relate to its attainment. Therefore, the aim of the current study is to compare the underlying hemodynamic and metabolic responses during self-paced and ramp treadmill GXT protocols.

Problem Statement

Previous research has briefly examined the utility of self-paced GXT protocols in eliciting VO_{2max}, yet there remains a need to further elucidate the underlying mechanisms that may support the utility of these protocols. Specifically, it has been shown that higher Q contributes to higher VO_{2max}; however, the limiting factors of Q (e.g., SV, diastolic filling, contractility) that explains the difference in Q between protocols is currently unknown. Furthermore, the self-modulation of power output may reflect differences in

metabolic thresholds (BLa^- and ventilation) that underscore performance during GXT protocols.

Purpose of the Study

The purpose of the current study was to determine the suitability of self-paced exercise and compare the hemodynamic (Q and SV) and metabolic (BLa^- and ventilatory threshold) responses between a self-paced and ramp treadmill GXT protocol.

Limitations

The following limitations were identified in this study:

1. The study sample consisted of healthy, active men aged of 18-45 years with 'Fair-Good' cardiorespiratory fitness. Therefore, the results of this study may not apply to females, individuals who are sedentary, have chronic disease, and are outside of the range for age and cardiorespiratory fitness.
2. All trials in the current study were performed on a treadmill. Therefore, the results of this study are not as applicable to other modes of exercise.

Assumptions

The following assumptions were identified in this study:

1. Prior to each visit, the participant did not perform any vigorous exercise for 24 hours, did not ingest caffeine or alcohol for 12 hours, consumed the same small meal 2-3 hours prior, and consumed enough water to maintain adequate hydration.
2. Each participant was familiar with the use of the RPE scale as a metric to self-regulate exercise intensity.

3. Each participant completed all GXT trials with maximal effort until volitional exhaustion.

Hypotheses

The following hypotheses were tested in this study:

Hypothesis 1: When examining VO_{2max} values on an individual basis, the self-paced protocol will elicit higher values compared to the ramp GXT protocol.

A self-paced exercise testing protocols has been shown to demonstrate higher VO_{2max} values using both treadmill and cycle ergometer (Mauger and Sculthorpe, 2012; Mauger et al., 2013; Astorino et al., 2015).

Hypothesis 2: The self-paced protocol will elicit a significantly ($p < 0.05$) higher Q compared to the ramp GXT protocol.

Self-paced exercise resulted in a higher maximal Q when compared to ramp exercise (Astorino et al., 2015)

Hypothesis 3: The self-paced exercise will not elicit a significantly ($p < 0.05$) higher maximal heart rate (HRmax) compared to the ramp GXT protocol.

Although self-paced exercise has resulted in a higher HRmax when compared to ramp exercise (Astorino et al., 2015; Hogg et al., 2015), most of the current literature suggests no difference in HRmax between self-paced and ramp exercise (Mauger and Sculthorpe; 2012; Mauger et al., 2013; Chidnock et al., 2013; Straub et al, 2014; Faulkner et al., 2015).

Hypothesis 4a: Stroke volume (SV) will reach a plateau during self-paced and ramp GXT.

To date, there has been no research on SV kinetics in response to self-paced GXT.

Hypothesis 4b: Stroke volume (SV) will reach a plateau at a higher submaximal intensity during self-paced compared to ramp GXT.

Stroke volume has been shown to frequently demonstrate a plateau during submaximal exercise in active individuals; however, a potential mechanism to explain the effectiveness of self-pacing could be the preservation of SV during maximal incremental exercise (Strickland et al., 2006; Stringer et al., 2005; Dufour et al., 2004; Zhou et al., 2001).

Hypothesis 5: The self-paced and ramp GXT will not differ in maximal BLa^- concentration.

It has been suggested that lower levels of BLa^- during exercise contribute to the lower levels of metabolic perturbations during self-paced exercise (Astorino et al., 2015); however, studies have shown no difference in maximal BLa^- concentration between protocols (Mauger et al., 2013; Chidnock et al., 2013; Faulkner et al., 2015; Straub et al., 2014).

Hypothesis 6: The self-paced and ramp GXT will not differ in the intensity ($\%VO_{2max}$) at which ventilatory thresholds are attained.

Although the self-selection of speed on the treadmill may lead to the ventilatory thresholds being reached at a later point during the test (DaSilva et al., 2011), there is currently no evidence to suggest that these thresholds will differ in the intensity at which they are reached.

Hypothesis 7: The self-paced and ramp GXT protocols will not differ in maximal arteriovenous oxygen difference ($a\text{-}vO_2$).

A previous study by Faulkner et al. (2015) suggests that the mechanism highlighting differences between self-paced and ramp exercise may be differences in a-vO_2; however, limited research has demonstrated no difference between the two protocol types (Astorino et al., 2015).

Scope of the Study

Sixteen healthy active males between the ages of 18 and 45 years with 'Fair-Good' cardiorespiratory fitness (40-79th percentile) completed three different GXTs to compare the hemodynamic (Q and SV) and metabolic (BLa^- and ventilatory threshold) responses between self-paced and ramp treadmill GXT protocols. To examine the influence of a learning effect, two of the trials were self-paced while the other was a speed-based traditional GXT. To fulfill these requirements, the initial trial was self-paced and the final two trials were completed in a randomized order. After the conclusion of each test, a supramaximal verification trial was performed to confirm the attainment of VO_{2max} within the accepted range of measurement error (3%). All hemodynamic measurements were collected continuously during exercise using impedance cardiography (ICG). Gas analysis was collected continuously using a metabolic cart, and BLa^- was analyzed before, immediately post-exercise, and 5-min post-exercise using a portable lactate analyzer.

Significance of the Study

This study compared the differences in hemodynamic and metabolic responses between a self-paced and speed-based traditional GXT protocol. Currently, there is a need to further investigate the suitability of self-paced exercise protocols to elicit VO_{2max}. In circumstances where a set duration is required to complete a test, any results

suggesting no differences between protocols indicates the potential use for self-paced protocols in clinical, research, or performance settings. Furthermore, recent evidence suggests a superior ability of self-paced exercise to elicit a greater Q response compared to ramp exercise; however, it remains unclear as to which components of Q contribute to such difference during speed-based treadmill GXT protocols. The results of this study will contribute to the growing understanding of the overall utility of self-paced GXT protocols and the physiological mechanisms that underpin any differences between self-paced and speed-based traditional GXT protocols

Definitions

Afterload: The threshold pressure which the left ventricle must reach before blood can be ejected from the left ventricle.

Arteriovenous oxygen difference (a-vO_2): The difference in oxygen saturation between arterial and venous blood representing oxygen extraction at the level of the tissue.

Blood lactate (Bla^-): A metabolic byproduct of glycolysis, indicative of exercise intensity.

Carbon dioxide production (VCO_2): The rate of carbon dioxide production as a result of metabolism.

Cardiac output (Q): The amount of blood ejected from the left ventricle per minute.

End-diastolic volume (EDV): The volume of blood in the left ventricle at the end of diastole.

Frank-Starling mechanism: The intrinsic mechanism within the myocardium by which an increase in stretch (from increased blood volume and pressure) results in an increase in left ventricular performance during systole.

Graded exercise test (GXT): An exercise test designed to increase in difficulty as they progress for examining maximal physiological responses and exercise tolerance.

Impedance cardiography (ICG): Noninvasive technology to measure the changes in electrical impedance across the thorax to assess hemodynamic variables.

Oxygen uptake (VO_2): The rate of oxygen consumption and utilization.

Preload: The amount of elastic tension stored within the cardiac myocytes because of increasing blood volume and pressure in the left ventricle.

Pulmonary ventilation (VE): The amount of air moved in and out of the lungs per minute.

Rating of perceived exertion (RPE): An affective measurement to indicate individual level of perceived exertion.

Respiratory exchange ratio (RER): The ratio of carbon dioxide production to oxygen consumption (VCO_2/VO_2), indicative of exercise intensity and substrate utilization.

Stroke volume (SV): The amount of blood ejected from the left ventricle per beat.

VO_2 plateau: A leveling or reduction in the rate of oxygen consumption despite the increase in work rate.

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CHAPTER 2

This chapter presents a review article, entitled “Graded exercise testing protocols for the determination of VO2max: Historical perspectives, progress, and future considerations” which has been published in the *Journal of Sports Medicine*. It is authored by Nicholas Beltz, Ann Gibson, Jeffrey Janot, Len Kravitz, Christine Mermier, and Lance Dalleck.

CHAPTER 2

Graded Exercise Testing Protocols for the Determination of VO_{2max}: Historical Perspectives, Progress, and Future Considerations

Nicholas M. Beltz[1], Ann L. Gibson[1], Jeffrey M. Janot[2], Len Kravitz[1], Christine M. Mermier[1], Lance C. Dalleck[3]

[1] *Department of Health, Exercise, and Sport Science, University of New Mexico, Albuquerque, NM*

[2]*Department of Kinesiology, University of Wisconsin-Eau Claire, Eau Claire-WI*

[3]*Recreation, Exercise & Sports Science Department, Western State Colorado University, Gunnison, CO*

Address for Correspondence: Nicholas M. Beltz; nbeltz@unm.edu

ABSTRACT

Graded exercise testing (GXT) is the most widely used assessment to examine the dynamic relationship between exercise and integrated physiological systems. The information from GXT can be applied across the spectrum of sport performance, occupational safety screening, research, and clinical diagnostics. The suitability of GXT to determine a valid maximal oxygen consumption (VO_2max) has been under investigation for decades. Although a set of recommended criteria exists to verify attainment of VO_2max, the methods that originally established these criteria have been scrutinized. Many studies do not apply identical criteria or fail to consider individual variability in physiological responses. As an alternative to using traditional criteria, recent research efforts have been directed toward using a supramaximal verification protocol performed after a GXT to confirm attainment of VO_2max. Furthermore, the emergence of self-paced protocols has provided a simple, yet reliable approach to designing and administering GXT. In order to develop a standardized GXT protocol, additional research should further examine the utility of self-paced protocols used in conjunction with verification protocols to elicit and confirm attainment of VO_2max.

1. Brief History of Graded Exercise Testing

The examination of the dynamic human physiological responses during incremental exercise has been an ever-evolving task for nearly 200 years. Beginning as early as the 18th century and continuing through the 19th century, pioneering physiologists such as Antoine Lavoisier and Nathan Zuntz have been credited with the first scientific examinations involving exercising humans under normal and hypoxic conditions. In 1918, Lambert described the use of a series of exercise tests to examine the impact on blood pressure to establish a reliable index of myocardial efficiency [1]. Inspired by Lambert and the foundational works of Francis Benedict, Goran Liljestrand, and August Krogh, British physiologist Archibald Vivian (A. V.) Hill conducted a fundamental series of experiments that remain the genesis of exercise physiology as an academic discipline [2]. Using Douglas bags to collect expired air samples, Haldane gas analyzers to determine fractional concentrations of oxygen and carbon dioxide, and a Tissot gasometer to measure air volumes, Hill and colleagues [3–6] repeated running trials of increasing speeds to plot the relationship between intensity and oxygen uptake (VO_2). Interestingly, it was concluded that a "ceiling" or upper limit in the maximal uptake of oxygen (VO_2max) existed [7]. It must be appreciated that a difference exists between VO_2peak and VO_2max and that these terms are often used interchangeably in the literature. That is, VO_2peak is the highest value attained during exercise and represents an individual's exercise tolerance while VO_2max represents the highest physiologically attainable value [8]. Interestingly, a VO_2max is always a peak but a VO_2peak is not always maximal. The difference between VO_2peak and VO_2max is often determined by the presence of a VO_2 "plateau," although the plateau depends on many protocol

variables. This review will further expound on this point. Hill's experiments and a century of observations before him led to the discovery of graded exercise testing (GXT), the gold standard for quantifying cardiorespiratory fitness (CRF) and measuring VO_2 during incremental to maximal exercise.

2. Applications of GXT

Graded exercise testing is used to observe the dynamic relationship between exercise workload and the integrated cardiovascular, pulmonary, musculoskeletal, and neuropsychological systems [9]. Protocols require a systematic and linear increase in exercise intensity over time until the individual is unable to maintain or tolerate the workload. Selected cardiovascular, pulmonary, and metabolic variables are collected during the test to evaluate exercise tolerance and represent the efficiency in which the cardiovascular system is able to deliver oxygenated blood to working skeletal muscle and the ability of muscle to utilize oxygen. Due to the widespread use of GXT in healthy populations, normative criteria have been established to help practitioners identify metabolic and ventilatory patterns. Moreover, these metabolic and ventilatory patterns may even assist in categorizing cardiovascular disease (CVD) states and prognoses [9].

The assessment of exercise tolerance has been used to establish the relationship between CRF, CVD, and all-cause mortality [10]. An early investigation by Blair et al. [10] examined the relationship between fitness and mortality in 32,421 men and women (20–80 years old) encompassing 264,978 living-years and 690 deaths. Their results were in agreement with a previous investigation [11] that found substantial strength and independence of low CRF as a predictor for all-cause mortality and future CVD. Similarly, a meta-analysis by Kodama et al. [12] showed that a 1-MET

(3.5 $mL \cdot kg^{-1} \cdot min^{-1}$) increase in VO_2max was associated with a 13% and 15% reduction in risk of all-cause mortality and CVD, respectively. Furthermore, a threshold to classify a substantially higher risk for all-cause mortality and CVD was established as low CRF (<7.9 METS) [12]. The evidence clearly demonstrates the influence of low CRF as an independent precursor to mortality and underpins the application of standardized protocols to monitor VO_2max in specific populations. These studies highlight the importance of accurate and reliable GXT results for minimal exercise tolerance and clinical evaluation of health status [10–12]. Furthermore, valid GXT results are relevant when interpreting studies using repeated measurements of VO_2max (and not VO_2peak) to establish a training effect or design exercise prescription.

Accurately capturing the dynamic physiological responses during GXT is essential to establish a valid VO_2max and to quantify CRF responses throughout various training interventions. Many independent factors contribute to the varying opinions surrounding the appropriateness of current standardized GXT guidelines, thereby limiting the ability to compare results between tests and population or apply them. The modes for administering GXT are traditionally limited to cycle and treadmill, each resulting in unique physiological responses. Protocol design variables such as stage length, workload increment per stage, and total test duration may individually limit the accuracy of GXT. Furthermore, the criteria used to confirm attainment of VO_2plateau are not consistent or universally applied among studies. This review will examine the limitations within the current recommendations for GXT and highlight the importance of the continuous search for identifying an optimal protocol.

3. Applications of the Fick Equation

The importance of seminal works by Hill et al. [4–6] is apparent considering that the fundamental basis for quantifying oxygen transport, utilization, and mitochondrial energy production remain the same today as they did nearly 100 years ago. The Fick equation states that VO_2 is equal to the product of cardiac output (Q) and the difference between arterial and venous oxygen content at the level of the capillary ($a\text{-}vO_{2diff}$).

$$VO2\ (mL \bullet kg^{-1} \bullet min^{-1}) = Q\ (L \bullet min^{-1}) \times (a\text{-}vO2diff)\ (mL \bullet L^{-1})$$

The equation can be expanded to represent as the product of heart rate (HR) and left ventricular (LV) stroke volume (SV), with SV being parsed into the difference between LV end-diastolic volume (EDV) and end-systolic volume (ESV).

$$VO2\ (mL \bullet kg^{-1} \bullet min^{-1}) =$$

$$HR\ (beats \bullet min^{-1})(EDV\ (mL \bullet beat^{-1}) - ESV\ (mL \bullet beat^{-1})) \times (a\text{-}vO2diff)\ (mL \bullet L^{-1})$$

Altogether, the components of Fick represent individual central (Q) and peripheral ($a\text{-}vO_{2diff}$) factors. The central component consists of factors that impact the diffusion of O_2 from the external environment into the arterial blood supply and transport of oxygenated blood to working skeletal muscle tissue. The peripheral component comprises various cellular and molecular mechanisms at the skeletal muscle level to diffuse $O_2$from arterial blood to the mitochondria for consumption in the process of ATP regeneration [13].

3.1. Implications of the VO_2max Protocol

It has been well established that increases at a linear rate similar to VO_2 upon the initiation of incremental to maximal exercise [14–18]. In response to metabolically

induced peripheral vasodilation in working skeletal muscle, blood pressure is maintained by increases in HR and SV [16]. Central medullary control of baroreceptors, chemoreceptors, and vascular tone contributes to the withdrawal of parasympathetic activity coupled with an increase in sympathetic drive. The result is an overall increase in chronotropic and inotropic characteristics of the heart.

Generally, HR increases linearly during incremental to maximal exercise; however, a breaking point (i.e., HR threshold) is eventually obtained after which the slope may increase or decrease until maximal heart rate (HRmax). The HR threshold is an individual phenomenon that may indicate chronotropic insufficiency [19]. Moreover, the significance of the flattened HR response following HR threshold, in particular, may be associated with a downregulation in beta-1 adrenergic receptor activation during greater exercise intensities. For instance, a study by Knight-Maloney et al. [19] examined HR responses during incremental to maximal exercise in 14 healthy individuals and found that eight subjects demonstrated a decelerated post-HR threshold response while six subjects showed an accelerated post-HR threshold response. These findings are in agreement with previous research that demonstrated the intersubject variability in HR responses [20–24].

Alongside the increasing inotropic response, shifts in sympathetic nervous system dominance raise chronotropic activity and influence central mechanical changes during incremental to maximal exercise. Neural drive enhances myocardial contractility, reducing ESV. Additionally, the intramuscular oscillations during exercise promote an increase in venous return to the heart. The improved blood flow to the LV enhances preload and promotes LV myocardial stretch, increasing elastic potential energy for

additional contractile force. This is known as the Frank-Starling mechanism [25]. The net effect is an increase in SV, contributing to an increase during incremental to maximal exercise. Contrary to traditional thought, SV within a healthy population may exhibit individual linear or plateau responses that are dependent on many factors.

Pioneering work by Astrand et al. [26] evaluating the SV response to incremental to maximal exercise established the widely accepted observation that SV plateaus are at approximately 40–50% of VO_2max [27, 28]. This finding was primarily attributed to tachycardiac limitations on diastolic filling time, therefore reducing EDV and blunting SV response [29]. More recently, a review by Vella and Robergs [30] underscores that the potential determinants of the intersubject variability in SV are more complex than originally established. Factors such as age, fitness level, and sex contribute to four main SV responses: the classic plateau, plateau with subsequent drop, plateau with subsequent rise, and gradual increase. While some studies have reported linear SV responses during incremental to maximal exercise in older individuals [31, 32] other studies showed that SV exhibited either a plateau or a subsequent drop at nonspecific points during incremental to maximal exercise [18, 33–35]. Although it may be logical to suggest that age has a negative impact on the maintenance of SV near maximal exercise due to reductions in myocardial compliance, the overall relationship between age and SV response remains unclear. Similarly, individual fitness level does not reliably predict the trend in SV during exercise. Some investigations showed a constant increase in SV up to maximal [36–39] and near maximal intensities [40] in trained individuals while others reported a plateau in both trained and untrained subjects [32, 41] and progressive increases until maximal exercise in untrained subjects [42–44]. One could postulate that

adaptations consequent to aerobic training enhance SV through combinations of the following: increased blood volume leading to greater EDV, increased LV chamber size, improved LV compliance, greater myocardial contractility, and reduced afterload may explain the individual ability to increase SV progressively through incremental to maximal exercise [30].

3.2. A-vO_{2diff} Implications of the VO_2max Protocol

It has also been accepted that an increase in was the sole component of maintaining VO_2 during the onset of exercise due to a potential lag between oxygen demand and venous return [45–47]. This was tested by Casaburi et al. [48], who found that pulmonary artery desaturation occurred as soon as four seconds after the onset of 150-Watt cycle exercise. The results reported by the Casaburi et al. [48] were questioned by De Cort et al. [45] and attributed to immobilized vena caval blood. Compared to Casaburi et al. [48], who measured a- upon exercise from rest, De Cort et al. [45] began measurements starting at the first increase in VO_2 after the abrupt increase in cycling intensity from a submaximal level. Although a-vO2diff may improve with aerobic training [49, 50], the cellular mechanisms contributing to oxygen extraction within skeletal muscle also increase at a predictable rate during incremental to maximal exercise. Recent meta-analyses by Montero et al. [51] and Montero and Diaz-Canestro [52] examined the effects of aerobic training on a-vO2diff in untrained or moderately trained healthy young (<40 years old), middle-aged, and/or older (≥40 years old) individuals. These studies concluded that the improvements in VO_2max from 5 to 52 weeks of endurance training were due to linear improvements in but not a-vO2diff.

Therefore, the peripheral mechanisms are viewed as a complement to the central mechanisms contributing to VO_2max.

4. VO_2max Protocol Critical Considerations

The foundational study by Taylor et al. [53] demonstrated that the sensitivity and reliability of physiological responses during GXT were limited by subject characteristics and GXT protocol design. In an attempt to investigate this issue, 115 healthy males (18–35 years) completed a wide range of GXT protocols under various conditions of physical stressors (caloric restriction, bed rest, temperature, and illness). It was the first comprehensive study to examine the sensitivity and reliability of VO_2max based on modality, fitness status, illness, environment, gas sampling rate, test duration, and speed/grade increments.

Since then, there has been a search for an optimal standardized protocol suitable for the entire spectrum of fitness abilities and testing goals. The two modalities commonly used in GXT are treadmill and cycle ergometry. While the treadmill appears to be the most widely used modality due to familiarity with upright locomotion and greater muscle mass utilization, cycling protocols present an opportunity to test individuals with coordination or orthopedic limitations. Furthermore, opting to use a cycle ergometer over treadmill may result in a more quantifiable workload (Watts) and provides an opportunity to use a progressive ramp protocol allowing for more reproducible outcomes [9]. However, VO_2max attained using treadmill protocols tend to produce up to 20% greater VO_2max values when compared to cycle protocols [54, 55]. This difference is attributed to a larger recruitment of exercising skeletal muscle mass,

and a-vO2diff, vascular conductance, and a lower rate of carbohydrate oxidation leading to a less severe development of metabolic acidosis at submaximal intensities [15, 56–60]. Realizing the need to investigate physiological responses to the earliest standardized GXT protocols, Pollock et al. [61] compared cardiopulmonary responses between four widely used treadmill testing protocols in 51 men (22 active, 29 sedentary): Balke [62], Bruce [63], Ellestad [64], and modified Astrand [65]. Each test differed in the method of increasing work rate in a step fashion (either speed or grade). The Balke protocol maintains a constant speed (3.3 mph) but increases grade by 1% each minute. The Bruce protocol increases speed and grade every 3 min. The Ellestad protocol increases speed each stage until the 10th minute upon introduction of a single increase in grade (to 5%) followed by subsequent increases in speed. Finally, the Astrand protocol maintains a constant running speed with increase in grade (2.5%) every 2 min. Pollock et al. [61] observed a similar VO_2max achieved between Balke, Bruce, Ellestad, and Astrand protocols (39.4, 40.0, 40.7, and 41.8 $mL \cdot kg^{-1} \cdot min^{-1}$, resp.) despite the difference in VO_2 plateau attainment (69%, 69%, 59%, and 80% of participants, resp.). Interestingly, this shows that the individual characteristics of similar protocols do not impact VO_2max but show inconsistencies in plateau. Their finding was one of the first to demonstrate the impact of protocol design characteristics on the attainment of VO_2max and a VO_2 plateau.

Early investigations by Whipp et al. [47] and Davis et al. [66] popularized the use of ramp protocols on electronically braked cycle ergometers. It was proposed that ramp cycle protocols would improve an individual's ability to reach VO_2max because the ramp increased work in a much more continuous fashion when compared to step increases in

work rate used in traditional treadmill protocols [67]. Since workload was deliverable in a linear fashion, attention turned to examining the slope of the VO_2-work rate ($\Delta VO_2/\Delta WR$) relationship. Buchfuhrer et al. [67] compared cycle tests of various changes in work rates (15 $W \cdot min^{-1}$, 30 $W \cdot min^{-1}$, and 60 $W \cdot min^{-1}$) and noted that ramping at an intermediate rate (30 $W \cdot min^{-1}$) produced the greatest VO_2max values; however, the work rate was dependent on fitness status. In a similar study, Zhang et al. [68] compared multiple work rates (15 $W \cdot min^{-1}$, 20 $W \cdot min^{-1}$, and 30 $W \cdot min^{-1}$) applied in continuous ramp versus step (1 min, 2 min, and 3 min) fashion. Interestingly, no differences were found in aerobic parameters (VO_2max, anaerobic threshold (AT), AT/VO_2max, and $\Delta VO_2/\Delta WR$) between any of the protocols. Furthermore, this study emphasized the importance of work rate increments independent of the stage length used. In contrast, Myers et al. [54] showed that ramp protocols (treadmill and cycle) represented a higher correlation between VO_2 and workload compared to step, thus reducing the error in predicting the metabolic cost at individual workloads. Muscat et al. [55] completed the most comprehensive investigation to date, comparing physiological responses (cardiometabolic function, gas exchange, breathing patterns, pulmonary function, and leg discomfort) between ramp treadmill and ramp cycle protocol matched for work increase (25 W/2 min) in 15 healthy young men. It was concluded that VO_2, VCO_2, respiratory exchange ratio (RER), HR, O_2 pulse, ventilation, and respiratory muscle effort (diaphragm) responses were greater at maximal and submaximal workloads for treadmill compared to cycle exercise; however, the responses in ventilatory equivalents and ventilatory thresholds were similar. Their results suggested that either

mode may be applied for purposes of evaluating mode-specific fitness and determining optimal training prescriptions when work rate increases are applied in a ramp fashion.

Similar to protocol mode (cycle versus treadmill), stage length, and work rate increments (ramp versus step), GXT protocol duration should be considered when comparing results. Buchfuhrer et al. [67] utilized 1-minute stage protocols to examine the impact of protocol duration on the achievement of VO_2max. Since VO_2max values were greater in protocols lasting between 8 and 17 min compared to tests outside these limits, the current duration recommendation of 8–12 min was established [67]. More recently, Yoon et al. [69] suggested that the Buchfuhrer et al. [67] study lacked appropriate statistical power and commented that overall test duration (5–14 min) may depend on age and training status [70–72]. Yoon et al. [69] compared VO_2max and incidence of VO_2 plateau across four protocols of different durations (5, 8, 12, and 16 min) using a cycle ramp protocol in moderate-to-highly trained individuals. They found that VO_2max was higher in men for the 8-minute protocol compared to 5-, 12-, and 16-minute protocols, while there was no difference in VO_2max in women. Further statistical analysis attributed this sex difference to lower fitness levels and perhaps more importantly the lower muscle mass (~45 kg in women versus ~67 kg in men) in women participating in the study. The impact of fitness level on test duration suggests that the steeper $\Delta VO_2/\Delta WR$ slope in shorter protocols may be disadvantageous to individuals of lower fitness. This could be due to increased reliance on nonmitochondrial energy systems, thus causing premature fatigue, as well as eliciting central cardiovascular limitations.

5. VO_2max Protocol Paradigm Shift

For the past 60 years the push to standardize GXT procedures has been essential to progress understanding of the complex and sensitive interaction between exercise and the integrated human physiological responses. In spite of the advancements that have given test administrators the ability to control fixed increments of intensities in an open-loop fashion (constant administrator testing variable manipulation without a fixed termination time), recent research has introduced an alternative approach to exercise protocols that allow the subject to self-pace the protocol in an incremental format [73–75]. In effect, this type of protocol would not negate past research that emphasized the role of the heart, lungs, circulatory, and other integrated systems in a limiting capacity but rather challenge the role of the brain as a potential simultaneous regulator. Although not entirely self-paced, studies by Pollock et al. [61, 76] used a model where speed was adjusted to accommodate individual movement efficiency and workload was increased by adding treadmill grade. In an effort to appreciate the evolution of self-paced protocols, the Pollock et al. [61, 76] investigations underscore the importance of identifying movement speeds that engage the greatest amount of muscle mass. Hagerman [77] was the first to report the ability of an individual to reach a greater VO_2 by self-pacing during a simulated competitive time trial compared to laboratory-based testing methods. The finding suggested that an individual's ability to self-regulate muscular power output may serve as the ultimate variable in maximizing physiologic responses. Intrigued by this finding, Foster et al. [78] compared the physiological responses between a self-paced laboratory 5-km cycle time trial and a GXT using a cycle ergometer. They found that maximal VO_2, HR, ventilation, and blood lactate (BLa^-) levels were significantly greater in the 5-km time trial compared to the cycle ergometer GXT. These initial findings raised

an important fundamental question regarding exercise testing protocols: if an individual possesses the ability to achieve a greater physiological ceiling when self-paced, does the search for achieving standardized protocol procedures serve a pragmatic purpose?

Eston and Thompson [73] used a closed-loop (no constant administrator manipulation and a fixed termination time) perceptually-regulated protocol guided by the 6–20 Rating of Perceived Exertion Scale (RPE) [79] to estimate maximal work rate in patients receiving β-blocker treatment. Using 4 × 3 min stages at an RPE of 9 ("very light"), 13 ("somewhat hard"), 15 ("hard"), and 17 ("very hard"), they reported that RPE could be used to predict maximal functional capacity. Similar RPE protocols using various stage lengths (2, 3, and 4 min) have been validated to accurately predict VO_2max [74, 80–83]. Using a similar perceptually regulated paradigm, Mauger and Sculthorpe [75] investigated a self-paced cycle exercise protocol in 16 untrained university students. The test design consisted of 5 × 2-min stages, totaling 10 min, at incremental intensities utilizing Borg's Rating of Perceived Exertion (RPE_{6-20}) Scale [79]. The protocol was designed as follows: stage one was clamped at an RPE of 11 ("fairly light"), stage two clamped at an RPE of 13 ("somewhat hard"), stage three clamped at an RPE of 15 ("hard"), stage four clamped at an RPE of 17 ("very hard"), and the final stage clamped at an RPE of 20 ("maximal exertion"). The unique aspect of the protocol was the additional stage of "maximal exertion" (RPE 20) to the established series of 2 min RPE-clamped stages as applied previously [80] in order to directly measure VO_2max. The participants achieved a significantly greater VO_2max (40 ± 10 versus 37 ± 8 $mL \cdot kg^{-1} \cdot min^{-1}$) and peak power output (273 ± 58 versus 238 ± 55 Watts) in the self-paced protocol compared to a traditional GXT despite the absence of significant

differences in HRmax, RERmax, VEmax, and mean power output. It is important to note that the results of Mauger and Sculthorpe [75] have received considerable criticism over methodology. Their results have been attributed to discrepancies in test duration between self-paced (10 ± 0 min) and traditional (13 ± 3 min) protocols and that direct GXT protocol comparison must require a match in total test duration [84, 85]. Interestingly, the closed-loop nature of the test elicited a motivation or "final push" during the final stage of the test similar to that expected toward the end of an athletic competition. Moreover, the results support the role of the brain during a closed-loop setting when the individual is able to vary work rate constantly, balancing discomfort with a maintainable power output and willingness to complete the test. The simplicity of the protocol design has produced many speculative explanations for the results. Mauger et al. [86] showed that a speed-based self-paced treadmill test elicited significantly greater VO_2max and HRmax values compared to those relative to a traditional test. This study also received criticisms over flawed methods and lack of control, attributing findings to using different modes (motorized versus nonmotorized treadmill) and neglect of measurement error to test their hypothesis [83, 87–89]. Follow-up studies have shown a higher VO_2max attainment during self-pacing using a cycle ramp protocol [90], similar VO_2max attainment using motorized treadmill [83, 91–93] and cycle protocols [84, 94], and a lower VO_2max attainment using an automated treadmill [89]. It is important to note that findings showing no difference between self-paced and traditional protocols demonstrate the potential utility for self-paced GXT protocols, particularly when considering protocol duration. While test duration is tightly controlled during self-paced testing and the incremental steps in oxygen cost between stages have been shown to fall within

recommended guidelines (1-2 METS) [95], physiological measurements that may distinguish self-paced from traditional protocols have yet to be adequately examined. It is purported that underlying variables that comprise the Fick equation, namely, and a-vO2diff, and the role of blood flow redistribution may underpin differences between self-paced and traditional protocols [92, 93]. More recently, Astorino et al. [90] showed that a self-paced cycle protocol elicited higher VO_2 compared to a ramp protocol (50.2 ± 9.6 versus 47.2 ± 10.1 $mL \cdot kg^{-1} \cdot min^{-1}$). Additionally, they were the first to compare central cardiovascular responses between protocols and showed a higher Q_{max} during self-paced compared to ramp (21.9 ± 3.7 versus 20.7 ± 3.4 $L \cdot min^{-1}$). It should be noted that their average test duration was not tightly controlled to 10 min (9.6 ± 0.8 min); therefore pacing was not restricted throughout the final 2-minute stage. Although initial results are intriguing, the investigation into the efficacy and suitability of self-paced protocols is in its infancy. Therefore, future researchers could choose to design studies to expound on the intertrial reliability using self-pacing protocols. Additionally, studies should examine the interaction between central, peripheral, and central regulating responses during self-paced exercise.

6. VO_2max Attainment Criteria

In order to increase the reliability and validity of a test, an undefined combination of standardized criteria must be met during the GXT including the following: VO_2 plateau, estimated HRmax, RER, BLa^{-}, and RPE. This widely accepted set of characteristics, or VO_2max criteria, has become a controversial topic of debate in recent years due to the high intersubject variability in attaining the criteria [96–99]. Furthermore, the number and type of criteria used to determine VO_2max are often

contingent on the preference of the researcher or clinician administering the test [96]. Along with protocol design, other factors such as metabolic data processing methods and participant effort make comparing the results for clinical or research purposes difficult [53, 99, 100].

7. Detection of a VO_2 Plateau

As with many of the principles used today in exercise physiology, the original reports of a slowing or "plateau" of oxygen consumption despite increasing muscular work can be attributed to Bassett Jr. and Howley [101]. Taylor et al. [53] later confirmed the existence of a VO_2 plateau in 9 of 13 men during a treadmill test of incremental speed (increasing 1 mph) and 108 of 115 (94%) men during treadmill tests of incremental grade (2.5%). Studies have since demonstrated that a VO_2 plateau can be detected in 17% to 100% of subjects tested, suggesting that its existence represents an inconsistent "phenomenon" [100]. Taylor et al. [53] were the first to apply the VO_2 plateau criterion of $\leq 150\ mL \cdot min^{-1}$, defined by a change in $VO_2 \leq 150\ mL \cdot min^{-1}$ despite a continuous increase in workload. This value, alongside the wide range of other values used as plateau criteria ($\leq 50\ mL \cdot min^{-1}$, $\leq 100\ mL \cdot min^{-1}$, $\leq 200\ mL \cdot min^{-1}$, and $\leq 280\ mL \cdot min^{-1}$), should not be applied universally since the criteria must reflect the expected rate of VO_2 increase per unit time relative to the protocol design [102]. Among the most important factors impacting the incidence of a VO_2 plateau are age [103], testing modality [104], and data analyses methodology [99, 105]. Astorino et al. [103] showed that the strongest predictor of VO_2 plateau among 30 men and women consisting of groups of endurance-trained (n=9), recreationally active (n=11), and strength trained (n=10) individuals was age, not training status, body composition, or training history. Gordon et al. [104] demonstrated

that treadmill testing (58%) was superior to cycle ergometer testing (8%) at eliciting a plateau. The diminished plateau in cycling was attributed to the increased metabolic cost of the eccentric skeletal muscle activity in treadmill running compared to the concentrically dominant cycle exercise. Furthermore, Astorino [105] confirmed that gas sampling rate impacted the incidence of a plateau. In this study, 13 sedentary, 48 recreationally active, and 47 competitive athletes completed a GXT using treadmill and cycle protocols. The group found that the incidence of plateau was greater using breath-by-breath (81%), 15-sec (91%), and 30-sec (89%) averaging compared to a longer sampling rate of 60-sec (59%). Collectively, these findings suggest that sampling rate in conjunction with the plateau threshold criteria may explain much of the discrepancy in incidence of VO_2 plateau across studies.

7.1. Heart Rate Response to the VO_2max Protocol

Due to its noninvasive nature, simplicity, and fairly predictable response to incremental to maximal exercise, HR is often used as a secondary criterion to VO_2 plateau. Much like plateau and other secondary criteria, the criteria for HR are highly variable. Typically, the threshold is established at a specific percentage using an age-predicted HRmax equation. Surprisingly, Fox et al. [106] created the 220-age equation by drawing an arbitrary best fit line from the observation of 10 studies [106]. Robergs and Landwehr [107] evaluated the Fox equation [106] and highlighted the fact that there were no statistical methods used to establish the regression equation from their data set. Instead, Fox and colleagues [106] summarize their methods by stating that “no single line will adequately represent the data on the apparent decline of HRmax with age. The formula, 220-age, defines a line not far from many data points.” Curious to

investigate the mystery of 220-age, Robergs and Landwehr [107] replicated the data set presented by Fox et al. [106], applied linear regression analysis, and found the equation to be 215.4–0.9147 (age) with a ±21 bpm error. Tanaka et al. [108] cross-validated an age-predicted HRmax equation by combining 351 studies (18,712 subjects) and a laboratory investigation using 514 subjects (18–81 years old). Their regression analysis established a new equation (208 − 0.7 × age) with a less, yet still substantial (±7–11 bpm), error range. It was concluded that the 220-age equation underestimates age-predicted HRmax in individuals over the age of 40 years. For these reasons, equations with less inherent error should be applied when using "±10 bpm of age-predicted HRmax" as a secondary VO_2max criterion.

7.2. RER Implication of the VO_2max Protocol

The RER is another secondary VO_2max criterion that is used to reflect the balance between bicarbonate buffering and hydrogen ion accumulation in the face of incremental exercise. Upon increasing metabolic acidosis, bicarbonate buffering leads to an increase in CO_2 production, increased ventilation, and a subsequent increase in RER. A series of studies by Issekutz et al. [109, 110] was the first to examine the relationship between RER and incremental exercise, VO_2max, and the metabolic state of the exercising human. Using a series of 4-5 min intermittent maximal exercise tests, they calculated the difference between CO_2 and the product of a metabolic respiratory quotient constant and VO_2 ($CO_2 - VO_2 \times 0.75$). This value was termed "excess CO_2" and used to reflect the change in substrate utilization and exercise intensity with the increase in VO_2 [109, 110]. Ultimately, they established the most widely used threshold for RER criteria (≥1.15) today. Much like HR and VO_2plateau criteria, a standard RER threshold value is not

consistently applied, using 1.0, 1.05, 1.08, 1.10, 1.12, 1.13, or 1.20 to verify VO_2max attainment [96, 97]. The high intersubject variability in RER responses due to inconsistent effort levels and training status makes higher RER values difficult to achieve for many individuals [97].

7.3. VO_2max and Blood Lactate Accumulation

Analysis of postexercise BLa^- compared to preexercise BLa^- has been used as a reliable marker to quantify exercise intensity. Hill et al. [4–6] were the first to establish a relationship between BLa^- and exercise intensity during vigorous to maximal exercise. Regardless of the debate for practical application or metabolic circumstances, it is agreed that BLa^- accumulation is related to an individual's ability to tolerate and sustain exercise; therefore, it is commonly used as a surrogate measure of the metabolic perturbations during maximal exercise and a secondary VO_2max criterion [111, 112]. The origins of the criterion date back to an investigation by Astrand [113] who used postexercise BLa^- concentrations to verify VO_2max in young (14–18 years) boys and girls. Despite the fact that only half of the subjects demonstrated a VO_2 plateau, it was noted that the individuals who exhibited a plateau had postexercise BLa^- concentrations between 7.9 and 8.4 mM (average $\geq$ 8 mM). It has since been determined that factors such as age, training status, sex, and overall effort may impact the level of BLa^-. This has led to the use of a $\geq$10 mM threshold or even a complete disregard for maximal BLa^-concentration due to the high intersubject variability (anywhere from 1.2 to 18 mM) in postexercise lactate [96, 98, 114].

7.4. RPE Assessment in a VO_2max Protocol

The simplest and most controversial measurement traditionally used as a VO_2max criterion was developed by Borg [79], known as Borg's Rating of Perceived Exertion (RPE). While RPE is not a direct measurement of physiological responses, the behavioral, motivational, and physical factors that an individual perceives during GXT contribute greatly to the overall validity of the test. Many studies have shown a strong relationship between RPE, HR, and VO_2 [82, 115–117]; however, others have demonstrated RPE to be less related to these variables in less active or sedentary individuals [118, 119]. Interestingly, Noakes [120] suggested that thresholds may not be as simple as limitations in central and peripheral components but rather controlled by a "central governor" that regulates self-pacing and overall effort throughout various points of a maximal exercise bout. Despite the relationship among RPE, HR, and BLa^-, inconsistencies in recent studies call to question the validity of RPE. Edvardsen et al. [114] examined the attainment of the commonly used RPE criterion of ≥17 in 840 individuals (20–85 years) and found that 84% of the subjects were able to achieve the criterion. Due to the variability and subjective nature of the criterion, Magnan et al. [121] examined the attainment of an RPE ≥18 in 240 inactive individuals (18–45 years) and found that 93.7% of the individuals reached the desired RPE threshold despite the fact that only 59% were demonstrating a VO_2 plateau. Overall, the assumptions in employing an RPE criterion depend on the subject's understanding of the scale and associated verbal descriptors, ability to differentiate between discomfort and physiological fatigue, and motivation.

8. Verification Protocols of VO_2max

Many of the current criteria used to determine VO_2max were established with technology that is no longer used today. Douglas bags and Tissot gasometers have been replaced by sophisticated metabolic analyzing systems and pneumotach and turbine flow measurement devices. Furthermore, the VO_2max criteria were developed using certain modalities (treadmill versus cycle) on relatively small samples of homogenous populations. These reasons and the overall variability of the criteria have resulted in some researchers rejecting secondary criteria altogether. Recent attempts have been made to establish new VO_2max attainment criteria through the introduction of a verification protocol [97, 122–126]. The origin of the verification protocol is believed to exist in a text by Thoden et al. [127], in which the first recommendation was made to include a bout of supramaximal exhaustive exercise (higher workload than achieved during GXT) following the completion of GXT. Since the inception of the verification protocol, there has been much effort to establish verification protocol intensity, duration, rest period after completing initial GXT, and the criteria used to verify VO_2max attainment. In follow-up guidelines, Thoden [128] recommended that the recovery phase between GXT and verification protocol should be between 5 and 15 minutes and a workload of one stage higher than the final completed stage during GXT should be used. Unfortunately, the recommendations by Thoden et al. [127, 128] were theoretical rather than research-based guidelines. The first study to directly examine the efficacy of a verification protocol was done by NiemelÄ et al. [129], who performed a verification protocol at a workload equal to the greatest workload achieved during initial GXT within a week of completing initial GXT. Using a ±5% repeatability range for VO_2max [130], they were able to confirm attainment in 8 of the 16 subjects. Day et al. [8] sought to observe the differences

between cycle GXT and a subsequent verification test in 38 healthy individuals (19–61 years) and reported no difference in VO_2max using a workload of ~90% W_{peak}. Follow-up studies commonly used verification workloads between 95 and 130% of peak workload achieved during initial cycle GXT or 0.5 1.6 $km \cdot hr^{-1}$ higher than the peak velocity achieved during initial treadmill GXT. The recovery time between tests ranged from the same day [122, 126, 131–134], 1–10 min active rest [122, 132, 133, 135], and 5–60 min passive rest [125, 135], to separate day testing [8, 123, 126]. Furthermore, tests were done on endurance-trained runners [122, 123], male athletes [135], recreationally active men and women [122, 125, 126], sedentary men and women [105], and middle-aged men and women [131]. Studies commonly revealed a nonsignificant mean difference in VO_2max between GXT and verification protocols when analyzed on an individual, rather than group mean, basis within the ±5% measurement error (accuracy of metabolic system when properly calibrated according to manufacturer) associated with VO_2 measurement [136–138]. Most recently, a study by Nolan et al. [125] investigated the impact of two verification intensities and rest periods. After an initial treadmill test, 12 active males and females completed each of the following four verification conditions: 105% maximal GXT workload, 20 min rest; 105% maximal GXT workload, 60 min rest; 115% maximal GXT workload, 20 min rest; and 115% maximal GXT workload, 60 min rest. Their results demonstrated a 100% success rate in verifying attainment of VO_2max when using 105% maximal GXT workload, regardless of the rest period between tests, and highlighted the current recommendation for intensity and rest period optimization during verification tests [125].

9. Error in VO_2max Measurement

The sophisticated metabolic systems used to collect and analyze data during exercise testing represent the most sensitive and reliable means for laboratory-based research. In order to compare results between studies, the data must be validated. Even if metabolic systems are appropriately maintained and calibrated, measurement error of ±5% is commonly accepted [136–138]. A study by Yule et al. [139] reported a 15% difference in VO_2max between three identical systems in the same laboratory. Furthermore, differences attributed to measurement error between 10 and 22% have been reported when comparing identical testing protocols using different metabolic systems [137]. A meta-analysis by Vickers [140] examined the test-retest reliability in maximal exercise testing and found that the average standard measurement error was 2.58 $mL \cdot kg^{-1} \cdot min^{-1}$. To account for total error, however, a source of biovariation reflecting the inherent biological fluctuations within an individual must also be considered [141]. Knowing that the underlying assumption for comparing VO_2max between and within studies is that biological variability must account for a portion of total error, Katch et al. [141] designed a study in which five participants completed an average of 16 maximal exercise tests over the course of a 2–4-week period. They found that, within the total error of ±5.6%, biological variability accounted for ~93% of the error while measurement error accounted for only ~7%. Meanwhile, recommendations by Balady et al. [138] report that the biological component of variability intrinsic to GXT is commonly accepted within 3-4%. Due to the numerous factors that contribute to biological variability and subsequent total error, an argument can be made against studies that consider only manufacturers' guidelines of measurement error when comparing tests [142]. This can be represented by a scenario comparing VO_2 responses to various

protocol designs, whereas an individual who falls within the accepted measurement error range between tests may have only done so based on the small contribution in variance from the system rather than the large contribution from biological variability due to heredity, homeostatic stress, training status, psychological stress, sleep, or nutrition [143]. For this reason, comparing attainment of VO_2max between protocols may be more reliable when considering total error (biological variability + measurement) rather than measurement error alone.

10. Conclusion

Due to the valuable information gathered and the wide spectrum of applications for the use of GXT, it has become an increasingly important objective to derive an optimal set of standardized procedures for the determination of VO_2max. Many years of observations examining the potential sources for individual variability in GXT responses are cited in the literature. Despite the pitfalls in physiological variability, standardized tests using traditional methods for VO_2max verification remain the most commonly employed. Furthermore, the methods in which these criteria have been previously established, as well as the comparison between studies evaluating the appropriateness of universal protocols, do not consider combined sources of inherent measurement and biological error. These reasons underpin the current suitability of more standardized GXT guidelines and subsequent methods for determining test validity. More recent approaches have highlighted alternative methods for measuring exercise capacity using a closed-loop, self-paced testing model. Future research directions should seek investigating perceptually regulated (RPE-clamped) protocols with verification protocols for the overall suitability and individualization of GXT.

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CHAPTER 3

This chapter presents a research manuscript, entitled “Hemodynamic and Metabolic Responses during Self-Paced and Ramp Graded Exercise Testing Protocols”. This manuscript is authored by Nicholas Beltz, Fabiano Amorim, Nathan Cole, Ann Gibson, Jeffrey Janot, Len Kravitz, Christine Mermier, Terence Moriarty, Tony Nunez, Sam Trigg, and Lance Dalleck. The manuscript follows the formatting and style guidelines of the journal *Medicine & Science in Sports & Exercise*. References are provided at the end of the chapter. Figures are provided after the references.

Hemodynamic and Metabolic Responses during Self-Paced and Ramp Graded Exercise Testing Treadmill Protocols.

Nicholas M. Beltz[1,2], Fabiano T. Amorim[1], Nathan Cole[1], Ann L. Gibson[1], Jeffrey M. Janot[2], Len Kravitz[1], Christine M. Mermier[1], Terence A. Moriarty[1], Tony P. Nunez[1], Sam Trigg[1], and Lance C. Dalleck[3].

[1] Department of Health, Exercise, and Sports Sciences, University of New Mexico, Albuquerque, NM

[2]Department of Kinesiology, University of Wisconsin-Eau Claire, Eau Claire-WI

[3]Recreation, Exercise & Sports Science Department, Western State Colorado University, Gunnison, CO

Running Title: Hemodynamic Response during Self-Paced Treadmill GXT

Address for Correspondence: Nicholas M. Beltz; nbeltz@unm.edu

Abstract

PURPOSE: Compare metabolic and hemodynamic responses between self-paced (SP) and ramp (RAMP) graded exercise testing (GXT) protocols. Given that SP is controlled for time while RAMP is not, similarities in physiological responses between protocols may support SP as a viable testing option from a time-management standpoint.

METHODS: Sixteen recreationally trained men (23.7±3.0 yrs) completed two separate treadmill GXT protocols. SP consisted of five 2-min stages (10 min total) of increasing speed based on the Borg RPE_{6-20} scale. RAMP increased speed by 0.16 km/hr every 15 s until volitional exhaustion. All testing was performed at 3% incline. Oxygen consumption (VO_2) was measured via indirect calorimetry; hemodynamic function was measured via thoracic impedance, and blood lactate (BLa^-) was measured via portable lactate analyzer. Differences between SP and RAMP protocols were analyzed as group means by using paired samples t-tests SPSS v. (R Core Team (2017))**. RESULTS**: Maximal values for SP and RAMP were similar ($p>0.05$) for VO_2 (47.1±3.4 vs. 47.4±3.4 $mL•kg^{-1}•min^{-1}$), heart rate (198±5 vs. 200±6 $beats•min^{-1}$), ventilation (158.8±20.7 vs. 159.3±19.0 $L•min^{-1}$), cardiac output (26.9±5.5 vs. 27.9±4.2 $L•min^{-1}$), stroke volume (145.9±29.2 vs. 149.8±25.3 $mL•beat^{-1}$), and arteriovenous oxygen difference (18.5±3.1 vs. 19.7±3.1 $mL•dL^{-1}$), and peak BLa^- (11.7±2.3 vs. 11.5±2.4 $mM•L^{-1}$), respectively. **CONCLUSION**: SP elicits similar physiological responses in comparison to RAMP. These results support SP as a feasible GXT protocol. Electing to employ SP may benefit clinicians and researchers from a time-management perspective.

Key Words: RPE-clamped, cardiac output, stroke volume, VO_{2max}

INTRODUCTION

Since its inception within the pioneering work of Hill, Long, and Lupton (14), the concept of maximal oxygen consumption (VO_{2max}) has been the most widely examined variable in the field of exercise physiology (26). Typically, VO_{2max} is obtained by administering a graded exercise test (GXT) to examine the dynamic relationship between exercise workload and the integrated cardiovascular, pulmonary, musculoskeletal, and neurophysiological systems (1). Altogether, the relationship depicts the upper limits of systems to collectively deliver oxygenated blood to working skeletal muscle and the ability of skeletal muscle to utilize oxygen for work. Therefore, results obtained from a GXT are the basis for a large spectrum of applications, ranging from clinical exercise tolerance and appropriateness for surgery to research on the efficacy of aerobic training programs in athletic performance.

Historically, GXTs were administered in a discontinuous fashion (34). That is, responses to exercise were evaluated by completing a series of single-stage exercise tests of incremental intensities on separate days. Taylor et al. (34) were the first to demonstrate the reliability of such testing, noting that modality, fitness status, test duration, and intensity increment were all important considerations when attempting to accurately assess VO_{2max}. In an effort to highlight inconsistencies between continuous GXT protocols, Pollock et al. (28) evaluated the ability of four popular treadmill protocols (Balke, Bruce, Ellestad, and Modified Astrand) to assess maximal cardiorespiratory responses. Although no differences were found between tests for VO_{2max}, the unique step-wise challenge applied by each protocol demonstrated the variability in ventilatory and metabolic responses across protocols. Investigations by Whipp et al. (37) and Davis et al.

(10) popularized the use of ramp protocols on electronically braked cycle ergometers. Given the workload was delivered in a linear rather than step fashion, observations examined the slope in VO_2-workload relationships and the optimal progression of work rates (6, 39). Since then, there has been a trend in research toward establishing an optimal and standardized GXT protocol suitable for all fitness levels and testing goals.

Despite the widespread importance of measuring VO_{2max}, the methodology in which graded exercise tests are applied and even the conceptualization of VO_{2max} itself has created recent controversy (7, 19-21). Hagerman (12) originally reported confounding evidence, highlighting the limitations of the continuous GXT by demonstrating the ability of individuals to reach a greater VO_{2max} during simulated competition compared to laboratory-based methods. These findings suggested that an individual and their ability to self-modulate muscular power output on a momentary basis may serve as the primary variable in maximizing physiological responses during exercise. Therefore, strategies in self-pacing similar to those exhibited during athletic competition may be the limiting factor in performance during a GXT. Subsequently, Noakes (26) introduced a three-fold model concerning the appropriateness and utility of continuous GXTs to elicit VO_{2max}. First, the test is administered in an “open loop” rather than “closed loop” fashion, meaning that the participant is unaware of when the test will end. Second, the nature of the exercise itself is not representative of natural exercise because it is delivered in predetermined and fixed increments. Lastly, the subjectivity of the test may limit reliability because the test only concludes upon the participant reaching volitional exhaustion.

These criticisms, coupled with the high inter-subject variability of the primary (VO_2 plateau) and secondary (rating of perceived exertion or 'RPE', blood lactate (BLa^-), respiratory exchange ratio, heart rate) criteria applied to verify the attainment of a "true" VO_{2max} have led to the recent application of self-paced GXT protocols coupled with supramaximal verification bouts. Mauger and Sculthorpe (21) developed a novel "closed loop" GXT design that does not rely on predetermined and fixed intensity increments by a test administrator, but rather allows the participant to self-select intensity throughout the bout via "RPE clamping". Their test design consists of five 2-min stages, totaling exactly 10 minutes at incremental intensities using Borg's Rating of Perceived Exertion (RPE_{6-20}) scale (Borg, 1982). Each 2-min stage is associated with an RPE value and its verbal cue, with stage one clamped at an RPE of 11 ("fairly light"), stage two at 13 ("somewhat hard"), three at 15 ("hard"), four at 17 ("very hard"), and five at 20 ("maximal"). Although the results of their study received widespread criticism surrounding research design, their results showed that untrained individuals were able to attain a greater VO_{2max} during a self-paced (SP) compared to traditional ramp GXT. In reference to Noakes' criticisms (26), the SP testing delivered exercise in a "closed loop" fashion that simulated the demands of a more natural form of exercise as evidenced by the final kick near the end of the test. Mauger and Sculthorpe (21) attributed this finding to the self-modulation of power output and subsequent feedback to regulate fatigue, presenting a willingness to endure greater levels of discomfort with an approaching test termination point. Other studies either confirm (2, 20) or question (7, 11, 13, 15, 31, 33) the utility of the SP GXT model to elicit greater VO_{2max}. However, the underlying mechanism(s) that may explain any differences between protocols have yet to be fully

elucidated. It has been reported that left ventricular (LV) volumes and twist mechanics limit performance during incremental exercise (32). Furthermore, these limiting characteristics within the LV have a close relationship with cardiac output (Q) and stroke volume (SV) (32). Recently, Astorino et al. (2) examined Q responses during SP and ramp GXT cycle protocols and found that the greater VO_{2max} during SP compared to ramp exercise was attributed to a greater Q. Currently, there remains a need to further examine Q and characterize SV kinetics in SP compared to traditional ramp treadmill GXT protocols. Furthermore, additional examinations should seek to describe the appropriateness of these protocols to elicit VO_{2max} and the underlying metabolic mechanisms (BLa^-, ventilatory threshold (VT) attainment) that relate to its attainment. Therefore, the aim of the current study was to compare the underlying hemodynamic and metabolic responses during SP and ramp treadmill GXT protocols.

METHODS

Sixteen males (age 23.6±3.0 years; height 175.2±5.7 cm; weight 81.5±11.0 kg; and body fat 17.1±5.0%) participated in the current study. All subjects were active (30 min/d, 3 d/wk, for at least 3 consecutive mo), had “Fair” to “Good” cardiorespiratory fitness based on age as categorized by the American College of Sports Medicine (22), and were between the ages of 18 and 45 years. As confirmed by the health-history questionnaire, all subjects were free of known cardiovascular, metabolic, respiratory, or renal disease, orthopedic limitations, and any other condition that would preclude safe maximal treadmill exercise. The study was approved by the University of New Mexico Institutional Review Board and all subjects provided written informed consent prior to participating in the study.

Experimental Overview

The study design included three separate visits to the laboratory over a 2- to 3-wk period. To avoid variation, all trials were performed under controlled environmental conditions and using the same equipment. Furthermore, trials were performed at the same time of day (± 2 hrs) and were separated by at least 48 hours but no more than seven days. Participants were told to refrain from vigorous exercise 24 hours, alcohol and caffeine 8 hours prior to all testing sessions. Volunteers were also instructed to consume the same small meal 2-3 hrs prior to each trial, arrive adequately hydrated, and maintain their current training routine throughout the duration of the study. During the first visit, participants were familiarized with the protocol and the Borg Rating of Perceived Exertion (RPE_{6-20}) Scale (5). A 5-min familiarization warmup was performed with the ramp (RAMP) protocol where each participant started at a walk and increased speed by 0.16 km/hr every 15 s. Participants then completed a SP graded exercise test on a treadmill followed by a verification bout of exercise to confirm the attainment of VO_{2max}. The verification bout has been reported in previous studies as a suitable alternative criterion compared to traditional secondary verification criteria to confirm attainment of VO_{2max} (2, 24, 27). Furthermore, the verification bout was employed following the completion of each GXT bout during the study. Following the completion of the first trial, screening was performed to determine further participation in the study as defined by the inclusion criteria of "Fair" to "Good" cardiorespiratory fitness. Prior to the second visit, the protocol order performed during the remaining two trials was randomized to prevent a learning effect. For example, if the subject performed SP on the second trial, they performed RAMP on the third trial and vice versa.

Exercise Testing

Each session began with the barefoot measurement of height (HR-200, Tanita Corporation of America, Inc., Arlington Heights, IL, USA), body weight (Model 884, Seca, Hamburg, Germany), resting blood pressure (Diagnostix 703, ADC, Hauppauge, NY, USA), and resting BLa^- (Lactate Plus, Nova Biomedical, Waltham, WA). Throughout the study, all blood lactate measurements were obtained in duplicate and averaged. During the first session, body density was estimated using standardized skinfold (Lange, Beta Technology, Ann Arbor, MI, USA) measurement procedures at the chest, abdomen, and thigh and then converted to body fat (16).

Prior to the GXT, subjects performed a 5-min warm-up at a self-directed pace on the motorized treadmill (c966, Precor, Woodinville, WA, USA). Upon completion of the warm-up, subjects then completed either SP or RAMP in the order previously described. Immediately following the initial GXT bout, the mouthpiece was removed and the subject rested quietly for 10 min. Blood lactate was collected immediately post-exercise and 5 min into the rest period. After the rest period, each subject completed a verification trial to confirm attainment of VO_{2max}. The verification trial began with the subject exercising at 30% max speed attained during the GXT (V_{max}) for 2 min, 40-45% V_{max} for 1 min, and 105% V_{max} until volitional exhaustion. The total time to exhaustion during the 105% V_{max} stage is typically 90-180 s (2). The attainment of VO_{2max} was confirmed if the VO_{2max} during the verification trial did not exceed the accepted measurement error (3%) of the ParvoMedics True One 2400 metabolic system (Sandy, UT, USA) (24). VO_{2max} was determined in all tests to be the mean of the two highest 15-s values during any 30-s

period of the test. Work rates were calculated for all protocols using the appropriate ACSM walking and running equations and converted to METs (22).

Self-Paced GXT

Prior to SP, each subject was familiarized with the RPE_{6-20} scale and to the self-paced nature of the maximal exercise test. The SP was a 10-min test comprised of five 2-min stages. Each stage corresponded to a value on the RPE_{6-20} scale and a verbal cue, where stage one is clamped at an RPE of 11 (“fairly light”), stage two at 13 (“somewhat hard”), three at 15 (“hard”), four at 17 (“very hard”), and five at 20 (“maximal”). Participants were instructed to modify their running speed on a moment-by-moment basis so that their effort reflected the desired RPE intensity and associated verbal cue. Running speed was adjusted by pressing the up or down arrow on the motorized treadmill, and the grade was kept at 3% throughout all exercise trials. Participants were blinded to the speed during exercise.

Ramp GXT

To determine the starting RAMP speed, the VO_{2max} attained during the familiarization session was multiplied by a factor of 1.15 based on the ~15% overestimation of the ACSM equation for the metabolic cost of treadmill running (18) and served as the 10-min endpoint goal. The endpoint workload was reduced by 0.16 km/hr every 15 sec until reaching the zero timepoint. The workload during RAMP was delivered in a continuous ramp fashion, where speed was increased by 0.16 km/hr every 15 sec until the subject reached volitional exhaustion. Identical to the SP, grade was maintained at 3% throughout all exercise trials. RPE_{6-20} was collected during the final 15 seconds of each stage.

Metabolic Gas Collection

During all exercise trials, metabolic gases were continuously measured using breath-by-breath sampling to obtain all respiratory variables (VO_2, expired carbon dioxide (VCO_2), pulmonary ventilation (V_E)). The metabolic cart was calibrated before each GXT trial in accordance with manufacturer guidelines.

Hemodynamic Variable Collection

Hemodynamic variables (Q, SV) were measured continuously throughout all exercise trials using impedance cardiography (ICG) (PhysioFlow Enduro, Manatec, Strasbourg, France). A detailed description of ICG and the underlying principles and assumptions can be found in previous literature by Richard et al. (29). Prior to exercise, six sites were prepared (two neck, sternum, ribcage, and two back) and electrodes were placed in accordance with manufacturer guidelines and connected to the PhysioFlow leads. Calibration was also performed before each exercise test with the subject in a standing position as recommended by manufacturer guidelines. Similar to the methods reported by Astorino et al. (2), all variables were averaged and recorded every 15 sec during exercise. Arteriovenous oxygen difference (a-vO_{2diff}) was determined by calculating the quotient of (VO_2/Q).

Data Processing

To obtain VT, all respiratory gas data (VO_2, VCO_2, V_E) were processed using an 11-breath averaging technique (30). VT was established using bisegmental piecewise linear regression where the breakpoint in the V_E-VCO_2 relationship (4) was the VCO_2 value with the least mean square error. To identify breakpoints in SV, data were analyzed

as 30-sec averaged values and then plotted against time. Breakpoints in the time-SV relationship were established using identical methods to VT breakpoints.

Statistical Analysis

All data are presented as mean ± SD and 95%CIs. Data were initially evaluated at the univariate level for conformance with the assumptions for *t-test* processing. A paired samples *t-test* was used to examine the differences in gas exchange and hemodynamic variables between protocols. A two-way (protocol x time point) repeated-measures ANOVA was used to identify differences in BLa^- across time points (pre, immediate-post, 5-min post) and differences in workload (METs) and oxygen consumption (VO_2) across stages between SP and RAMP protocols. A *post hoc* Bonferroni correction was applied when a significant difference was observed. Day-to-day variability of VO_{2max} was calculated between the familiarization and SP tests by taking the mean of the percentage change between trials, as absolute values, for each subject. A Bland-Altman 95% limits-of-agreement (LoA) was used to quantify the agreement (bias ± random error [1.96 x SD]) between the SP and RAMP VO_{2max} measurements. Linear regression analysis was used to examine the VO_2-time relationship for each protocol. Effect size was established using Cohen *d*, where 0.2, 0.5, and 0.8 represent a small, medium, and large effect, respectively (8) and η^2 where appropriate. Alpha was set *a priori* at $p < 0.05$ throughout all statistical analyses. Piecewise regression models were analyzed using the R Core Team (2017) statistical package. All other analyses were performed using the SPSS version 20 (IBM, Chicago, IL).

RESULTS

Table 1 highlights the individual rate of attainment for select criterion confirming VO_{2max}. Overall, criteria with the highest success rate for this sample were HR_{max} ± 10

bpm (32 of 32 total tests), RPE_{6-20} >19 (32 of 32), and verification VO_{2max} no more than 3% higher than GXT (32 of 32). A notable discrepancy was observed in the attainment of a VO_2 plateau ≤150 mL•min^{-1} during the last minute of exercise between SP (11 of 16) and RAMP (15 of 16). Verification VO_{2max} ±3% from GXT had the lowest rate of individual protocol (SP: 12 of 16); RAMP: 9 of 16) and total test total test (21 of 32) attainment.

INSERT TABLE 1 HERE

Differences in gas exchange, work rate, and metabolic data between protocols

The mean difference ± SD for dependent variables during SP and RAMP is shown in Table 2. Total test duration was not significantly different between SP (600 ± 0 s) and RAMP (606 ± 59 s) protocols; $p > 0.05$, 95% CI (-38 – 25), $d = .157$. The maximal speed (V_{max}) attained during the test was significantly higher in SP (14.5 ± 1.9 km•hr^{-1}) compared to RAMP (13.6 ± 0.8 km•hr^{-1}); $p < 0.05$, 95% CI (0.2 – 1.6), $d = .634$. The individual VO_{2max} differences between SP and RAMP for all 16 subjects is shown in Figure 1. VO_{2max} was mathematically higher in 11 of the 16 subjects during the RAMP compared to the SP protocol and the corresponding 95% LoA were 0.375 ± 2.783 mL•kg•min^{-1} (Figure 2). There were no differences revealed for relative VO_{2max} between SP (47.1 ± 3.4 mL•kg•min^{-1}) and RAMP (47.4 ± 3.4 mL•kg•min^{-1}) protocols; $p > 0.05$, 95% CI (-1.13 – 0.38), $d = .111$. Group data for VO_2-time relationship during SP and RAMP protocols are shown in Figure 3 and the workload-time relationship for each individual stage is highlighted in Figure 4. Linear regression showed strong linear VO_2-time relationships for SP ($VO_2 = 0.059$ (time) + 15.52; $r = 0.88$; adjusted $R^2 = 0.78$; $p < 0.05$) and RAMP ($VO_2 = 0.055$ (time) + 18.9; $r = 0.85$; adjusted $R^2 = 0.73$; $p < 0.05$).

Analysis revealed a significant stage x protocol interaction between SP and RAMP for workload ($p < 0.05$; $\eta^2 = .353$) and VO_2 ($p < 0.05$; $\eta^2 = .330$). There was no significant difference in VO_{2max} between the familiarization session SP (46.3 ± 3.2 mL•kg•min^{-1}) and the second SP and the day-to-day variability was calculated at 2.6%. No significant differences between SP (158.8 ± 20.7 L•min^{-1}) and RAMP (159.3 ± 19.0 L•min^{-1}) were observed for V_{Emax}; 95% CI (-3.23 – 2.25), *d* = .025. No significant difference was found in VT attainment between SP (78.2 ± 7.2%) and RAMP (79.0 ± 7.6%), represented as a percentage of VO_{2max}; $p > 0.05$, 95% CI (-5.7 – 4.2), *d* = .104. Additionally, there was no significant time x protocol interaction revealed between SP and RAMP for pre-test, immediate post-test, and 5-min post-test BLa^- (1.0 ± 0.3 vs. 1.0 ± 0.2 mM•L^{-1}; 10.3 ± 2.0 vs. 11.2 ± 2.3 mM•L^{-1}; and 11.2 ± 2.3 vs. 11.5 ± 2. mM•L^{-1}, respectively; $p > 0.05$, *d* = 1.03).

INSERT TABLE 2 HERE

INSERT FIGURE 1 HERE

INSERT FIGURE 2 HERE

Differences in hemodynamic responses between protocols

Maximal HR was generally higher during RAMP (200 ± 6 bpm) compared to SP (198 ± 5 bpm), although not statistically significant; $p > 0.05$, 95% CI (-5.7 – 4.2), *d* = .104. There was no difference in Q_{max} between SP (26.9 ± 5.4 L•min^{-1}) and RAMP (27.9 ± 4.2 L•min^{-1}); $p > 0.05$, 95% CI (-4.15 – 2.4), *d* = .219. Results also showed no difference between SP and RAMP protocols for SV_{max} and a-vO_{2max} (145.9 ± 29.2 vs. 149.8 ± 25.3 mL•beat^{-1} and 18.5 ± 3.1 vs. 19.7 ± 3.1 mL•dL^{-1}, respectively; $p > 0.05$).

Figure 5 shows the group SV-time relationship for SP and RAMP protocols as 30-sec averaged values. The SV response during SP demonstrated a breakpoint at a time-point corresponding to 78% VO_{2max}, in which the rate of change prior to the breakpoint (0.106 $mL \cdot s^{-1}$) was 2.9-fold greater than the change thereafter (0.036 $mL \cdot s^{-1}$). The SV-time relationship during RAMP showed a breakpoint at a time-point corresponding to 58% VO_{2max}, where the rate of change prior to the breakpoint (0.398 $mL \cdot s^{-1}$) was 19.3-fold greater than the rate of change thereafter (0.020 $mL \cdot s^{-1}$).

DISCUSSION

The current study sought to examine the suitability of a time-limited, perceptually-regulated GXT protocol by comparing differences in metabolic and hemodynamic responses between SP and RAMP. To our knowledge, this is the first study to characterize hemodynamic variables and SV kinetics associated with a SP on a treadmill. Furthermore, this study was designed to examine two speed-driven protocols that have been previously established as an effective means to elicit reliable VO_{2max} responses in high-fitness individuals (11, 15, 31); however, the suitability of a treadmill ramp protocol had yet to be determined for a homogenous group of individuals having a moderate fitness level.

The utility of SP in the current study was examined by comparing VO_{2max} values to those attained during RAMP; there was no significant difference between protocols. Previous studies have produced conflicting results, where SP has been shown to elicit higher (20), similar (11, 13, 15), or lower (31) VO_{2max} values during treadmill exercise. In an effort to reduce the need for manual control during SP, a recent study by Scheadler

and Devor (31) employed an automated treadmill to assess VO_2 responses during SP and modified-Astrand protocols in trained runners. The automated treadmill adjusted speed based on the location of the runner on the belt and corresponding distance from a sonar range finder located behind the treadmill. They reported a significantly lower VO_{2max} in SP (63.4 ± 7.8 mL•kg•min^{-1}) compared to the modified-Astrand (64.9 ± 8.2 mL•kg•min^{-1}). Unlike Scheadler and Devor (31), our study design contained a SP protocol during the initial familiarization session. The reason for the initial SP was two-fold: 1) to familiarize the recreationally-active but moderately-fit men to the effort required in maximal testing, and 2) to conceptualize the 'pacing strategy' during the test. Although the difference between the familiarization SP and RAMP were not significantly different, they trended toward a lower (P =.06) familiarization SP trial value. The highly-trained individuals in the Scheadler and Devor (31) study should have been familiar with 'pacing' but the lack of maximal SP during the familiarization session may have attributed to their findings. Moreover, the ingenuity of implementing a radar system could have shortcomings in the way of a processing lag between the sensing and feedback in the system, resulting in unwarranted oscillations in running speed.

Despite no difference for VO_{2max} in the current study, previous studies showing higher VO_{2max} in SP have not gone without criticism. Mauger and Sculthorpe (21) were the first to establish a higher (8%) VO_{2max} in cycle SP compared to traditional methods; however, follow-up studies (7, 33) have have established opposing results and attribute their findings to a difference in total test duration (10 ± 0 min in SP vs. 13 ± 3 min). Previous literature suggests that total test duration may impact peak physiological responses and, therefore, must be considered when directly comparing protocols (6, 38).

The current study was designed to control for test duration, resulting in analogous total test time between SP (10.0 ± 0 min) and RAMP (10.1 ± 1 min). Moreover, all 16 participants completed RAMP within the recommended duration of 8-12 minutes suggesting that any differences between protocols were physiological rather than methodological. Mauger et al. (20) completed the only study to show higher VO_{2max} utilizing a treadmill design, but have been criticized for their methods in comparing SP on a nonmotorized treadmill and traditional GXT on a motorized treadmill. Protocol design was a unique feature of this study knowing that individuals are given full autonomy to change speed at any point throughout the SP test. During pilot testing, individuals regularly adjusted speed in 15 s-1 min increments during SP. Therefore, our design applied more frequent and smaller increments in work rate (0.16 $km•h^{-1}$ every 15 s) during traditional GXT than protocols used in studies by Hanson et al. (13) (3-min stages), Scheadler and Devor (31)(2-min stages), Faulkner et al. (11) (1 $km•h^{-1}$ every 2 min), and Hogg et al. (15) (1 $km•h^{-1}$ every 2min). Work rate delivered in a ramp fashion allows for comparison to studies utilizing cycle ergometers.

INSERT FIGURES 3 and 4 HERE

The VO_2 and work rate responses in Figures 2 and 3 are a visual representation of the pacing strategy involved during SP exercise. As expected, both work rate (calculated by speed) and the measured VO_2 were lower ($p < 0.05$) in SP throughout each of the first three stages of the test. The significantly higher change in work rate ($p < 0.05$) from stages 2 to 3 is reflected in a lag in O_2 kinetics and the higher change in VO_2 from stages 3 to 4 until the hallmark characteristic of SP, the ‘kick’ or end-spurt that highlights their likeness to a competitive scenario. Maximal velocity (Vmax) attained during SP was

higher ($p < 0.05$) compared to RAMP (14.5 ± 1.9 vs. 13.6 ± 0.8 km•hr^{-1}) despite no differences in VO_{2max}. This finding aligns with those reporting higher Vmax attainment during SP compared to traditional treadmill protocols (15, 31). It has been hypothesized that the lower work rates during SP mitigate the disturbance within the metabolic milieu by modulating power output from select motor units and allowing an individual to access more type II fibers during the final stage, thus demonstrating a final burst of exertion (2, 31). Although the current study did not measure motor unit activation, differences in central motor command and motor unit dominance were related to key metabolic markers (VT and BLa^-). Accordingly, there is potential that a proposed 'central governor' model may tightly modulate work rate during SP via peripheral tissue afferent feedback and reflect a delayed onset of peripheral fatigue and such metabolic disturbances (muscle metabolite accumulation, VT) resulting in a greater activation of anaerobic fibers during the maximal stage (25). Our results do not support this hypothesis as no differences were observed between SP and RAMP for VT or peak BLa^- (see Table 2).

Explanations for varying physiological responses between protocols have often been purported to involve underlying mechanisms within blood flow and oxygen extraction. Astorino et al. (2) recently investigated the role of maximal oxygen transport and extraction as a means to augment the VO_2 response during cycle SP and RAMP across individuals of different fitness status. Their findings suggest that the higher VO_{2max} in SP (50.2 ± 9.6 mL•kg•min^{-1}) compared to RAMP (47.2 ± 10.2 mL•kg•min^{-1}) was enhanced by higher HR and Q responses during the SP exercise. The hemodynamic results of the present study are not in alignment with those reported by Astorino et al. (2) and may be explained by our initial finding of no difference in VO_{2max}. Interestingly, the

participants in the current study exhibit VO_{2max} values similar to those in the moderate fitness group in Astorino et al. (2); however, Q and a-vO_{2diff} in the current study more closely resemble those in the high fitness group in Astorino et al. (2). The difference in testing modalities between study design may explain this contrast in the VO_2, Q, and a-vO_2 relationship where treadmill exercise places a greater demand on blood flow and extraction than cycling. Original studies investigating SP trials suggest that differences in VO_{2max} may be explained by differences in peripheral oxygen extraction (15, 20). Similarity of VO_{2max} values found between protocols in this study is supported by no difference in blood delivery and extraction. It is worthwhile to note that the similarities in SV_{max} support the findings by Astorino et al. (2), but their study was not intended to describe and characterize differences in SV kinetics throughout SP and RAMP. Therefore, the SV responses described in this study may provide useful insight regarding pacing and components of central blood flow regulation. It has been accepted that SV exhibits a plateau ~40-50% VO_{2max} in response to the decrease in diastolic filling time throughout incremental exercise. A review by Vella and Robergs (35) suggests that SV kinetics can be characterized as having one of four responses to incremental exercise: 1) progressive increase; 2) plateau with secondary increase; 3) plateau, or 4) plateau with drop. Fitness level is believed to have the greatest influence on SV response but mode, age, and sex may all play important roles. Figure 5 depicts the SV response during SP and RAMP. It appears that protocol design may influence SV behavior at submaximal exercise intensities. Stroke volume remains lower in SP at submaximal intensities, reflected in the lower work rates and VO_2, but observation of the individual slopes show a plateau occurring at a time-point relating to 58% and 78% VO_{2max} in RAMP and SP,

respectively. Furthermore, the overall SV response during RAMP may be characterized as a plateau while SP exhibits a final 'kick' in SV (plateau with secondary increase), evidenced by a greater change in work rate during the final stage in SP but not VO_{2max}. In this case, we would likely not observe a lag in VO_2 kinetics and subsequently greater VO_{2max} values had the test continued for an additional stage. This can be reasoned with a degree of confidence given that each individual completed a supramaximal test after SP and none of the 16 subjects exhibited VO_{2max} values greater than the measurement error of the metabolic cart (3%). Therefore, pacing during SP may not be as modulated by peripheral metabolic disturbances to the degree that is a conservation and regulation of blood flow.

INSERT FIGURE 5 HERE

A discrepancy between achieving *significant* and *practical* difference should be considered when evaluating VO_{2max} as group means rather than on an individual basis. The expected range of variation owed to measurement error of a metabolic system is typically 3-4% (3) between tests. The 2.6% day-to-day variability in SP found in the current study is similar to that reported by Scheadler and Devor (31) (2.3%) and Straub et al. (33) (3%), but the concept of total error (measurement + biological variability) cannot be ignored when comparing separate-day tests. Katch et al. (17) found that the expected total error of separate day tests is ±5.6%, with 93% of the total error accounted for in biological variability and 7% in measurement error. This means that although statistically different, any two tests within 5.6% can be considered similar while tests outside of that range are meaningfully different. Only one of 16 subjects in the current study was outside the acceptable range of total error (5.7%), which supports the use of SP as a valid testing

protocol.

This study is not without its limitations. First, even though the two SP tests were not statistically different, there was a degree of novelty for the moderately-trained individuals to complete a test in which they needed to pace themselves. Although they were familiarized with the RPE_{6-20} scale, their strategies changed drastically between the familiarization SP and second SP in that their approach during the second test represented a much more gradual and ramp-like pace. For this reason, the reliability of underlying data other than VO_{2max} collected during SP may rely on at least one familiarization session, and multiple familiarization sessions may be advantageous for lesser-trained individuals. Second, the criteria to determine VO_{2max} has yet to reach universal application. The current study used the criteria recommended by Midgley et al. (24) and was verified in all 32 tests used in analyses; however, two participants needed to repeat trials due to unmet criteria. If more strict criteria were applied such as $HR_{max} \leq 4$ bpm (23) or verification and GXT $VO_{2max} \pm 3\%$ (9, 36) then our participants would not have initially met criteria. Studies should aim to detail verification criteria and individual success rates so that future studies may know which criteria apply to their specific study population. Finally, although none of the participants reported treadmill speed as a limiting factor during testing, performing the tests at slightly higher inclines may have moderated this limitation and resulted in different VO_{2max} responses.

CONCLUSION

This study is the first to compare hemodynamic responses during SP and RAMP treadmill graded exercise testing protocols. Specifically, there were no differences in

maximal Q, SV, or a-vO_{2diff} between protocols. Moreover, there were no differences in VO_{2max} values between protocols when analyzed as group means as well as when considering total error (biological + measurement) on an individual basis. The findings in the current study showing no difference in VO_{2max} attainment, maximal hemodynamic response, or maximal BLa^- may seem unfavorable; however, the authors view them as the contrary. These results further support the application of SP as a valid treadmill GXT protocol and demonstrated a difference in SV response between SP and RAMP. The time-regulated and open-loop protocol design of SP may mitigate error associated with unsatisfactory protocol duration. Future research should continue to evaluate the utility of SP on various populations (age, sex, fitness status, disease state) and even examine the responses during SP protocols of various stage length, test duration, and RPE assignments.

ACKNOWLEDGEMENTS

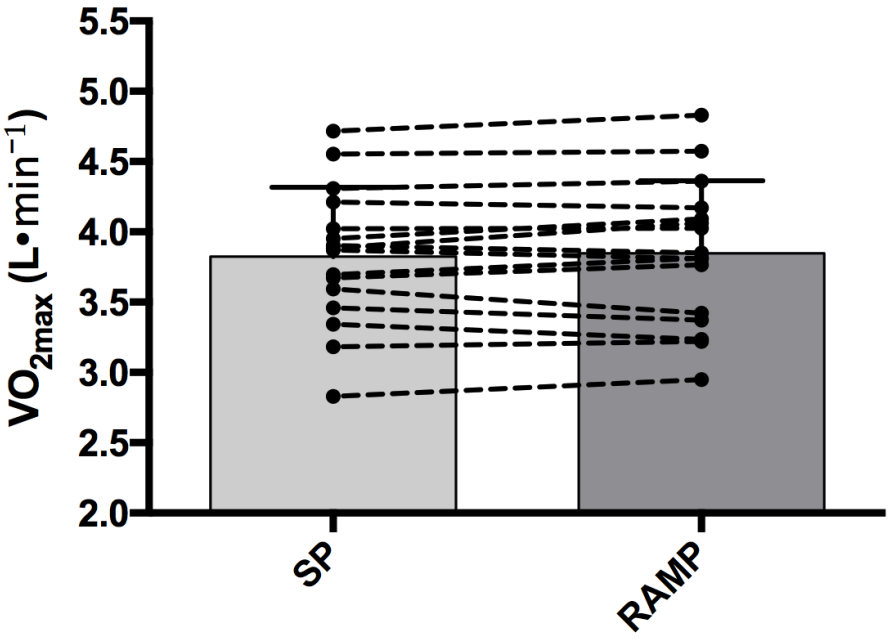

Figure 1. Comparison of mean absolute maximal oxygen uptake (VO_{2max}) values for self-paced (SP) and ramp (RAMP) in all 16 subjects. Individual data are represented by the dotted line graph. Statistical analysis revealed no difference in VO_{2max} between SP and RAMP protocols ($P < 0.05$).

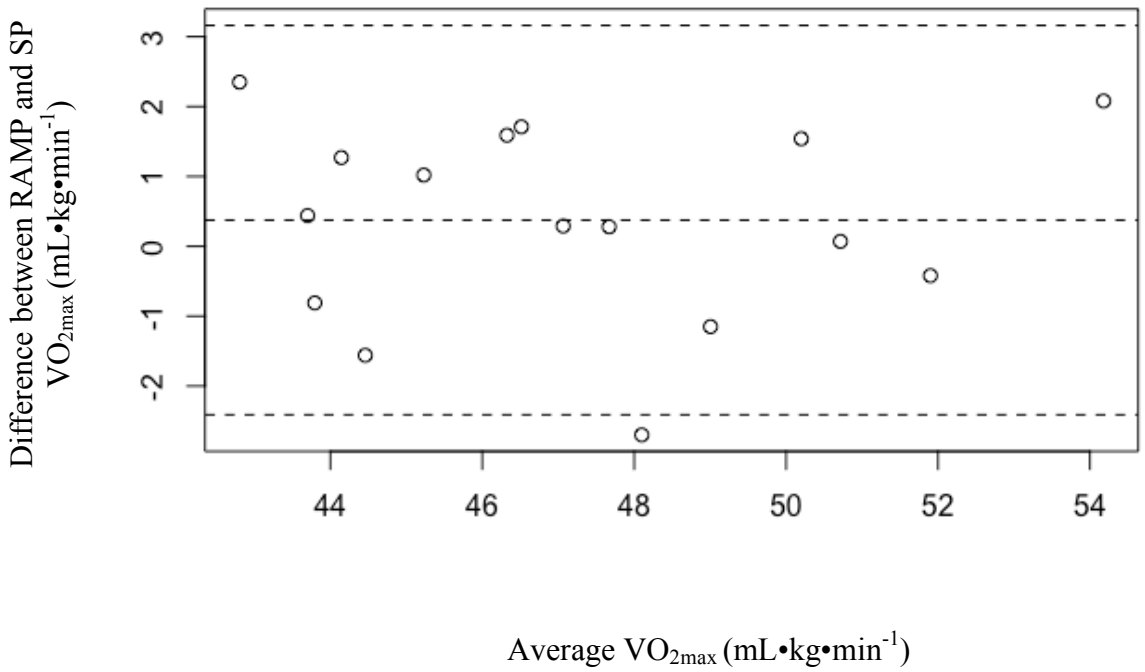

Figure 2. Narrowest 95% limits of agreement (bias ± [1.96 x SD_{diff}], mL•kg•min^{-1}) between ramp and self-paced protocols.

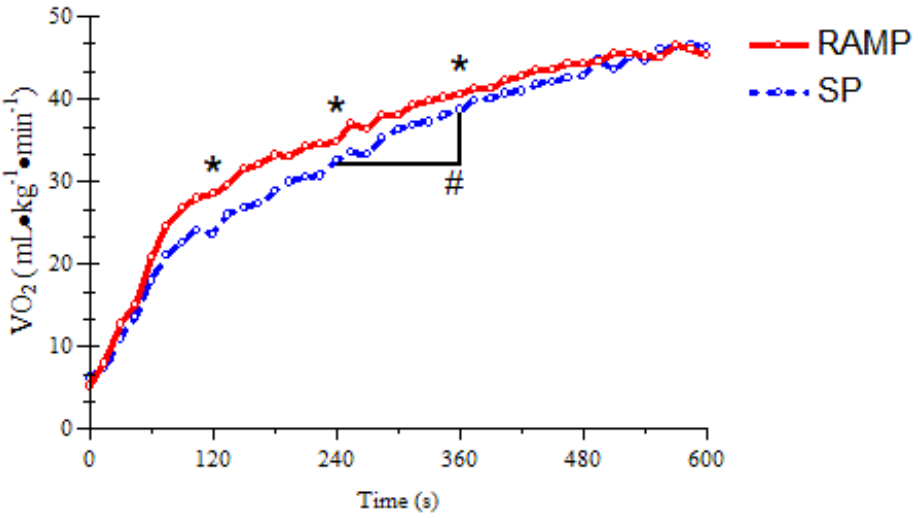

Figure 3. Group mean oxygen uptake (VO_2) data for self-paced (SP) and ramp (RAMP) throughout the 10-min test duration.
*-significant difference ($P < 0.05$) at time point between protocols.
#-significant difference ($P < 0.05$) in within-stage change between protocols.

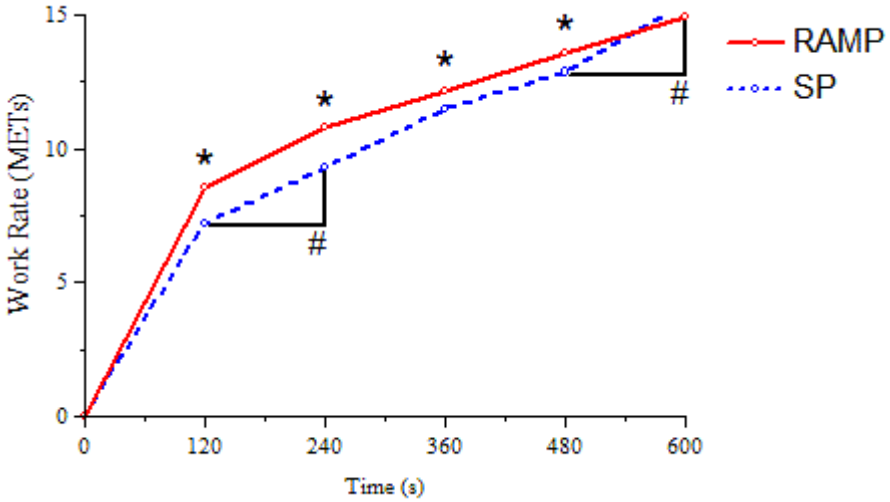

Figure 4. Group mean work rate (METs) data for self-paced (SP) and ramp (RAMP) throughout the 10-min test duration.
*-significant difference ($P < 0.05$) at time point between protocols.
#-significant difference ($P < 0.05$) in within-stage change between protocols.

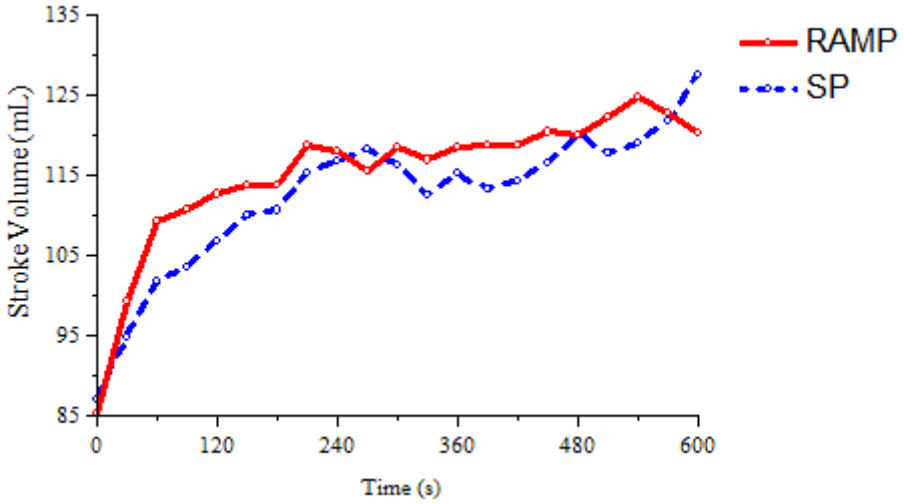

Figure 5. Group stroke volume responses over time for ramp (RAMP) and self-paced (SP) graded exercise testing protocols. Plateau was identified using bisegmental linear regression. Plateau for RAMP occurred at ~90s and 58% VO_{2max}, where the rate of change prior to the breakpoint (0.398 $mL \cdot s^{-1}$) was 19.3-fold greater than the rate of change thereafter (0.020 $mL \cdot s^{-1}$). Plateau for SP occurred at ~300s and 78% VO_{2max}, where the rate of change prior to the breakpoint (0.106 $mL \cdot s^{-1}$) was 2.9-fold greater than the change thereafter (0.036 $mL \cdot s^{-1}$).

Table 1. Results of individual VO_{2max} attainment criteria for all tests.

Criterion	SP	RAMP	Total (%)
VO_2plateau ($\leq$150 mL·min^{-1})	11/16	15/16	81.3
HR (± 10 bpm)	16/16	16/16	100.0
RER ($\geq$1.15)	13/16	14/16	84.4
BLa^- (>8mmol)	15/16	15/16	93.8
RPE (>19)	16/16	16/16	100.0
Verification ±3% from GXT	12/16	9/16	65.6
Verification no more than 3% higher than GXT	16/16	16/16	100.0

Table 2. Differences in gas exchange, hemodynamic, and metabolic parameters between protocols (mean ± SD).

Variable	SP	RAMP	95%CI	p-value	*d*
VO_{2max} ($mL \cdot kg \cdot min^{-1}$)	47.1 ± 3.4	47.4 ± 3.4	(-1.13 - 0.38)	.308	.111
VO_{2max} ($L \cdot min^{-1}$)	3.83 ± 0.5	3.85 ± 0.5	(-0.08 - 0.03)	.398	.044
HR_{max} (bpm)	198 ± 5	200 ± 6	(-3.4 - 0.1)	.065	.297
V_{Emax} ($L \cdot min^{-1}$)	158.8 ± 20.7	159.3 ± 19.0	(-3.23 - 2.25)	.71	.025
VT (%VO_{2max})	78.2 ± 7.2	79.0 ± 7.6	(-5.7 - 4.2)	.746	.104
Q_{max} ($L \cdot min^{-1}$)	26.9 ± 5.4	27.9 ± 4.2	(-4.15 - 2.04)	.479	.219
SV_{max} ($mL \cdot beat^{-1}$)	145.9 ± 29.2	149.8 ± 25.3	(-19.9 - 12.3)	.62	.140
a-vO_{2max} ($mL \cdot dL^{-1}$)	18.5 ± 3.1	19.7 ± 3.1	(-3.4 - 0.9)	.24	.399
Time (s)	600 ± 0	606 ± 59	(-38 - 25)	.664	.157
V_{max} ($km \cdot hr^{-1}$)	14.5 ± 1.9*	13.6 ± 0.8	(0.2 - 1.6)	.015	.634
BLa^- ($mM \cdot L^{-1}$)				0.081	
Pre	1.0 ± 0.3	1.0 ± 0.2	(-0.1 – 0.2)		
IPE	10.3 ± 2.0**	11.2 ± 2.3**	(-1.9 – 0.2)		
5 min	11.2 ± 2.3***	11.5 ± 2.4	(-0.6 – 1.0)		

VO_{2max}: maximal oxygen consumption; HR_{max}: maximal heart rate; V_{Emax}: maximal ventilation; VT: ventilatory threshold; Q_{max}: maximal cardiac output; SV_{max}: maximal stroke volume; a-vO_{2max}: maximal arteriovenous oxygen difference; V_{max}: maximal velocity; BLa^-: blood lactate concentration; IPE: immediately post exercise

*-significant difference ($P < 0.05$) between SP and RAMP

**-significant ($P < 0.05$) change from baseline

***-significant difference ($P < 0.05$) from IPE

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CHAPTER 4

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This research team was the first to examine and compare the hemodynamic responses to self-paced GXT protocols and ramp treadmill protocols performed on a treadmill in a homogenous group of young, moderately-trained males. Specifically, we investigated: 1) the overall utility of SP by comparing attainment of VO_{2max} to that of RAMP, 2) differences between central (Q and SV) and peripheral (arteriovenous oxygen difference) hemodynamic variables between protocols, 3) stroke volume kinetics to highlight potential central command mechanisms, and 4) variables associated with the level of metabolic disturbances during these protocols. The published review manuscript found in Chapter 2 entitled "Graded Exercise Testing Protocols for the Determination of VO_{2max}: Historical Perspectives, Progress, and Future Considerations" encompasses all relevant peer-reviewed literature to date examining the responses during and the overall utility of perceptually-regulated self-paced graded exercise testing protocols. This paper demonstrates that, though SP may serve as a valid alternative to more traditional protocols, the physiological responses during SP that underpin mixed results remain to be fully elucidated.

The research manuscript entitled, "Hemodynamic and Metabolic Responses during Self-Paced and Ramp Graded Exercise Testing Treadmill Protocols" found in Chapter 3 provides evidence that SP may serve as an alternative to RAMP treadmill testing protocols in college-aged, moderately-trained men. Furthermore, the SV response exhibited during self-paced exercise may provide insight concerning in central command as it relates to 'pacing' strategy during maximal graded exercise.

Conclusions

Perceptually-regulated self-paced graded exercise tests are a reliable alternative to a ramp graded exercise test protocol. Given the consideration for total test duration when designing a graded exercise test, a self-paced protocol might be particularly beneficial from a time-management standpoint. We conclude the following regarding our study of hemodynamic and metabolic responses during self-paced and ramp graded exercise testing treadmill protocols:

1. Self-paced and ramp protocols do not differ in maximal oxygen uptake.
2. Measures of maximal blood flow (cardiac output and stroke volume) and peripheral oxygen extraction (arteriovenous oxygen difference) are similar between protocols; however, the stroke volume patterns exhibited during protocols may highlight characteristics relating to central command.
3. There are no differences in markers of metabolic disturbance (blood lactate accumulation and ventilatory threshold) between protocols.

Recommendations

Several questions remain regarding the value of self-paced protocols. We did not find any differences between self-paced and ramp protocols regarding hemodynamic or metabolic responses; however, we suggest future research apply a design that examines the utilities of these protocols in homogenous samples (age, sex, fitness level, disease status). These studies should seek to preemptively control for differences in ergometers to mitigate the chance of poor study design as an explanation for significance of findings. Furthermore, it would be interesting to see more advanced statistical analyses to describe potential differences in hemodynamic responses by examining individual rather than

group responses. Finally, there remains to be a study manipulating variables other than speed within a self-paced protocol. Total time duration could be increased or decreased or the rating of perceived exertion (RPE) 0-10 scale could be used in place of 6-20. It was clear throughout the course of this study that familiarization with the RPE_{6-20} scale and concept of 'pacing' was crucial for individuals lacking prior experience with them. Practically speaking, it may benefit the time-efficient nature of self-paced protocols if methods were tested to reduce the importance of familiarization sessions during self-paced exercise.

APPENDICES

A. Informed Consent

B. Flyer

C. Health History Questionnaire

D. Email Script

E. Data Collection Sheets

APPENDIX A.

Comparison of hemodynamic responses between self-paced and standard ramp protocols.
Consent to Participate in Research

01/05/17

Purpose of the study: You are being asked to participate in a research study that is being done by Dr. Len Kravitz, who is the Principal Investigator and his associates from the Department of Health, Exercise, and Sports Sciences. This study will not be funded by any organization. The purpose of this study is to compare the cardiovascular responses between two different graded exercise tests with males. You are being asked to take part in this study because you are a healthy individual between the ages of 18 and 45 years old, who engages in > 30 minutes physical activity on at least 3 days per week. Up to 40 people will take part in this study at the University of New Mexico. All study visits will take place in the Exercise Physiology Lab at the University of New Mexico.

This form will explain what to expect when joining the research, as well as the possible risks and benefits of participation. If you have any questions, please ask one of the study researchers.

What you will do in the study:

- You will be asked to visit the Exercise Physiology Lab in Johnson Center at the University of New Mexico, Albuquerque, NM on three separate occasions.
- All visits will be separated by a minimum of 2 days and a maximum of 7 days with all visits completed within a 3-week time frame.
- Prior to each visit, you will be asked not to perform any exercise 24 hours before the visit, not ingest caffeine or alcohol 24 hours prior to the visit, and to consume at least a pint of water as well as a small meal 2-3 hours before the visit.
- The first visit will take up to 90 minutes and the remaining two visits will take up to 60 minutes.
- <u>During your first visit you will do each of the following:</u>
    - Complete physical activity/health history questionnaire. You will be asked to fill out this questionnaire about your health and that of your close relatives in order to screen for any issues that could cause additional risk to you by being a part of the study.
    - Your height and weight will be measured, and body fat will be estimated via skinfold (SKF) measurements. The SKF measurements are done using a skinfold caliper to measure a double layer of skin and underlying (subcutaneous) fat. The sum of the SKF measurements is used to estimate body density (Db). The determined Db is used to estimate percent body fat.
    - You will then be asked to perform a maximal oxygen uptake test (VO_2 max) on either cycle ergometer or treadmill (based on personal preference) for approximately 10 minutes. This test will require you to pedal on a

stationary cycle ergometer or run on a treadmill with an increasing intensity (controlled by the participant) until you cannot continue. You will have a mouthpiece in your mouth (somewhat like a snorkeling breathing device) and a nose clip on your nose. This equipment is connected to a gas analyzer to measure your exhaled oxygen and carbon dioxide throughout each trial.

- During exercise, an alcohol swab will be used to prep your skin and pairs of electrodes will be placed at specific sites on your neck, chest (just below the collarbone next to the sternum), trunk (just below the bottom of the sternum), and back. This equipment uses electrical signals (non-invasive and similar to a 12-lead EKG) to measure the function of your heart. For quality assurance, the electrodes may need to be secured using tape. You will be asked about allergies to glue prior to applying tape.
- We will be measuring the level of lactate in your blood. To do this the researcher will collect a drop of blood from your ear 3 times during each visit. This will occur before you begin to exercise, immediately after you finish exercising, and 5-minutes post exercise.
- In the self-paced protocol, you will gradually increase the intensity of the cycle or treadmill throughout the test by manually changing the workload in accordance to your rating of perceived exertion (how hard you feel you are working) on a scale of 6-20. This rating scale will be explained to you in detail. The entire test will consist of five 2-minute stages.
- Upon completing the graded exercise protocol, the mouthpiece will be removed and you will recover for 20 minutes. After a light exercise (walk) recovery period, you will begin to warm-up for 2-minutes. At this point, the intensity will be set to a level 5% greater than the maximal intensity achieved during the initial graded exercise test. You will be asked to exercise at that level until you can no longer continue. This usually takes between 1.5-3min. If this test does not confirm VO_2 max, you may need to return for additional testing.
- If this verification trial does not confirm VO_2max, you have the option to withdraw from the study and not return for additional trials or testing.

- <u>During your second visit and third visits the following will occur:</u>
  - At visit 2- The exercise test type (either self-paced or ramp) that you will be asked to perform will be randomly chosen for you by a flip of a coin.
  - At visit 3- You will perform the exercise test type that was not done at visit 2. For example, if self-paced exercise was performed at visit 2 you will do the ramp exercise testing or vice versa.
  - Before the start of the warm-up, you will be re-familiarized with the procedures of the graded exercise test.
  - In the case of performing a ramp exercise protocol, you will complete a different type of test on the same mode (cycle or treadmill) than you performed on the first visit.
  - In the case of performing a self-paced protocol, you will repeat the same graded exercise protocol that you completed during the first visit. The self-paced protocol is a 10-min exercise test and requires that you (the

participant) will gradually increase the exercise intensity throughout the test based your own perception of effort. The test will conclude once you have reached a point where you cannot continue.

- During the ramp protocol, you will complete a maximal oxygen uptake test (VO_2 max) for approximately 8-12 minutes. This test will require you to pedal on a stationary cycle ergometer or run on a treadmill with an increasing intensity until you cannot continue. This test is different in that it will not be controlled by your rating of exertion (how hard you feel you are working). Instead, the researcher will control the gradual increase in exercise intensity until you reach a point where you cannot continue.
- All other procedures will be the same as the first trial, other than the way the intensity of the exercise increases.
- Your rating of perceived exertion (how hard you feel you are working) on a scale of 6-20 will be recorded at the end of each 2-min exercising stage
- Blood lactate will be measured via a total of 3 pinpricks to the ear to collect a sample of a drop of blood before you begin exercising, immediately post-exercise, and 5-minutes post exercise.
- At visit 2 you will be asked to schedule your final visit, which will take place at least 2 days but no more than 7 days after the completion of visit 2.

Participation in this study will take a total of 3.5 hours over a period of three separate visits. The first visit will last approximately 90-minutes while the second and third visits will last approximately 60-min each.

Risks: Every effort will be made to protect the information you give us. However, there is a risk of loss of privacy and/or confidentiality that may result in hardship or inconvenience. There are risks associated with maximal/submaximal exercise testing including the following: brief feelings of nausea, lightheadedness, muscle cramps, or dizziness. According to the American College of Sports Medicine, the risk of a cardiac event in normal healthy individuals during a maximal exercise test is minimal, 0.0006% (6 in 10,000). The mouthpiece/headgear/nose clip can be uncomfortable, especially during exercise. We will adjust the gear to be as comfortable as possible. There is a risk of discomfort when we prick your ear to obtain a drop of blood for the measurement of lactate, which will happen eight times per visit. There is a very small risk of infection when blood is drawn, however, sterile techniques and trained personnel will be used to limit this risk. There are no known risks associated with the placement of electrodes and the measurement of heart function. There are risks of stress, emotional distress, injury, and inconvenience associated with participating in this study.

Benefits: There will no direct benefits to you. However, it is hoped that information gained from this study will help us understand the suitability of self-paced protocols for the purpose of assessing VO_2max. For your personal information, you will be given the results of your maximal exercise tests, your VO_2max, and body fat percentage within two weeks of completing the study.

Confidentiality of your information: Your name and other identifying information will be maintained in locked files, available only to authorized member of the research team for the duration of the study. For any information entered into a computer, the only identifier will be a unique study identification (ID) number. If you decide to withdraw from the study, all data which has been previously collected will be destroyed immediately. Any personal identifying information and any record linking that information to study ID numbers will be destroyed at the conclusion of the study. Information resulting from this study will be used for research purposes and may be published; however, you will not be identified by name in any publications.

We will take measures to protect the security of all your personal information, but we cannot guarantee confidentiality of all study data. The University of New Mexico Institutional Review Board (IRB) that oversees human subject research may be permitted to access your records. Your name will not be used in any published reports about this study.

Payment: Upon completing all trials for this study, you will be compensated with a gift card equal to $60 for your time and inconvenience. Compensation will not be pro-rated by trial. Therefore, you must successfully complete all trials to receive compensation.

Research related injury:

If you are injured or become sick as a result of this study, any emergency treatment will be at your cost. No commitment is made by the UNM to provide free medical care or money for injuries to participants in this study.

It is important for you to tell the Principal Investigator immediately if you have been injured or become sick because of taking part in this study. If you have any questions about these issues, or believe that you have been treated carelessly in the study, please contact the Office of the IRB at (505) 277-2644 for more information.

Right to withdraw from the study: Your participation in this study is completely voluntary. You have the right to choose not to participate or to withdraw your participation at any point in this study without penalty. Any such data which may have been previously collected will be destroyed if you do decide to withdraw from the study. You will be given the results of all testing you completed before withdrawal.

If you have any questions, concerns, or complaints about the research study, please contact:

Len Kravitz, Ph.D.
University of New Mexico Department Health, Exercise and Sport Sciences
Johnson Center, MSC04 2610

1 University of New Mexico
Albuquerque New Mexico 87131

(505) 277-4136
lkravitz@unm.edu

If you would like to speak with someone other than the research team or have questions regarding your rights as a research participant, please contact the IRB. The IRB is a group of people from UNM and the community who provide independent oversight of safety and ethical issues related to research involving people:

UNM Office of the IRB, (505) 277-2644, irbmaincampus@unm.edu. Website: http://irb.unm.edu/

CONSENT

You are making a decision whether to participate in this study. Your signature below indicates that you have read this form (or the form was read to you) and that all questions have been answered to your satisfaction. By signing this consent form, you are not waiving any of your legal rights as a research participant. A copy of this consent form will be provided to you.

I agree to participate in this study.

_____	_____	_____
Name of Adult Participant	Signature of Adult Participant	Date

Researcher Signature (to be completed at time of informed consent)

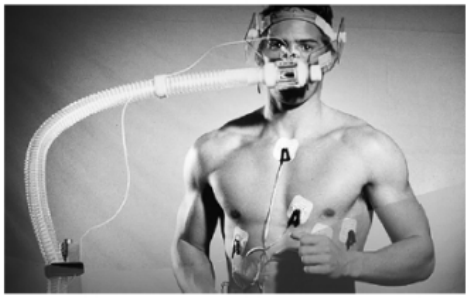
I have explained the research to the participant and answered all of his/her questions. I believe that he/she understands the information described in this consent form and freely consents to participate.

_____	_____	_____
Name of Research Team Member	Signature of Research Team Member	Date

APPENDIX B.

Participants needed for Research Study

Are you a healthy, fit individual interested in learning more about VO_2max testing ??



The exercise physiology lab will be assessing two exercise testing protocols to compare the differences in cardiovascular responses and blood lactate concentrations. This study will involve three different laboratory visits, totaling 3.5 hours of required time.

Participant requirements:

- 18-45 years old
- Males and Females welcome
- Free of any illness/medical conditions

If interested contact: Nick Beltz nbeltz@unm.edu
Or Dr. Len Kravitz lkravitz@unm.edu

APPENDIX C.

HEALTH HISTORY QUESTIONNAIRE (RESEARCH ONLY 5/20/02)

Subject #____________________________ Date___/___/___

*Phone #: home*_______________________ *cell*______________

Date of Birth ___/___/___Age____ Gender____ Ethnicity_______ Phone _________

Address
(home)____zip________

email____________________________________

Primary health care provider and health insurance________________________________
(Only for information/emergency contact)
Person to contact in case of emergency:
name____________________________**phone #**______________________________

♦♦♦

MEDICAL HISTORY

Self-reported: Height______ Weight______
Physical injuries:____

Limitations____

Have you ever had any of the following cardiovascular problems? Please check all that apply.

Heart attack/Myocardial Infarction	____	Heart surgery	____	Valve problems	_____
Chest pain or pressure	____	Swollen ankles	____	Dizziness	_____
Arrhythmias/Palpitations	____	Heart murmur	____	Shortness of breath	_____
Congestive heart failure	____				

Have you ever had any of the following? Please check all that apply.

Hepatitis/HIV	_____	Depression	_____	Cancer (specify type)	__________
Rheumatic fever	_____	High blood pressure	_____	Thyroid problems	_____
Kidney/liver disease	_____	Obesity	_____	Total cholesterol >200 mg/dl	_____
Diabetes (specify type)	_____	Asthma	_____	HDL cholesterol <35 mg/dl	_____
Emphysema	_____	Stroke	_____	LDL cholesterol >135 mg/dl	_____
				Trygylcerides>150 mg/dl	_____

Do immediate blood relatives (biological parents & siblings **only**) have any of the conditions listed above? If yes, list the problem, and family member age at diagnosis.

____

Is your mother living? Y N Age at death______ Cause___________________
Is your father living? Y N Age at death______ Cause___________________

Do you currently have any condition not listed that may influence test results? Y N

Details____

____

Indicate level of your overall health. Excellent ____ Good ____ Fair ____ Poor_____

Are you taking any medications, vitamins or dietary supplements now? Y N

If yes, what are they?____

Do you have allergies to any medications? If yes, what are they?

____

Are you allergic to latex? Y N

Have you been seen by a health care provider in the past year? Y N

If yes, elaborate

____

Have you had a prior treadmill test? Y N. If yes, when?______________ What were the results?

____

____

Have you ever experienced any adverse effects during or after exercise (fainting, vomiting, shock, palpitations, hyperventilation)? Y N If yes, elaborate.____

♦♦

LIFESTYLE FACTORS

Do you now or have you ever used tobacco? Y N If yes: type ________________

How long?______ Quantity____/day Years since quitting______________

How often do you drink the following?

Caffeinated coffee, tea, or soda _______oz/day Hard liquor _______oz/wk Wine _______oz/week

Beer _______oz/wk

Indicate your current level of emotional stress. High____ Moderate ____ Low____

♦♦

PHYSICAL ACTIVITY/EXERCISE

Physical Activity

Minutes/Day (*Weekdays*) Minutes/Day (*Weekends*)

_____/_____ average _____/_____ average

Do you train in any activity (eg. jogging, cycling, swimming, weight-lifting)?

Y N

How well trained are you? _____

Vigorous Exercise (>30 Minute sessions)

_____Minutes/hours a week

APPENDIX D.

My name is Nick Beltz and I am a researcher in the Health, Exercise, and Sports Sciences Department at the University of New Mexico. I am emailing you to notify you of a research study we are conducting in which you may be an eligible participant. The research study is investigating the differences in hemodynamic responses between two different graded exercise testing protocols. If you are a healthy male between the ages of 18 and 45 years who is physically active (> 30 minutes of activity at least three days per week) you may be eligible to participate. Throughout the study you will have your body fat %, oxygen consumption and hemodynamic measurements taken (via non-invasive cardiac impedance). Additionally, a drop of blood will be collected at multiple time points to measure blood lactate. This study will require 3 visits to the research laboratory and require you to be at the laboratory a total of 3.5 hours. Please contact Nick Beltz at nbeltz@unm.edu or Len Kravitz at lkravitz@unm.edu if you are interested in participating and would like to learn more about the study.

APPENDIX E.

Subject #: _____ Date: _____

Height (cm): _____ Weight (kg): _____ Age (yrs): _____

Vig Ex? Alcohol? Caffeine? Hydrated? Food?

Visit #: _____ Protocol: _____

	Chest	Abd	Thigh	Total	BD	BF%
SKF 3 site	/	/	/			

BLa	Pre	IPE	5min
	/	/	/

Traditional

Speed	Time	RPE
	0-2	
	2-4	
	4 - 6	
	6 - 8	
	8 - 10	
	10 - 12	
	End Time:	

Max Speed: _____

VO2 max (ml/kg/min): _____

HRmax (bpm): _____

Verification Speed (mph): 30% _____ (2min)

40-45% _____ (1min)

105% _____

Verification VO2 max (ml/kg/min): _____

Verification Duration (min:sec): _____

Confirm (±3%) ?? Y N

RPE

Time	Speed
:30	
1:00	
1:30	
2:00	
2:30	
3:00	
3:30	
4:00	
4:30	
5:00	
5:30	
6:00	
6:30	
7:00	
7:30	
8:00	
8:30	
9:00	
9:30	
10:00	

BP Pre	BP Ver