CONSTRUCTING QUASI-LINEAR OXYGEN UPTAKE RESPONSES FROM NON-LINEAR PARAMETERS

by

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Abstract

Purpose: Oxygen uptake (VO2) has been shown to be controlled by a nonlinear system, yet the VO2 response to ramp style exercise appears linear. We tested the hypothesis that an integrative model incorporating nonlinear parameter values could accurately estimate actual VO2 responses to ramp style exercise. **Methods:** Six healthy, men completed three bouts of varying ramp rate exercise (slow ramp (SR): 15 W/min, regular ramp (RR) 30 W/min, fast ramp (FR) 60W/min) and four bouts of extended-step incremental exercise, where each step lasted 5-15 min or until volitional fatigue on a cycle ergometer on separate days. The step-responses were then fit with a simple monoexponential starting at time zero (MONO) or allowing a time delay and using only the first 5 min of data (5TD). The resulting VO2 parameters from the step protocol were incorporated into an integrative model for the estimation of the VO2 response to each of the rates of ramp incremental exercise. The parameters from the actual and model ramp protocols were compared with 2 way repeated-measures ANOVAs. **Results:** Both Gain (G) and Mean Response Time (MRT) (or time constant) values increased significantly across work rate transitions (mean±SD; Gain:10.0±0.9, 11.6±1.1, 13.1±1.3, 17.6±3.3 ml O_2/min/W; MRT:39.4±7.7, 54.0±5.4, 79.6±15.0, 180.1±56.2 s). Up to maximal VO2 the models over-estimated the actual VO2 response for FR (Gain: ACT 8.7±1.0, MONO 9.9±0.4, 5TD 10.3±0.3 ml O_2/min/W). Up to 80% maximal VO2 the models accurately predicted the actual VO2 response across all ramp rates (Gain: ACT 10.7±1.1, 10.2±0.5, 9.2±1.0; MONO 11.0±0.8, 10.3±0.6, 9.2±0.5; 5TD 10.4±0.4, 10.2±0.3, 9.8±0.2 ml O_2/min/W, values are listed SR,RR,FR). **Conclusions:** When variable parameter values (G and either MRT or time constant and time delay) were utilized by an integrative model, accurate estimations of the VO2 response to ramp

incremental exercise were possible regardless of ramp rate (up to 80% maximal VO2). The increases in both G and MRT (or time constant) appear to balance each other to produce the quasi-linear VO2 responses.

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List of Symbols/Abbreviations

α	Probability of a type I error
ACT	Actual Data
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
β	Probability of a type II error
CO_2	Carbon Dioxide
CK	Creatine Kinase
CP	Critical Power
Δ	Change
δ	Time Delay
η	Efficiency
FR	Fast Ramp (60 W/min)
G	Gain
H^+	Hydrogen Ion
H_20	Water
HCO_3	Bicarbonate Ion
θ_c	Critical Metabolic Rate
$\hat{\theta}_L$	Lactate Threshold (Estimated)
θ_L	Lactate Threshold
La^-	Lactate Ion
$\mu\dot{V}O_2$	Maximal Oxygen Uptake
MONO	Full Stage without Time Delay Model
MRT	Mean Response Time
O_2df	Oxygen Deficit
ϕ_1	Phase One (Cardiodynamic Phase)
ϕ_2	Phase Two (Fundamental or Primary Phase)
ϕ_3	Phase Three (Steady-State or Slow Component)
PCr	Phosphocreatine
$\dot{Q}$	Blood Flow
RR	Regular Ramp (30 W/min)
SR	Slow Ramp (15 W/min)
ss	Steady-State
S1	Segment 1 (<lactate threshold)
S2	Segment 2 (>lactate threshold)
t	Time
τ	Time Constant
V_{venous}	Volume of Venous Blood
$\dot{V}CO_2$	Carbon Dioxide Production
$\dot{V}_E$	Minute Ventilation
$\dot{V}O_2$	Oxygen Uptake
$\dot{V}O_2sc$	Slow Component of Oxygen Uptake
VTD	Venous Time Delay
WR	Work Rate
5TD	5 Minute with Time Delay Model
[ ]	Concentration

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Chapter 1 - Introduction

$\dot{V}O_2$ has previously been described as a first-order linear system (Henry 1951, Whipp *et al.* 1981). First-order linear systems are those in which a given input provides a predictable output according to a single, constant transfer function. A linear system will adhere to the Principle of Superposition, which is well described by Douglas Riggs:

"The principle of superposition states that in any linear system the output produced by applying several inputs simultaneously is equal to the sum of the outputs produced by applying each input separately" (Riggs 1976)

For a linear system to be first-order requires that the transfer function have no more than one derivative of the first-order and none of a higher order (Rossiter 2011). In the system of respiratory metabolism, WR is the input and the output is $\dot{V}O_2$. The transfer function for step transitions in WR is exponential (Henry 1951, Hill *et al.*1924, Margaria *et al.* 1933) and the response is described by the equation:

$$\dot{V}O_2(t) = \Delta\dot{V}O_{2ss} \cdot (1-e^{-t/\tau})$$

where t is time, $\dot{V}O_2(t)$ is the oxygen uptake for a given t, $\Delta\dot{V}O_{2ss}$ is the difference between the steady-state and baseline $\dot{V}O_2$ values, and τ is the time constant (i.e. inverse of the rate constant). This is the case for moderate exercise intensities (< lactate threshold).

With higher intensity exercise (> lactate threshold), the first-order linearity of $\dot{V}O_2$ kinetics is lost due to the addition of a slow component (Poole *et al.* 1988, Whipp and Wasserman 1972) and asymmetries in the on- and off-kinetics (Özyener *et al.* 2001, Paterson and Whipp 1991). The slow component imparts an additional $\dot{V}O_2$ to the monoexponential response, which is not described by the constant transfer function. The asymmetry in the on-

and off-kinetics violates the Principle of Superposition (Rossiter 2011). Even within the moderate intensity domain the linearity of $\dot{V}O_2$ has been strongly challenged by studies which found increasing values for τ and/or Gain (G) ($G = \Delta\dot{V}O_{2ss}/\Delta WR$) when exercise was initiated from an elevated baseline WR (Brittain 2001, di Prampero *et al.* 1970, Hultman *et al.* 1967). These changing parameters would necessitate additional derivatives in the transfer function. Therefore, the system would no longer be first-order. These examples indicate that the $\dot{V}O_2$ system is thus more complicated than a first-order linear system.

Ramp exercise testing, where the WR is gradually increased, is frequently used in a variety of conditions, from clinical evaluation of patients to testing of elite athletes. The ramp function is the integral of the step function (Fujihara *et al.* 1973a). Thus, the $\dot{V}O_2$ response from a ramp function should be the integral of the step function, if the $\dot{V}O_2$ system is first-order linear (Whipp *et al.* 1981). Given the above deviations from first-order linearity, it is surprising that the $\dot{V}O_2$ response to ramp exercise is so well described by the integrated $\dot{V}O_2$ transfer function for step exercise (Whipp *et al.* 1981). That this is the case raises additional questions concerning the control of $\dot{V}O_2$. Recently, Rossiter has provided a putative mechanism to reconcile these incongruences in which the $\dot{V}O_2$ response to ramp incremental exercise was predicted using either MRTs (Mean Response Time) or Gs which increased with WR while the other parameter was held constant (Rossiter 2011). The resulting $\dot{V}O_2$ was near, or quasi-linear.

However, while the parameter values used by Rossiter were based on values from previous studies, only the linearity of the model was assessed (Rossiter 2011). The accuracy of the estimations of such an approach needs to be validated against observed data.

Therefore, the purpose of this study was to determine if parameters empirically derived from submaximal exercise can be incorporated into an integrative model to accurately estimate actual $\dot{V}O_2$ responses from ramp incremental tests. We hypothesized that 1) both MRT and G would increase with WR, 2) the estimated response produced by the model would not be statistically different from the actual response for a range of ramp rates, 3) a more sophisticated model accounting for a time delay and limiting the inclusion of the slow component of $\dot{V}O_2$ ($\dot{V}O_2sc$) would more accurately estimate the actual $\dot{V}O_2$ response.

Chapter 2 – Literature Review

Overview of Oxygen Uptake

For muscular contraction to take place, adenosine triphosphate (ATP) must supply the energy needed for a plethora of cellular processes such as actin-myosin release, ion pumping, glucose activation, etc. As the body's stores of free ATP are utilized, additional ATP must be resynthesized for the muscular activity to continue. The body can resynthesize ATP through multiple pathways, each of which has its own advantages and drawbacks. Phosphocreatine (PCr) offers an immediately available energy source, but prolonged activity relying solely on this energy source would quickly deplete the body's stores. Lactic acid fermentation can support longer bouts of activity, but is also of limited supply, promotes acidosis, and produces fewer ATP per glucose molecule. Aerobic metabolism can maintain elevated energy expenditure for an extended length of time. However, the rise in energy output from aerobic metabolism is much slower than either lactic acid fermentation or phosphocreatine catabolism. This slower increase is due to aerobic metabolism functioning through a much more complicated series of linked pathways which terminates with the utilization of O_2.

While PCr breakdown and lactic acid fermentation can occur in an anoxic environment, oxygen (O_2) is needed as a substrate for aerobic metabolism. At the end of the electron transfer chain, where most ATPs from aerobic metabolism are formed, O_2 is used as the terminal electron acceptor and is converted to water. This utilization of O_2 requires a continuous O_2 supply for aerobic metabolism to continue. The quantity of O_2 consumed, the O_2 uptake ($\dot{V}O_2$), can thus provide an estimate of mitochondrial energetics.

$\dot{V}O_2$ is often used as an indicator of energy expenditure, but there are fundamental simplifications in this interpretation. First, O_2 is used in the resynthesis of ATP. Therefore, what is being estimated is not the energy expended, but, conversely, the energy produced. However, as [ATP] remains remarkably constant across exercise bouts (86), it is accepted that ATP expenditure and production are approximately equal. Additionally, aerobic metabolism is only one of many available methods for resynthesizing ATP. $\dot{V}O_2$ data may provide some insight concerning the extent of these other processes, but this requires many assumptions (beyond those necessary in estimating $\dot{V}O_2$ itself) and $\dot{V}O_2$ is thus best interpreted as a very general estimate of energy expenditure. Furthermore, aerobic metabolism is an elaborate and extensive process consisting of many reactions spread throughout many parts of the cell. This results in some "inertia" when altering the rate of aerobic metabolism. As aerobic metabolism (as indicated by $\dot{V}O_2$) increases toward, but does not yet attain, the required level, the quicker processes of PCr breakdown and lactic acid fermentation (and free ATP) must provide the remaining energy necessary. Therefore, at the onset of exercise, $\dot{V}O_2$ by itself grossly underestimates energy expenditure. $\dot{V}O_2$ can only directly provide information about the rate of aerobic metabolism. However, that does not erode the value of $\dot{V}O_2$ measurements, which have been used to predict exercise tolerance

(Burnley and Jones 2007, Murgatroyd *et al.* 2011), classify exercise intensity (Gaesser and Poole 1996, Whipp 1996), and reveal important physiologic phenomena in both health and disease (reviews: Hansen *et al.* 2012, Jones and Poole 2005, Poole and Jones 2012).

Constant Work Rate Kinetics

Our modern understanding of $\dot{V}O_2$ kinetics is based on the 1913 pioneering work of Krogh and Lindhard. In this experiment they obtained three ventilatory samples during the first minute of cycling exercise and a varying number of samples for a few minutes afterwards. They found that following the onset of exercise $\dot{V}O_2$ did not increase immediately, but rather gradually, up to a steady-state value (Krogh and Lindhard 1913). Intriguingly, the next major steps in evaluating $\dot{V}O_2$ kinetics were also steps down (i.e. over the next four decades conclusions were based on examination of the $\dot{V}O_2$ response at the offset of exercise (recovery) with an assumption of a symmetrical response for the onset). Thus, the next advancement was the work by A.V. Hill and colleagues who were the first to describe $\dot{V}O_2$ kinetics as exponential (Hill *et al.* 1924). Hill described the off-kinetics with two exponentials which related to the oxidation of lactic acid from within the muscle (the quicker component) and from throughout the body (the slower component). After work from other physiologists emerged demonstrating that oxidation of lactic acid could not be the only contributing factor to the gradual off-kinetics (Owles 1930), Hill's colleagues at the Harvard Fatigue Lab framed the simultaneous double-exponential off-kinetics as a sum of slower "lactacid" and faster "alactacid" portions (Margaria *et al.* 1933). Importantly, in the work of both Hill and Margaria the double-exponential model was only needed for higher WRs (those that resulted in elevated blood lactate concentrations), while a single-exponential model

adequately described the response to lower WRs. Thus, exercise intensity plays an important role in the determination of $\dot{V}O_2$ kinetics and must be addressed.

Exercise Intensity-Domains

Exercise intensity has been described as varying in degree of intensity for millennia (Whipp *et al.* 1998). However, the use of specific physiologic measurements to describe exercise intensity is a much more recent development. At least by 1924, exercise intensity was described by A.V. Hill as either severe or moderate depending on whether blood lactic acid levels rose or not (Hill *et al.* 1924). This early lactic acid-based schema has now been replaced by one which is defined by $\dot{V}O_2$ (N.B. there is another schema that is not discussed here but see reference Whipp 1996).

In the exercise intensity-domain schema proposed by Gaesser and Poole, there are four domains of exercise intensity, defined by their relationships to three physiologic parameters (Gaesser and Poole 1996). The domain of lightest exercise intensity is the Moderate domain. The Moderate domain includes any WR below the lactate threshold (Θ_L). The Θ_L is the metabolic rate at which lactate ion concentration [La^-] of the blood increases (Koyal and Beaver 1973, Wasserman and McIlroy 1964). Although technically this represents a point at which La^- appearance exceeds disappearance, it has traditionally been accepted to represent a dramatic increase above the baseline rate of lactic acid fermentation. Lactic acid fermentation produces lactic acid, which immediately dissociates to produce La^- and H^+. The H^+ ions are buffered so that initially only a slight decrease in pH occurs. However, the buffering of H^+ ions by the hydrogen carbonate ion (i.e. bicarbonate or HCO_3^-) produces CO_2.and H_20 via the carbonic anhydrase reaction. This increase in CO_2 drives an increase in

ventilation ($\dot{V}_E$) (Wasserman 1978). Therefore, θ_L is often estimated ($\hat{\theta}_L$) by an increase in $\dot{V}CO_2$ vs. $\dot{V}O_2$(v-slope), the ventilatory equivalent of O_2 ($\dot{V}_E/\dot{V}O_2$) and/or the ratio of $\dot{V}CO_2/\dot{V}O_2$ (respiratory exchange ratio or RER) (Beaver *et al.* 1986).

θ_L serves as the lower boundary of the Heavy intensity domain; the upper boundary is critical power (CP). CP is the power asymptote of the power-duration relationship, i.e. the highest WR which could theoretically be maintained indefinitely (Monod and Scherrer 1965, Moritani *et al.* 1981). CP is exercise mode specific, but the metabolic rate elicited by exercising at CP is invariant, providing a 'critical' metabolic rate (θ_c) (Barker *et al.* 2006).

Above θ_c and up to maximal $\dot{V}O_2$ ($\mu\dot{V}O_2$) is the Severe intensity domain. $\mu\dot{V}O_2$ is the highest aerobic metabolic rate possible for any given mode of exercise. As the Severe domain is above θ_c, a steady state in $\dot{V}O_2$ is not possible unless it is at $\mu\dot{V}O_2$ (which would be only briefly sustainable during constant WR exercise). With sufficient duration, any WR within the Severe domain will elicit $\mu\dot{V}O_2$ (Poole *et al.* 1988). The Extreme intensity domain encompasses those highest WRs where fatigue limits exercise duration to the point that $\dot{V}O_2$ is unable to attain $\mu\dot{V}O_2$. The changes in $\dot{V}O_2$ which determine and are described by the exercise intensity domains can be helpful as diagnostic and therapeutic measures; however, the ultimate utility of that measurement will be determined by its accuracy and accessibility.

Pulmonary Versus Muscular $\dot{V}O_2$

For the subject, measuring pulmonary $\dot{V}O_2$ is a simpler, and certainly safer, method for investigating $\dot{V}O_2$ than measuring muscular $\dot{V}O_2$. Unfortunately, pulmonary $\dot{V}O_2$ is necessarily distanced from and different than the $\dot{V}O_2$ directly required for the work done, i.e. muscular $\dot{V}O_2$. As described above, O_2 is utilized during aerobic metabolism. During bouts

of exercise, aerobic metabolism will be increased primarily in the myofibers of the active skeletal muscle. As pulmonary $\dot{V}O_2$, this response will necessarily be convoluted by the intervening myoglobin, blood, tissue, and lung oxygen stores, changes in blood flow, transport delays, and mixing of venous blood from the rest of the body (Barstow *et al.* 1990, Barstow and Molé 1991, Whipp *et al.* 2005). Until recently, technical limitations have precluded more direct measurements of muscle $\dot{V}O_2$ in humans.

$\dot{V}O_2$ kinetics from pulmonary data has been assessed for over a century (Krogh and Lindhard 1913). As technology has advanced since then, measurements of $\dot{V}O_2$ have been able to focus nearer to the point of O_2 consumption. Specifically, $\dot{V}O_2$ has been measured or calculated across the exercising limb (Bangsbo *et al.* 2000, Grassi *et al.* 1996), solitary active muscles (Behnke *et al.* 2002) and myofibers (Kindig *et al.* 2003). The data from myofibers and muscle utilized a phosphorescence quenching technique to calculate $\dot{V}O_2$. Their results show that with constant WR exercise, muscle $\dot{V}O_2$ increases from the onset of exercise with no appreciable time delay (<2 s; 8, Kindig *et al.* 2003) up to a steady state value. These results stand in contrast to the results from exercising limb and whole body exercise.

In the exercising limb, $\dot{V}O_2$ is calculated using arterial and venous O_2 content and blood flow measurements via direct application of the Fick equation. In these cases, due to an intervening volume of blood between the active muscle and the site of measurement, a discernable time delay occurs between exercise onset and the rise in $\dot{V}O_2$ due to increases in aerobic metabolism (Bangsbo *et al.* 2000, Barstow *et al.* 1990, Barstow and Molé1991, Grassi *et al.* 1996, Whipp *et al.* 1982). Although not caused by metabolic activity, pulmonary $\dot{V}O_2$ does, however, increase at the onset of exercise.

The initial increase in pulmonary $\dot{V}O_2$, which takes place before the more deoxygenated blood from the active skeletal muscle arrives at the lungs, is called the cardiodynamic phase, or ϕ_1. This cardiodynamic effect has been postulated since the work of Krogh and Lindhard (Krogh and Lindhard 1912). At the onset of exercise there is an almost immediate rise in cardiac output (Buchanan 1909, Krogh and Lindhard 1912). Blood flow through the lungs thus increases, causing an increase in pulmonary $\dot{V}O_2$, as seen by the relationship in the Fick Principle:

$$\dot{V}O_2 = \dot{Q} \cdot (C_aO_2 - C_{\bar{v}}O_2) \qquad \text{Eq. 1}$$

where $\dot{Q}$ is blood flow, C_aO_2 and $C_{\bar{v}}O_2$ are the O_2 contents of arterial and venous blood, respectively. The blood flowing through the lungs during ϕ_1 will be the venous volume between the exercising muscle and lungs as well as venous return from the rest of the body. This blood will therefore have a C_vO_2 that is similar to resting values. The rate of rise of pulmonary $\dot{V}O_2$ during ϕ_1 has been shown to be dependent only on the rate of change of pulmonary blood flow (Barstow and Molé 1987), while the duration of ϕ_1 (or venous time delay (VTD)) can be determined by the following equation

$$VTD = \frac{V_{venous}}{\dot{Q}} \qquad \text{Eq. 2}$$

Where V_{venous} is the venous volume between the active muscle and the lungs. After ϕ_1, blood from the exercising muscle reaches the lungs with a decreased C_vO_2 reflecting a greater O_2 extraction with exercise.

Phase 2 (ϕ_2) pulmonary $\dot{V}O_2$ kinetics have been shown to be very similar to muscle $\dot{V}O_2$ kinetics (Bangsbo *et al.* 2000, Barstow and Molé 1987, 1991, Grassi *et al.* 1996). As

cardiac output increases with the onset of exercise, the more deoxygenated blood from the working muscle will flow through the lungs more rapidly than it did through the muscle. Thus, the pulmonary ϕ_2 kinetics will be slightly temporally displaced from the muscular $\dot{V}O_2$ kinetics. Therefore, to better describe the muscular $\dot{V}O_2$ kinetics when fitting pulmonary $\dot{V}O_2$ kinetics, the first 20 s of data are excluded to account for ϕ_1 (Barstow and Molé 1987, Linnarsson 1974, Whipp *et al.* 1982). The resulting kinetics of $\dot{V}O_2$ during ϕ_2 have been shown by the modelling of Barstow and Molé (Barstow and Molé 1987, 1991) to be accurate to those of the contracting muscle(s) to within 10%. This conclusion from modelling predictions was later verified in humans undergoing large muscle mass exercise (Bangsbo 2000 *et al.*, Grassi *et al.* 1996).

The similarity between ϕ_2 pulmonary $\dot{V}O_2$ and the exercising muscle was further established by the similarities noted between changes in $\dot{V}O_2$ and metabolic phosphate concentrations, notably PCr (Whipp and Mahler 1980). As muscular work necessitates hydrolysis of ATP a decrease in [ATP] should be expected, however this was found to not be the case (Fleckenstein *et al.* 1954, Hill 1950, Mommaerts 1954). Instead, increases in creatine (Mommaerts *et al.* 1962) and decreases in PCr (Infante *et al.* 1964, Infante and Davies 1965) were found. Through the creatine kinase reaction, the decrease in [ATP] was buffered as the phosphate group from PCr was transferred, via the creatine kinase enzyme, to adenosine diphosphate (ADP), producing ATP (Cain and Davies 1962, Infante *et al.* 1965). Experimental evidence revealed that the expected relationship between $\dot{V}O_2$ and [creatine] held true for a range of steady state WR transitions (Piiper *et al.* 1968). Subsequent studies showed that the time course of the decline in PCr were similar to that of the increase in $\dot{V}O_2$ (Dydynska and Wilkie 1966, Harris *et al.* 1976, Hultman *et al.* 1967, Mahler 1985, Piiper and Spiller 1970).

Later, with the use of non-invasive phosphorus-31 magnetic resonance spectroscopy, Rossiter was able to simultaneously measure [PCr] and pulmonary $\dot{V}O_2$ and observed that the [PCr] and ϕ_2 $\dot{V}O_2$ changed at rates which were not significantly different (Rossiter *et al.* 1999, 2000, 2002). The kinetics of ϕ_2 pulmonary $\dot{V}O_2$ can thus be used as a proxy to examine the control of metabolic processes in exercising muscle.

First-Order Linear Systems

The application of control systems analysis aids the interpretation of data by quantifying the role(s) of physiologic mechanism(s) (or at least our understanding of them) affecting physiologic processes. Control systems analysis examines the transfer function, which characterizes an output for a known input stimulus. The transfer function can then either be used to help develop theories of control which can then be tested or as a method of testing a theory of control.

A linear system is one in which all of the operators are linear. In a linear system any input, whether that input is a derivative, integral, or arithmetic alteration of another input, will produce a predictable output according to a constant transfer function. That this is the case may be tested by the Principle of Superposition, which is superbly explained by Riggs:

> "The principle of superposition states that in any linear system the output produced by applying several inputs simultaneously is equal to the sum of the outputs produced by applying each input separately" (Riggs 1976)

For a linear system to also be first-order, there cannot be any derivatives higher than first-order and there can only be one first-order derivative. In such a system, there is one variable input for the system, while all parameters are constant.

Many physiologic mechanisms are first-order linear systems controlled by the concentration of one substrate (e.g. Michaelis-Menten kinetics). The response of these systems to a single-step change in the concentration of that substance is exponential. The differential equation for such a system is given below:

$$-\frac{dy}{dx} = k \cdot y(x) \qquad \text{Eq. 3}$$

where y is a variable which decreases as a function of x, k is the rate constant, and x is the independent variable. This demonstrates that the rate of change in y is solely dependent on the value of y at any given x (which is then modified by a constant parameter, k). A more common mathematical form of this equation would be:

$$y(x) = y_s\, e^{-kx} \qquad \text{Eq. 4}$$

where y_s is the starting value from which y projects. In these examples, y decreases as a function of x towards zero and the rate of that decline is dependent on the value of y. The difference between any value of y and zero may be considered the "error signal". The system acts to reduce the error signal. A similar system where the y increases exponentially to a steady-state (y_{ss}) will be described by the equation:

$$y(x) = y_{ss} - y_{ss}e^{-kx} \qquad \text{Eq. 5}$$

where $y_{ss}e^{-kx}$ represents the error term. This can be simplified to

$$y(x) = y_{ss}(1 - e^{-kx}) \qquad \text{Eq. 6}$$

These equations describe an increasing exponential by subtracting the decreasing error term from the steady-state value.

$\dot{V}O_2$ as a first-order linear system is described by the following equation:

$$\dot{V}O_2(t) = \Delta\dot{V}O_{2ss}(1-e^{-t/\tau}) \qquad \text{Eq. 7}$$

In this equation, t represents time, $\dot{V}O_2(t)$ is the $\dot{V}O_2$ at a given time, $\Delta\dot{V}O_{2ss}$ is the steady-state $\dot{V}O_2$ value (Δ is used when the baseline value is not zero), and τ is the time constant. The τ is the inverse of the rate constant (i.e. $\tau = 1/k$) and is thus equal to the amount of time necessary for the response to proceed ~63% toward the steady-state value.

With the idea of a first-order linear system, physiologic control mechanisms could be investigated. In 1955, Chance and Williams published a landmark study which identified the concentration of free ADP as the most probable factor controlling aerobic metabolism (Chance and Williams 1955). In this study, novel biochemical techniques allowed the estimation of $\dot{V}O_2$ from isolated mitochondria with the addition of ADP. The authors found that [ADP] tightly controlled $\dot{V}O_2$ in a manner that followed Michaelis-Menten kinetics. This supported the idea that $\dot{V}O_2$ kinetics is a first-order linear system with [ADP] as the controlling variable. However, it has been observed that [ATP] remains essentially constant with the onset of exercise (Carlson 1963, Crow and Kushmerick 1982, Edwards *et al.* 1972, Rossiter *et al.* 1999) and offset (Edwards *et al.* 1972, Rossiter *et al.* 1999). Therefore the role of CK was considered, first by Chance and colleagues (Chance *et al*. 1962) and later refined by Whipp and Mahler (Whipp and Mahler 1980). This model of control has been supported by studies in isolated muscle (Mahler 1985, Piiper *et al.* 1968), isolated mitochondria (Glancy *et al.* 2008), and later by *in vivo* human studies (Rossiter *et al.* 1999, 2000, 2002). These studies demonstrated that the τ of $\dot{V}O_2$ was proportional to the concentration of PCr. The interaction between CK and the kinetics of $\dot{V}O_2$ was further examined by Meyer and

colleagues who saw $\dot{V}O_2$ kinetics as analogous to a simple resistor-capacitor circuit (Meyer 1988). In this model, the CK reaction and PCr stores were the capacitor, mitochondrial function was a resistor, and ATP flux was current. The electrical circuit model was consistent with the results of the study; PCr changes are linearly related to WR, just as $\dot{V}O_2$ seemingly is. Thus, the addition of CK in the control of $\dot{V}O_2$ could still allow $\dot{V}O_2$ kinetics to be a first-order linear system in submaximal exercise. However, future research would reveal other complications to the concept of $\dot{V}O_2$ kinetics being controlled as a simple first-order linear system.

Slow Component

The slow component has its roots in the double exponential model used by A.V. Hill in 1924 (Hill *et al.* 1924), the model of Margaria, Edwards, and Dill (Margaria *et al.* 1933) for recovery kinetics, and the 1951 article from Henry where he mentions that there may be deviations away from first-order kinetics, especially when O_2 availability for the exercising muscle may become limiting (Henry 1951). However, most attribute the concept of the slow component to the 1956 article from Henry and DeMoor, which expanded on the skepticism of first-order linearity by noting that a simultaneous double-exponential was needed to describe greater increases in WR where more time was needed to reach a steady-state in $\dot{V}O_2$ (Henry and DeMoor 1956). The delay in attainment of steady-state $\dot{V}O_2$ was displayed again in a study from Wasserman in 1967 (Wasserman *et al.* 1967). In this study, it was found that below the "anaerobic threshold" (now the θ_L) the speed of $\dot{V}O_2$ kinetics was invariable, but above the θ_L attainment of a steady-state in $\dot{V}O_2$ was delayed. The results of this study were confirmed in work five years later by Wasserman and Whipp (Whipp and Wasserman 1972). In their study, six subjects each completed six six-minute bouts of various exercise

intensities on a cycle ergometer. Within the Moderate domain there was no increase in $\dot{V}O_2$ over the last three minutes of exercise. However, above θ_L there was a significant increase in $\dot{V}O_2$ over the last three minutes of exercise. This late-onset additional $\dot{V}O_2$ was termed the "slow component". Linnarsson determined through the use of constant-WR bouts during which either intensity or oxygen concentration was altered that this slow component was dependent not only on the absolute WR, but the relative WR (i.e. same % $\mu\dot{V}O_2$) as well (Linnarsson 1974). The observance of the slow component violates the Principle of Superposition and thus serves as evidence that $\dot{V}O_2$ kinetics is not linear for exercise intensities above θ_L.

The exact mechanisms driving the slow component are still being debated. It is well accepted that the majority of the slow component comes from the exercising muscle. This was first determined by Poole and colleagues who measured pulmonary $\dot{V}O_2$ while simultaneously measuring $\dot{V}O_2$ across the exercising lower limb (Poole *et al.* 1991). They found that ~90% of the slow component seen in the pulmonary $\dot{V}O_2$ data was attributable to the slow component of $\dot{V}O_2$ measured at the lower limb. This study helped discredit many of the other mechanisms reported to cause the slow component (e.g. an increasing temperature and work of breathing). To what degree this excess $\dot{V}O_2$ results from the increased recruitment of less efficient fibers (Borrani *et al.* 2001, Crow and Kushmerick 1982, Krustrup *et al.* 2004), decreasing efficiency in active fibers (Rossiter *et al.* 2000, 2002), or excessive metabolic needs for fatiguing, or recovering fibers (Burnley *et al*. 2002, Saunders *et al.* 2000) is undetermined. Thus, the slow component forces a non-linearity of $\dot{V}O_2$ for metabolic rates $>\theta_L$, but the linearity of $\dot{V}O_2$ $<\theta_L$ also needs to be assessed.

On-Off Asymmetries

The Law of Superposition states that the summed outputs of two inputs will be equal to the output of the two individual inputs summed. This must hold true even if one or both of the inputs are negative. Thus, if $\dot{V}O_2$ is a first-order linear system, the on-kinetics should be the mirror image of the off-kinetics. Cerretelli and colleagues showed that this was not the case in 1977. They noticed that for cycle ergometer exercise at about 80% of the subjects' $\mu\dot{V}O_2$, the on-kinetics were markedly slower than the off-kinetics (Cerretelli *et al*. 1977). These findings were expanded upon by Paterson and Whipp who found that the disparity between on- and off-kinetics holds only when WRs exceed $\hat{\theta}_L$ (Paterson and Whipp *et al.* 1991). A study ten years later would elaborate on the effect of WR intensity. Özyener and colleagues demonstrated that on-kinetics displayed a slow component in the Heavy and Severe domains; whereas for off-kinetics, a slow component was only apparent in the Severe and Extreme domains (N.B. the results of the study have been translated into the more commonly used exercise intensity schema) (Özyener *et al.* 2001). Furthermore, the authors found that in the Severe domain, the slow component for the off-kinetics was significantly larger than the slow component of the on-kinetics. Thus, for exercise in the Heavy, Severe and Extreme domains on- and off-kinetics are disparate. This is thus additional evidence that, at least for exercise intensities $>\theta_L$, $\dot{V}O_2$ kinetics is not controlled by a first-order linear system.

Elevated Baseline

The change in the parameters of $\dot{V}O_2$ at various work rates, both above and below θ_L, is further evidence against a first-order linear control of $\dot{V}O_2$. The first example of the effect of an elevated baseline WR was performed by di Prampero and colleagues (di Prampero *et*

al. 1970). They found that τ was shorter when the subject performed a step increase in work rate from a baseline of light exercise rather than when the baseline was rest. These results were corroborated by other studies (Davies *et al.* 1972, Diamond *et al.* 1977). These studies compared the kinetics of a step from a low baseline WR and a step from rest. A transition from rest would contain a large cardiodynamic phase which would both make the overall change appear extremely rapid and misrepresent the response at the muscle. Several studies comparing a higher baseline WR to a low baseline WR (about 20 W) found that τ increased with baseline WR during exercise steps to Heavy or Severe work rates (DiMenna *et al.* 2010, Hughson and Morrissey 1982, Wilkerson *et al.* 2004, Wilkerson and Jones 2007) and entirely within the Moderate domain (Brittain *et al*. 2001, MacPhee *et al.* 2005, Spencer *et al.* 2011, Bowen *et al.* 2011, Wüst *et al.* 2014).

The evidence is less disparate for a changing G. G has largely been found to increase with increasing baseline WRs (Bowen *et al.* 2011, Brittain *et al*. 2001, MacPhee *et al.* 2005, Spencer *et al.* 2011, Wilkerson *et al.* 2004, Wilkerson and Jones 2007). However, one study found no change in G (Hughson and Morrissey 1982). To account for increasing G and τ with elevated WRs would require the transfer function to contain additional derivatives (i.e. the G and τ would need to be calculated before they could be correctly used to calculate $\dot{V}O_2$), which clearly disobeys the first-order linearity of $\dot{V}O_2$ kinetics.

Ramp Incremental Kinetics

Although non-linearities have been demonstrated in the $\dot{V}O_2$ response it is still often treated as a linear system for pragmatic reasons (e.g. utility of the derived parameters and apparent linearity of data set). Whipp saw that four important parameters of $\dot{V}O_2$ could be

gleaned from a single ramp-incremental protocol (Whipp *et al.* 1981). In a ramp incremental protocol the WR is changed continuously (or at least appreciably faster than the response time of the system, becoming in effect, a series of small step increments in WR). As the ramp increment is the integral of the step increment, the $\dot{V}O_2$ response to ramp incremental exercise should be the integral of the $\dot{V}O_2$ response to step increment exercise, assuming $\dot{V}O_2$ to be a linear system (Fujihara *et al.* 1973a, Rossiter 2011, Whipp *et al.* 1981). This is described in the equation for the $\dot{V}O_2$ response to ramp-incremental exercise:

$$\Delta VO_2(t) = \Delta VO_{2ss}[t - MRT\left(1 - e^{-\frac{t}{MRT}}\right)] \qquad \text{Eq. 8}$$

where t is time, $\Delta\dot{V}O_2(t)$ is the change in $\dot{V}O_2$ at a given t, $\Delta\dot{V}O_{2ss}$ is the amplitude, and MRT is the mean response time. MRT is the τ if the function is forced to start from time zero and is equal to the sum of the τ and time delay from an exponential fit to a step increment. Importantly, the ramp incremental protocol allows the determination of four physiologic parameters: $\mu\dot{V}O_2$, $\hat{\theta}_L$, MRT, and efficiency (η). Here η is the ratio of WR to $\Delta\dot{V}O_{2ss}$ (as joules). This is essentially the reciprocal of the G (with $\dot{V}O_2$ converted to energy). Together these parameters describe well the $\dot{V}O_2$ system for an individual and have been used to interpret the mechanisms of the $\dot{V}O_2$ response. However, the use of a single, unchanging transfer function implies first-order linearity; as discussed above, this is not the case and those non-linearities need to be accounted for in the fitting strategies if they are to elucidate the physiologic mechanisms behind $\dot{V}O_2$ kinetics.

Quasi-Linear System

The linearity of physiological systems across different exercise forcing functions has previously been tested with the use of integrative models. In 1973 Fujihara and colleagues

had subjects perform impulse, step and ramp-incremental exercise protocols on a cycle ergometer. The parameters describing the kinetics of ventilation and heart rate from the impulse exercise were used to predict the physiologic responses from a step function (via a model incorporating a single-integral) and a ramp-incremental function (via a model incorporating a double-integral). The authors found that the predicted models were able to reliably match the actual responses within one standard error of the actual data (Fujihara *et al.* 1973a, b). In a 2011 review of $\dot{V}O_2$ kinetics, Rossiter modelled the independent effect of different MRT and G values on the $\dot{V}O_2$ response to ramp incremental exercise (Rossiter 2011). Briefly, longer MRTs delayed the onset of the linear portion of the $\dot{V}O_2$ response, while larger Gs increased the slope of the $\dot{V}O_2$ response ($\Delta\dot{V}O_2/\Delta WR$). Rossiter noted that previous studies had demonstrated that the MRT and G increased proportionally to each other as WR increases (as described above in the Elevated Baseline section). Taking these changing parameters into consideration, Rossiter predicted the $\dot{V}O_2$ response to ramp incremental exercise and found this estimation appeared quasi-linear. The $\dot{V}O_2$ response to ramp incremental exercise was thus best described not as a single linear system, but as the integration of several kinetic responses from multiple sources (Rossiter 2011, Whipp *et al.* 2002). The simplicity of the system controlling $\dot{V}O_2$ is thus invalidated, but this more complicated model should allow for a more accurate and thus useful description of $\dot{V}O_2$ kinetics.

Significance

The value of $\dot{V}O_2$ kinetics lies in its ability to help elucidate the controlling factor(s) behind exercise (in)tolerance. $\mu\dot{V}O_2$ and θ_L have often been used to estimate endurance exercise performance (reviews: Bassett and Howley 2000, Joyner and Coyle 2008). These

correlations may serve to predict exercise performance, but they fail to explain the underlying mechanisms which lead to fatigue. A greater understanding of the mechanisms causing fatigue would help by providing direction for more effective interventions to alleviate or delay fatigue. This greater understanding may be achieved through the use of control systems analysis to describe and test our understanding of physiological mechanisms and the relationships among them.

Whether the difference in exercise tolerance is milliseconds separating an Olympic gold-medalist from the silver-medalist, or the result of exercise training allowing a heart failure patient to leave his chair and walk across the room, $\dot{V}O_2$ kinetics can have a great effect on a person's quality of life. The effect of G may be more easily appreciated when considering its reciprocal value $WR/\dot{V}O_2$, or "efficiency". When considering the case of two individuals exercising at the same steady-state level of $\dot{V}O_2$, it is clear that the person with the higher efficiency, i.e. lower G, would be exercising at a higher WR (Coyle 1999). Perhaps more important to consider is the case of the same individual pre- and post-training where improvements in "efficiency" have been observed (Jones 2006, Santalla *et al.* 2009).

The role of MRT in exercise (in)tolerance may be less apparent initially, but may be seen in the difference of the amount of O_2 deficit incurred with step exercise. For constant WR exercise, the O_2df is calculated by:

$$O_2df = \Delta\dot{V}O_{2ss} \cdot MRT \qquad \text{Eq. 9}$$

where O_2df is the O_2 deficit, $\Delta\dot{V}O_{2ss}$ is the difference between the baseline and steady state $\dot{V}O_2$, and MRT is the mean response time. When faced with the same increase in WR, two individuals with the same $\Delta\dot{V}O_{2ss}$ but different MRTs would have different O_2df's. As the O_2df

represents the amount of energy needed for exercise, but not supplied by oxidative metabolism, this would require the use of PCr stores and/or lactic acid fermentation which, as described earlier, are of limited supply and may produce fatigue inducing metabolites. Therefore, the individual with the longer MRT would fatigue earlier. The parameters MRT and G and their relationship to each other can thus provide important insights into the role of $\dot{V}O_2$ and its kinetics in fatigue. These insights can then direct interventions to more effectively alleviate or delay fatigue.

$\dot{V}O_2$ kinetics have revealed much about the control of aerobic metabolism, but there is much they still conceal. Although initially understood as a simple first-order linear system, more recent studies inspecting the $\dot{V}O_2$sc, asymmetries between on- and off-kinetics, and elevated baselines have shown the system to have markedly non-linear characteristics. The focus of this study is to determine how non-linear parameters combine to result in $\dot{V}O_2$ responses that appear strikingly linear or at least quasi-linear. This will be accomplished by empirically determining the G and either MRT or τ and δ at several WR using the $\dot{V}O_2$ response from an extended-step incremental protocol. These parameter values will then be used in an integrative model to estimate the $\dot{V}O_2$ response to a ramp protocol. An accurate estimation of the $\dot{V}$O2 response by a model incorporating parameter values which change with WR will demonstrate how the non-linear control system which determines $\dot{V}O_2$ can produce a linear response. This understanding will then aid in the interpretation of ramp protocols which have been used as a diagnostic and evaluative tool for both patient and elite athletic populations (Jones and Poole 2005, Hansen *et al.* 2012).

Chapter 3 – Methods

Participants

Six healthy men [(mean ± SD) age: 23.5 ± 2.4 years; height: 178.2 ± 5.7 cm; weight: 77.6 ± 9.2 kg] participated in this study (Table 1). All participants were nonsmokers and free of known cardiovascular, respiratory, and metabolic diseases as indicated by medical questionnaire which was distributed before testing. All participants gave their written, informed consent for the study as approved by the Kansas State University Institutional Review Board and adhered to the *Declaration of Helsinki*. All participants were instructed to not consume caffeine or food for at least two hours before each test and to avoid strenuous exercise for the 24 hours before each test.

Measurements

Height and weight were recorded for each subject. Breath-by-breath gas exchange was measured using an open circuit metabolic system (Ultima CardiO_2, Medical Graphics, St. Paul, MN, USA). The system measured pulmonary oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and minute ventilation ($\dot{V}_E$). Before each testing session, the flow transducer was calibrated using a 3 liter syringe and the oxygen and carbon dioxide sensors were calibrated using precision-analyzed gases.

Exercise Protocol

All participants adjusted the seat height and handlebars on the cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) to their own specifications. The handlebar and seat positions were then recorded and reset to the same values for every test. Each participant completed seven bouts of exercise to volitional exhaustion over a

period of three to four weeks. There was at least 48 hours between each exercise bout. The first three bouts were ramp incremental exercise of varying ramp rates and the last four bouts were extended step-incremental. Participants were instructed to maintain a pedal cadence between 70 and 75 rpm. The ramp rates were either slow at 15 W/min (SR), regular at 30 W/min (RR), or fast at 60 W/min (FR). Each participant completed one test at each ramp rate, the order of which was randomized. Each ramp test began with five minutes of pedaling at a baseline WR of 25 W. The initiation of the ramp was not indicated to the participant and the ramp increase in WR continued until the participant failed to maintain 70 rpm for five seconds despite vigorous verbal encouragement. The WR was then reduced to 25 W for five minutes of recovery.

The gas exchange data from the ramp incremental tests provided the $\dot{V}O_2$ at baseline ($\dot{V}O_{2\,BL}$), maximal oxygen uptake ($\mu\dot{V}O_2$), and the estimated lactate threshold ($\hat{\theta}_L$). $\mu\dot{V}O_2$ was determined as the highest 15 s bin-averaged value across the three ramps and validated as similar $\dot{V}O_2$ values (<150 ml O_2/min difference) were reached in both the SR and RR conditions. $\hat{\theta}_L$ was determined via V-slope method (Beaver *et al.* 1986) in combination with examination of ventilatory equivalents and end-tidal pressures of O_2 and CO_2 and the respiratory exchange ratio (Koyal and Beaver 1973). These parameters were then used to establish the WRs for the extended-step incremental protocol (Figure 1). Each step incremental test began with five minutes of cycling at 25 W. The WRs for the next five steps were based on the $\dot{V}O_2$ at baseline WR, $\hat{\theta}_L$, and $\mu\dot{V}O_2$ values for each subject. Each target $\dot{V}O_2$ value (described below) was paired to the closest five-breath mean value from the RR. 20 W were then subtracted to account for a 40 s MRT (Hughson and Morrissey 1982). Using that procedure, the target $\dot{V}O_2$ values for each of the five steps were: 1) 45% of the

difference between $\dot{V}O_{2\,BL}$ and $\hat{\theta}_L$ (WR 1), 2) 90% of the difference between $\dot{V}O_{2\,BL}$ and $\hat{\theta}_L$ (WR 2), 3) 20% of the difference between $\hat{\theta}_L$ and $\mu\dot{V}O_2$ (WR 3), 4) 40% of the difference between $\hat{\theta}_L$ and $\mu\dot{V}O_2$ (WR 4), and 60% of the difference between $\hat{\theta}_L$ and $\mu\dot{V}O_2$ (WR 5). Each stage below $\hat{\theta}_L$ lasted five minutes while each stage above $\hat{\theta}_L$ lasted 15 minutes or until volitional exhaustion. Stages >$\hat{\theta}_L$ were held for 15 minutes to allow the slow component to fully develop for $\dot{V}O_2$ to reach a steady state if possible (i.e. if below CP or θ_c). Four repetitions of this extended step-incremental protocol were performed by each participant on separate days.

Data Processing

Offline, all gas exchange data were corrected for bad breaths. Bad breaths were defined as those outside three standard-deviations of the middle-five-of-seven breaths (i.e. the breath in question and the three preceding and subsequent to it). These values were then replaced with the mean value of those same middle-five-of-seven breaths. These breath-by-breath data were then interpolated to second-by-second values. The ramp data were then averaged into 30 s bins, while the step-incremental data were separated into each step. The four bouts for each stage were then time-aligned and averaged together. These second-by-second data were then averaged into 5 s bins. The final 30 s of the previous stage was used as the baseline value for the next stage.

These averaged stages were then fit using non-linear regression with minimization of the residual sum of squared errors (SigmaPlot 12.5, Systat Software, San Jose, CA, U.S.A.) to each of two monoexponential models (which are graphically described in Figure 2): 1) full stage with no time delay (MONO), 2) first five minutes of each stage with a time delay

(5TD). The parameters (G and either MRT or τ and δ) determined for each of these stages were then assigned to the WR halfway between the previous and current stages. These parameters were then point-to-point linearly interpolated to provide values for these parameters across the entire range of WR performed during the ramp tests (Figure 3). The parameter values from the first step of the extended-step incremental protocol were extrapolated as constant values back to the baseline WR (25 W). The parameter values from the highest useable step of the extended-step protocol (40%Δ) were extrapolated as constant values up to the peak WR for each ramp. Parameter values were thus available for the range of WRs encountered during each ramp test.

These parameters were then used in a series of integrative models to estimate $\dot{V}O_2$ responses to each ramp test. There were two models used, corresponding to the fitting models described above. The integrative models were designed to emulate the actual ramp tests already performed. Therefore, each test began with a 25 W baseline. Every 30 s a new monoexponential component commenced with parameters set by the corresponding WR. For every 30 s all active monoexponential components were summed and added to the baseline $\dot{V}O_2$ value determined by the average $\dot{V}O_2$ over the last 30 s of the 25 W baseline from the corresponding actual ramp test (Figure 4). This method provided an estimated value every 30 which could then be compared to the actual 30 s values from each ramp style (Figure 5).

Each model estimate and actual response were then graphed as a function of a time. For the computation of G, the first minute of data were excluded. For the computation of MRT, the first minute and data above $\hat{\theta}_L$ were excluded. A linear regression was then

applied to each plot. The values of the slope and y-intercept were then used to calculate the G and MRT. G was calculated from the following equation

$$G = \left(\frac{slope}{ramp\ rate}\right) \cdot 1000 \qquad \text{Eq. 10}$$

Where slope is given in l O_2/min/s and ramp rate is given in W/s to ultimately give G in units of ml O_2/min/W (Figure 6).

MRT was calculated, as previously described (47), from the following equation

$$MRT = \frac{(VO_{2BL} - b)}{slope} \qquad \text{Eq. 11}$$

Where $\dot{V}O_{2BL}$ is the baseline $\dot{V}O_2$ value and b is the y-intercept of the linear regression. This provided a MRT value in units of s. For the calculation of MRT, the first minute of data and all data above $\hat{\theta}_L$ were excluded (i.e. only S1).

Statistics

To evaluate whether G and MRT increased over the range of WRs used in the step protocol, a one-way repeated measures ANOVA was completed for each fitting strategy. To evaluate the accuracy of each model's estimation (for any subset of data), a two-way repeated measures ANOVA was completed for each calculated parameter from the linear-regression (i.e. G and MRT) using ramp-rate and estimation model as the factors being assessed. As the investigation of multiple factors decreases the power of the test, paired-t tests with Bonferroni correction were used to further investigate the change in neighboring parameter values from each step of the extended step-incremental protocol.

As we did not obtain parameter values for WRs all the way up to $\mu\dot{V}O_2$, we also compared the models only up to 80%$\mu\dot{V}O_2$ to evaluate the models over a range of WRs for which we did have parameter values. Furthermore, previous studies have shown a difference in the G of the $\dot{V}O_2$ responses below versus above $\hat{\theta}_L$ (i.e. S1 and S2) across different ramp rates. We therefore compared the models using data from only S1 (excluding data above $\hat{\theta}_L$ and within the first minute) and S2 (using only data above $\hat{\theta}_L$ and below 80%$\mu\dot{V}O_2$). The same two-way repeated measures ANOVAs were used for these datasets. Statistical significance was determined at the α=0.05 level. However, we include any p-value below 0.10 for the consideration of the reader.

Chapter 4 – Results

Ramps

$\hat{\theta}_L$ was determined only from the RR data. For the group, $\hat{\theta}_L$ was 2.13±0.26 (mean±SD) (Table 2). Peak $\dot{V}O_2$ values were determined for each ramp rate and were not significantly different for the SR and RR ramp rates (SR: 3.98±0.40, RR: 3.90±0.41 l/min) nor for RR and FR (FR: 3.75±0.55 l/min). This confirmed that the peak $\dot{V}O_2$ value was indeed the $\mu\dot{V}O_2$. However, the SR peak $\dot{V}O_2$ values were significantly greater than for FR (p=0.037) (Figure 7).

Extended-Step Protocol

The actual end-stage $\dot{V}O_2$ values for the extended-step protocol were not significantly different than the target $\dot{V}O_2$ values (the $\dot{V}O_2$ value which determined each WR in the extended step-incremental protocol) for WRs below $\hat{\theta}_L$ (Table 3). Above $\hat{\theta}_L$, the actual $\dot{V}O_2$ values were significantly greater than the target $\dot{V}O_2$ values (p=0.002 and p<0.001 for

WR 3 and 4, respectively). Although the end stage $\dot{V}O_2$ from the highest stage at which $\dot{V}O_2$ parameters were measureable (WR 4) was significantly different than $\mu\dot{V}O_2$ ($p=0.420$), the end $\dot{V}O2$ from the extended-step protocol was not significantly different than the $\mu\dot{V}O_2$.

For both data-fitting strategies (MONO and 5TD), the Gs of the extended-step protocol transitions were significantly greater with higher WRs ($p<0.001$ and $p=0.003$ for MONO and 5TD, respectively) (Table 4). For MONO, the G of WR 4 was significantly greater than the lower three transitions ($p<0.001$ for all). The G of WR 3 was significantly greater than WR 1 ($p=0.013$), but was not significantly different than WR 2. The G of WR 2 was not significantly greater than WR 1. For 5TD, the G of WR 4 was significantly greater than WR 1 ($p=0.002$) and WR 2 ($p=0.036$). No other significant differences in G were found with the two-way repeated measures ANOVA. However, for MONO the paired-t tests showed significant increases in G from WR 1 to 2 ($p=0.003$), WR 2 to 3 ($p=0.009$), and WR 3 to 4 ($p=0.003$). The same tests for 5TD only showed a significant increase from WR 1 to 2 ($p=0.003$) but not for WR 2 to 3 nor for WR 3 to 4.

For both data-fitting strategies, the MRT or τ of the extended-step protocol steps was significantly greater with higher WRs ($p<0.001$ and $p=0.026$ for MONO and 5TD, respectively) (Table 5). For MONO, the MRT of WR 4 was significantly greater than the lower three transitions ($p<0.001$ for all). No other significant differences in MRT were found. For 5TD, WR 4 was significantly greater than WR 1 ($p=0.024$). No other significant differences in τ were found. However, for MONO the paired-t tests showed no significant increase in MRT from WR 1 to 2 ($p=0.029$; N.B. Bonferroni correction for three hypotheses lowers the p-value threshold of significance to $p=0.0167$) but significant increases for both

WR 2 to 3 and WR 3 to 4 (p=0.008 and 0.002, respectively). The same tests for 5TD showed a significant increase from WR 1 to 2 (p=0.001) but not for WR 2 to 3 nor for WR 3 to 4.

Accuracy of MRT Estimations

As a main effect, the MRT estimations from the models were not significantly different than ACT (Table 6). For the MON model, the estimated MRT was significantly different than ACT for SR (p=0.019), but not for RR nor for FR. For the 5TD model, the estimated MRTs were not significantly different than ACT for any ramp rate (p=0.096 for SR). As MRT is calculated using only the data after one minute and up to $\hat{\theta}_L$, these values will be the same for the S1, $\mu\dot{V}O_2$, and 80%$\mu\dot{V}O_2$ data subsets. MRT was not calculated for S2 segments.

Accuracy of G Estimations Up to $\dot{V}O_2pk$

Within ACT, the G was not significantly different between SR and RR (SR: 11.3±1.2, RR: 10.5±0.8) (Table 6). However, the G of FR was a significantly less than both SR (FR: 8.7±1.0; p<0.001) and RR (p=0.009). Up to $\mu\dot{V}O_2$, the Gs of both model estimates were not significantly different than the G of the ACT data (p=0.064 for MONO). There were no significant differences between the Gs estimated by either model and ACT for SR nor RR. However, for FR the Gs predicted by both models were significantly different than ACT (p=0.004 and p<0.001 for MONO and 5TD, respectively). The Gs predicted by the two models were significantly different from each other for SR (p=0.028) but not for RR nor FR.

Accuracy of Gain Estimations Up to 80% Maximal $\dot{V}O_2$

Up to 80%$\mu\dot{V}O_2$, the ACT G for FR was significantly less than SR (FR: 9.2±1.0, SR: 10.7±1.1; p<0.001) and RR (RR: 10.2±0.5; p=0.001). Whereas SR and RR were not

significantly different (Table 7). *T*he G estimates from both models were not significantly different than the ACT G. G was not significantly different between the model estimates and ACT for any ramp rate.

Accuracy of Gain Estimations Within Segment 1

In ACT, there were no significant differences for the G of the S1 region across ramp rates (SR: 10.1±0.9, RR: 9.7±0.9, FR: 9.7±1.7) (Table 8). In addition, within S1, the G estimates from both models were not significantly different than the ACT G. The G estimates from both models were not significantly different than the ACT G for any ramp rate.

Accuracy of G Estimations Within S2

The G of the S2 segment in ACT was not significantly different between SR and RR (SR: 11.1±2.0, RR: 10.5±1.2; $p=0.079$); however, the G of the S2 region was less for FR than both SR and RR (FR: 8.4±1.6; $p<0.001$, for both) (Table 8). As a main effect, within S2, the G estimates from both models were not significantly different than the ACT G. The G estimates from the MONO model were not significantly different than the ACT G for any ramp rate ($p=0.069$ for FR). The G estimates from the 5TD model was significantly different than the ACT G for FR ($p=0.003$) but not for RR nor for SR.

Chapter 5 – Discussion

This study demonstrated a significant increase in G and τ or MRT with increased WR for both the MONO and 5TD fitting strategies, consistent with hypothesis one. Up to $\mu\dot{V}O_2$ the integrative models produced inaccurate estimates of the actual $\dot{V}O_2$ response to a ramp incremental protocol. However, when the models were run to work rates up to which

actual parameter values were available (i.e. 80%μ$\dot{V}O_2$), the models were accurate for a range of ramp rates, consistent with hypothesis two. However, in contrast to hypothesis three, the quality of ramp response estimation was not improved by the inclusion of a time delay and limiting the influence of the $\dot{V}O_2$sc.

Gain and τ or MRT Related to WR

Our data demonstrate a positive correlation between WR and both G and MRT or τ. These findings agree with previous research which shows that both end-exercise G and τ increase with WR. Several studies have shown that the end-exercise G increases with WR (Brittain *et al*. 2001, Özyener *et al.* 2001, Paterson and Whipp 1991, Scheuermann and Barstow 2003, Spencer *et al.* 2011, 2013, Wilkerson *et al.* 2004). Below $\hat{\theta}_L$ several studies have shown that the G of the primary phase increases with increases in baseline WR (Bowen *et al.* 2011, Brittain *et al*. 2001, MacPhee *et al.* 2005, Spencer *et al.* 2011, 2013). Above $\hat{\theta}_L$, the G of the primary phase has been shown to decrease, yet the end-exercise G continues to increase (Carter *et al*. 2002, Pringle *et al.* 2003, Scheuermann and Barstow 2003, Wilkerson *et al.* 2004). This occurs primarily as a result of the increasing O_2 cost mediated by the $\dot{V}O_2$sc (Scheuermann and Barstow 2003, Wilkerson *et al.* 2004). Thus, for a simple monoexponential response, as modelled in this study, the (end-exercise) G has been shown to increase with WR.

Previous research has also shown that an increasing WR produces longer τ or MRT values. Below $\hat{\theta}_L$, τ of the primary response has been shown to be longer for higher baseline WRs (Bowen *et al.* 2011, Brittain *et al*. 2001, Hughson and Morrissey 1982, MacPhee *et al.* 2005, Spencer *et al.* 2011). Studies have also shown a greater τ of the

primary response with exercise above $\hat{\theta}_L$ compared to exercise below $\hat{\theta}_L$ (DiMenna *et al.* 2010, Koga *et al.* 2001, Koppo *et al.* 2004, Paterson and Whipp 1991, Wilkerson and Jones 2006, 2007) although some studies suggest otherwise (Koga *et al.* 1999, Özyener *et al.* 2001, Scheuermann and Barstow 2003). To help clarify these mixed results, Jones and Poole combined the mean results of 25 studies and found that the τ of the primary response was typically about 20% longer for exercise above $\hat{\theta}_L$ (Jones and Poole 2005).

The mechanism(s) behind an increase in τ with increasing WR are still under debate. Two mechanisms have received the most attention: 1) muscle fiber recruitment patterns (Brittain *et al*. 2001, Hughson and Morrissey 1982) and 2) muscle blood flow kinetics (Breese *et al.* 2012, Goodwin *et al.* 2012, Hernandez *et al.* 2010, MacPhee *et al.* 2005). As exercise intensity increases, myofibers with progressively slower τ and lower efficiencies are recruited. (Henneman *et al.* 1965, Crow and Kushmerick 1982, 1983, Coyle *et al.* 1992). The responses of these active fibers will then be summed to provide a pulmonary (or muscle) $\dot{V}O_2$ response which will appear to be simply a mono- or double exponential response (Brittain *et al*. 2001, Whipp 2002). Muscle blood flow kinetics were purported to slow $\dot{V}O_2$ by limiting O_2 availability at the muscle (Hughson and Morrissey 1982, MacPhee *et al.* 2005). However, recent evidence suggests that these two mechanisms are not the only mediators of the increase in τ (Wüst 2014). This study used a canine gastrocnemius preparation in which a pump was used to control blood flow and the sciatic nerve was activated maximally. Therefore, blood flow kinetics and muscle unit activation were both controlled and there was still a slowing of τ.

In the current study, both WRs 3 and 4 were above $\hat{\theta}_L$, thus the $\dot{V}O_2$sc should be present and would be expected to increase both τ or MRT and G as compared to WRs 1 and 2, which were below $\hat{\theta}_L$ (Özyener *et al.* 2001, Paterson and Whipp 1991, Poole *et al.* 1988). We thus expected the differences between WRs 4 and 1, but were surprised that there were not more significant differences between WRs above versus below $\hat{\theta}_L$. We believe this dearth of differences is due to our analyses being statistically under-powered (power analysis showed n=38 for G and n=23 for MRT). Although many WR differences did not reach statistical significance, for each individual, all parameter values increased with WR (except for the MRT in one subject which went from 49 s during WR 1 to 45.6 s during WR 2). Therefore, we believe that the analyses were under-powered for a two-way repeated measures ANOVA and the addition of more subjects would have resulted in statistically significant increases in both G and MRT or τ with increasing WRs. To reduce the factors being evaluated (and thereby increase the power of the tests), we also ran paired-t tests on the neighboring WR data. These tests support our conclusion that although the 2-way repeated measures ANOVAs did not show many statistically significant differences between the work rates, this was likely due to the tests being under-powered and not a reflection of truly consistent physiological underpinnings. Regardless of the statistical analysis of the data, the models utilized parameter values based on the exact empirically derived parameter values for each subject, which did increase with WR.

Estimated Versus Actual Results

When the data up to $\mu\dot{V}O_2$ were compared, the models produced estimates that were significantly different than the ACT responses. When compared to the ACT response, the MONO model produced significantly different parameter values for both G and MRT

and the 5TD model produced a significantly different G (MRT was p=0.057) (Figure 8 and Table 6). The ramp-rate by model comparison within the two-way repeated measures ANOVA performed was under-powered for the test on MRT (β=0.889; power analysis shows n=63). This is primarily due to the considerable imprecision in calculating MRT using linear regression, especially from single ramp incremental responses (Hughson and Inman 1986, Markovitz *et al.* 2004). Much of this imprecision is due to the leveraging of data during linear regression where minute changes in G can result in extreme changes in MRT (Hughson and Inman 1986). Furthermore, differences in baseline $\dot{V}O_2$ values can provide another source of imprecision. All ramps and extended step protocols were started from a baseline WR of 25 W and a notable intrasubject variability in $\dot{V}O_2$ was observed (range mean was 128 ml O_2/min). That discrepancy in baseline $\dot{V}O_2$ alone could explain 13 s of variability in the SR. Moreover, these discrepancies will be magnified within the SR condition where, due to the expansion of the scale of the abscissa, small differences in the calculation of G or baseline $\dot{V}O_2$ would result in larger differences in time. With these sources of variability in mind, we believe these results should be interpreted with caution and do not consider the lack of fidelity in MRT values between the models and ACT in the SR alone to evince a shortcoming of the model. However, a discrepancy in the G data would cast serious doubt upon the efficacy of these models.

It is more important that the G, or the slope, of the models estimate the ACT response well. The objective of the model was to accurately estimate the changing $\dot{V}O_2$ over a ramp incremental protocol given increasing parameter values. The G (more precisely ΔG) describes exactly this change in $\dot{V}O_2$ over a change in WR without specific regard for the baseline $\dot{V}O_2$ value. When the data up to $\mu\dot{V}O_2$ were compared, the main effect for G was not

significantly different between either model and ACT. However, this finding is weighted by the SR and RR tests as the G was significantly different for both models within the FR ramp rate. In both cases, the models over-estimated the G compared to the ACT response. The quality of the estimation is inversely related to the ramp rate (Figure 9). As ramp rate increases, so does the over-estimation of the models.

This over-estimation could be due to the model using parameters that were not obtained at the highest $\dot{V}O_2$ values. Although the protocol had a 60%Δ stage, four of the six subjects fatigued before sufficient data were collected for fitting of the $\dot{V}O_2$ response. Therefore, the highest stage for which we obtained parameter values was WR 4 (40%Δ). However, the $\dot{V}O_2$ value at the end of the WR 4 stage was significantly different than $\mu\dot{V}O_2$ and only 86.5±5% of the subjects' $\mu\dot{V}O_2$. As a WR, the WR 4 stage was much lower than the peak WR from each ramp (86.0±3.5%, 74.3±3.6%, and 65.2±2.9% for SR, RR, and FR, respectively). In this model, for WRs above WR 4 the parameter values were kept constant. Thus, for the FR, the models estimate $\dot{V}O_2$ values for the highest 35% of WRs based on the same values for G and MRT.

Previous studies have shown that at WRs associated with peri-maximal $\dot{V}O_2$s MRT stays relatively constant while G decreases (Scheuermann and Barstow 2003, Wilkerson *et al.* 2004). These values were obtained using multiple step transitions from a single, lower baseline WR. A stage at these highest WRs was not possible with this extended-step protocol as task-failure occurred at stages with a lower WR. However, a decreasing G at higher WRs would decrease the estimated $\dot{V}O_2$ value at the highest WRs and thus ameliorate the over-estimations of the models. As we did not have exact WR values at

which this decline should begin, nor a reliable factor by which to decrease the G from our highest WR, we were unable to directly apply this concept in our model.

To better understand the accuracy of the model for WRs at which we had parameter values which were empirically derived, we ran the models only up to 80% of $\mu\dot{V}O_2$. Although the r^2 values decreased, removing the values from the top 20% of $\dot{V}O_2$ resulted in much more accurate estimations of the data (Figure 10). The decrease in r^2 values in this case is not a reflection of more disparate estimated values, but instead is a result of the decrease in the ranges of values. Using these datasets, the G was not significantly different between either model and ACT for any ramp rate (Table 7 and Figure 11). As MRT is calculated by truncating the dataset to only include values up to $\hat{\theta}_L$, the MRT values did not change by cutting the dataset down to 80%$\mu\dot{V}O_2$. However, as stated earlier, we do not believe that the discrepancy between the models' and ACT values for MRT in SR alone evinces a failure of the model.

Reliability Across Ramp Rates

Testing a variety of ramp rates was important as previous studies have shown that both the MRT (Boone *et al.* 2008, Scheuermann *et al.* 2002, Swanson and Hughson 1988) and G (Boone *et al.* 2008, Hansen *et al.* 1988, Scheuermann *et al.* 2002, Swanson and Hughson 1988) decrease with increasing ramp rate (although see Davis *et al.* 1982 for contrary results). The current data support those findings as G decreased significantly with increasing ramp rate. However, in the current study, MRT was found to be significantly shorter for SR than for RR and FR (as may be seen in the ACT data in Table 6). This shorter MRT in the SR condition is heavily weighted by two trials which resulted in negative values.

The MRT was determined from only data below $\hat{\theta}_L$ and the first minute was excluded, however if the response had not yet reached its "linear" portion, a shorter MRT would be calculated. We attempted removing the first 120 s of data, but some values were still negative. This reflects the unreliability of MRT calculations from single ramp responses that has been previously reported (47, 68).

S1 vs S2

The model fits up to 80%$\mu\dot{V}O_2$ provided close estimates of G; however, several previous studies have further separated the ramp response into segments below (S1) and above (S2) θ_L. This separation is important to consider as the $\dot{V}O_2$sc begins to develop at WRs above θ_L (Henry 1951, Paterson and Whipp 1991, Poole *et al.* 1988, Whipp and Wasserman 1972). A slower ramp rate allows more time for the $\dot{V}O_2$sc to develop and manifest itself in the whole-body $\dot{V}O_2$ response, which would increase the G of the S2. Within our data, the average time spent above θ_L during SR was 590 s whereas in the FR it was only 195 s. Studies have shown that there is an appreciable time delay (~90 s) before the manifestation of the $\dot{V}O_2$sc during constant WR exercise above $\hat{\theta}_L$ (Barstow *et al.* 1990, Henry 1951, Poole *et al.* 1988, Whipp and Wasserman 1972). There is thus, on average, less than two minutes for the $\dot{V}O_2$sc to manifest during FR whereas the SR allows nearly 12 minutes.

Previous studies have shown a significant increase in G for S2 (compared to S1) with ramp rates under 20 W/min (Hansen *et al.* 1988, Scheuermann *et al.* 2002, Takaishi *et al.* 1992 (although not significant)). For the SR in the current study, the group difference between S1 and S2 was not significantly different (p=0.078), although four of the six

subjects showed an increase in G for S2, as seen in Table 8. For ramp rates between 20 and 40 W/min, the literature is more ambiguous as the data from Takaishi show a larger S1 while the data from Hansen shows a larger S2 (Hansen *et al.* 1988, Takaishi *et al.* 1992). For RR in the current study, the difference between S1 and S2 was not significantly different ($p=0.158$), although four of the six subjects showed an increase in G for S2 (not the same subjects as for SR). Previous studies of ramp rates greater than 40 W/min have shown either no significant difference between S1 and S2 (Hansen *et al.* 1988) or a significantly smaller S2 (Scheuermann *et al.* 2002). For FR in the current study, the group mean difference between S1 and S2 was not significantly different ($p=0.101$), although four of the six subjects showed a decrease in G for S2 (the same subjects as for RR. For all ramp rates, the number of subjects displaying a greater S1 or S2 was always split. These discrepancies greatly reduce the power of the statistical tests for sectional differences across ramp rates In all two-way repeated-measures ANOVA comparisons of S1 and S2 the tests were underpowered ($\beta= 0.950, 0.866, 0.376$ and power analysis showed $n=87, 50, 34$ for SR, RR, and FR, respectively). Interestingly, the two subjects with both the highest relative $\mu\dot{V}O_2$ (both above 55 ml/kg/min) and $\hat{\theta}_L$ (both above 30 ml/kg/min) were the subjects whose S2 was greater than S1 across all ramp rates.

Both models tracked the directional differences between S1 and S2 that occurred in the ACT responses, but the MONO model did so more accurately. The Gs from the MONO model responses were not significantly different than ACT for either S1 or S2 within any ramp rate (Table 8). The Gs from the 5TD model responses were not significantly different than ACT for either S1 or S2 within any ramp rate, except S2 in FR where the 5TD model produced a greater G.

The G of S1 in this study was not significantly different across ramp rates. Previous studies have been ambiguous as to whether the G of S1 decreases as ramp rate increases. Hansen and colleagues' data show no difference between ramp rates of 15 and 30 W/min but a decrease at a ramp rate of 60 W/min (Hansen *et al.* 1988). Scheuermann's data show a greater G for S1 for 8 W/min than for 64 W/min (Scheuermann *et al.* 2002). Takaishi's data show no difference in the G of S1 across ramp rates of 10, 20, 30, and 40 W/min (Takaishi *et al.* 1992). Some of this ambiguity is likely due to the low quantity of data points for the higher ramp rates. To increase certainty of the actual external WR for cycle exercise, many studies start at a low WR near 25 W/min (Brittain *et al*. 2001, Özyener *et al.* 2001). This baseline WR requires a baseline $\dot{V}O_2$ of about 800 ml O_2/min, which, when combined with a $\hat{\theta}_L$ of about 2000 ml O_2/min and a G of 10 ml O_2/min/W, only provides about 120 seconds of data in a 60 W/min ramp protocol. Ideally, only the "linear" portion of the response would be fit, so at least the initial minute would be removed. There is thus only about one minute of data which may be fit, which leaves very few data points available for fitting (especially as several seconds may pass between breaths at these lower WRs and the data are then often bin-averaged). In the current study, the fit of the S1 portion of the FR used only about 4 points.

Relationship Between MRT or τ and Gain

The quasi-linear $\dot{V}O_2$ response to ramp incremental exercise seems to be due to the relationship between the non-linear parameters of G and MRT or τ. Both the end-exercise G and MRT were seen to increase with WR. As the WR increases in a ramp incremental protocol, these parameters balance each other to maintain an overall quasi-linearity. An increase in G means that the subject is becoming less efficient, i.e. using more oxygen per

minute per W. This would result in a growing upward shift in the $\dot{V}O_2$ response to ramp incremental exercise, as seen in Figure 12. An increase in MRT means that the system is taking longer to exhibit a given increase in $\dot{V}O_2$. As the increase in $\dot{V}O_2$ is slowed, the $\dot{V}O_2$ at a given time is less than it would be if MRT was constant. This appears as a growing downward shift in the $\dot{V}O_2$ response to ramp incremental exercise. Therefore, as both parameters increase, the upward shift caused by an increasing G is balanced by the downward shift caused by the increasing MRT.

Comparing the change in G to the change in MRT reveals this balance, as demonstrated in Figure 13. When the parameter values for all subjects from the extended step incremental are plotted against each other as a percent change from WR 1, it becomes apparent that although there is some intersubject variability, these parameters increase at a consistent proportion. The regression for the group data reveals that about 83% of the variation in MRT can be explained by the variation in G. Furthermore, for the group, a larger change in MRT is needed to balance a smaller change in G to maintain linearity. Therefore, a change in G has a stronger relative influence on the $\dot{V}O_2$ response.

Limitations

The extended step incremental protocol was designed to segment the $\dot{V}O_2$ response to specific regions. We believed that allowing each stage to reach a steady-state would more distinctly describe the $\dot{V}O_2$ response to each increase in WR. However, during that time, the milieu of the myofibers undoubtedly changes. Therefore, the state of the cells may be different than that at the same WR during a ramp incremental test. Additionally, only six participants were used in this study, which left some comparisons under-powered.

Furthermore, the protocol involved several smaller steps, which resulted in smaller $\dot{V}O_2$ responses (only a few hundred ml O_2). This resulted in a small signal to noise ratio for the fitting of the $\dot{V}O_2$ responses. Furthermore, our metabolic cart does not calculate alveolar gas exchange. We took measures to decrease the noise such as correcting bad breaths, averaging four replicate transitions, and bin-averaging the data. However, despite these efforts, we were unable to fit the data with a double-exponential model. If $\dot{V}O_2$ was measured more precisely, then a double-exponential (or monoexponential plus linear component) model may be applied and the role of the $\dot{V}O_2$sc could be determined. This would necessitate an additional component in the model, but could reveal important additional insights to the control of oxygen uptake. Nonetheless, use of the simplest monoexponential function over all the data permitted accurate prediction of the actual $\dot{V}O_2$ response in each subject.

Conclusions

We sought to test whether an integrative model incorporating increasing parameter values for G and either MRT or τ and δ empirically derived from constant WR exercise could accurately estimate the $\dot{V}O_2$ response to ramp incremental exercise. Previous research has been ambiguous but, we found that both G and MRT (or τ) increased with WR in an extended-step incremental protocol. Through WRs at which these parameters were derived (80%$\mu\dot{V}O_2$), the models accurately estimated the actual $\dot{V}O_2$ response regardless of ramp rate and even when segmenting the response into S1 and S2 phases. It therefore appears that the increasing values of G and MRT (or τ) balance each other to produce the quasi-linear responses seen with ramp incremental exercise with G exerting a stronger

proportionate influence. Furthermore, the addition of a time delay and a limiting of the impact of the $\dot{V}O_2$sc did not significantly improve the ramp response estimates.

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Tables

Table 1: Subject Parameters

Subject	**Age (years)**	**Height (cm)**	**Mass (kg)**
1	24	173	76.5
2	27	177	81.0
3	25	175	64.1
4	20	174	83.2
5	23	183	89.8
6	22	188	89.8
AVG ± SD	*24 ± 2*	*178 ± 6*	*77.6 ± 9.2*

Table 2: $\dot{V}O_2$ Parameter Values from Ramps

Subject	$\hat{\theta}_L$ **(from RR)**	$\mu\dot{V}O_2$		
		SR	**RR**	**FR**
1	1.75	3.91	3.76	3.50
2	2.10	3.39	3.52	3.14
3	2.20	3.88	3.93	3.72
4	2.00	4.06	3.87	3.92
5	2.20	4.01	3.64	3.47
6	2.55	4.65	4.69	4.74
AVG ± SD	*2.13 ± 0.26*	*3.98 ± 0.40*	*3.90 ± 0.41*	*3.75 ± 0.55*

Values in l O_2/min

Table 3: Actual Versus Target $\dot{V}O_2$ Values for Extended-Step Incremental

	WR 1	**WR 2**	**WR 3**	**WR 4**	**END**
Target $\dot{V}O_2$	1.45±0.15	1.97±0.25	2.51±0.27	2.89±0.29	4.02±0.41
Actual $\dot{V}O_2$	1.47±0.12	2.06±0.16	2.77±0.24	3.47±0.27	3.88±0.34
Difference	*0.02±0.04*	*0.09±0.11*	*0.25±0.11**	*0.58±0.18**	*-0.15±0.26**

values in l O_2/min * - significantly different ($p<0.05$)

Table 4: Gain Values from Extended-Step Incremental

Gain (ml O_2/min/W)					
MODEL	**SUBJECT**	**WR 1**	**WR 2**	**WR 3**	**WR 4**
MONO	**1**	9.4	11.2	11.5	16.5
	2	9.9	10.9	14.1	20.5
	3	10.4	12.4	14.4	16.6
	4	11.4	13.5	14.2	22.0
	5	8.7	11.4	13.1	17.4
	6	10.3	10.5	11.5	12.7
	AVG ± SD	*10.0 ± 0.9*	*11.6 ± 1.1*[t]	*13.1 ± 1.3* [1t]	*17.6 ± 3.3* [123t]
5TD	**1**	9.3	10.7	10.9	13.5
	2	9.7	10.9	13.6	12.5
	3	10.2	12.1	13.0	13.6
	4	11.1	13.3	14.5	22.8
	5	8.5	11.3	12.0	14.0
	6	10.1	10.5	10.9	11.2
	AVG ± SD	*9.8 ± 0.9*	*11.5 ± 1.1*	*12.5 ± 1.5* [1t]	*14.6 ± 4.1* [12*t]

significantly different ($p<0.05$) than: [1] – WR 1, [2] - WR 2, [3] - WR 3, * - MONO via two-way repeated measures ANOVA; [t] – the previous WR via paired-t test

Table 5: MRT or Time Constant and Time Delay Values from Extended-Step Incremental

MRT or τ and δ (s)					
MODEL	**SUBJECT**	**WR 1**	**WR 2**	**WR 3**	**WR 4**
MONO (MRT)	**1**	27.8	62.8	68.6	158.5
	2	46.7	51.3	86.7	213.9
	3	32.7	57.4	77.2	138.9
	4	47.1	47.8	86.1	272.2
	5	41.2	53.7	100.7	180.4
	6	41.1	51.0	58.1	116.7
	AVG ± SD	*39.4 ± 7.7*	*54.0 ± 5.4[t]*	*79.6 ± 15.0*	*180.1 ± 56.2 [123]*
5TD τ (TD)	**1**	19.6 (9.6)	35.6 (20.0)	47.6 (12.3)	87.0 (0.0)
	2	33.7 (10.2)	50.0 (0.0)	77.2 (0.0)	47.2 (3.2)
	3	15.1 (17.2)	43.2 (9.1)	54.9 (0.0)	74.8 (10.1)
	4	25.7 (20.5)	36.2 (11.5)	89.1 (0.0)	280.2 (0.0)
	5	22.4 (15.4)	52.7 (0.0)	76.1 (0.0)	111.8 (0.0)
	6	24.0 (16.7)	47.0 (3.1)	42.5 (10.7)	70.2 (5.4)
	AVG ± SD	*23.4 ± 6.3 (14.9 ± 4.3)*	*44.1 ± 7.1[t] (7.3 ± 7.8)[t]*	*64.6 ± 18.8 (3.8 ± 6.8)[1]*	*111.9 ± 85.1 [12]* (3.1 ± 4.1)[1]*

significantly different ($p<0.05$) than: [1] – WR 1, [2] - WR 2, [3] - WR 3, * - MONO via two-way repeated measures ANOVA; [t] – the previous WR via paired-t test

Table 6: Parameter Comparisons Across Models Up to Maximal $\dot{V}O_2$

	Model	SR	RR	FR
G	**ACT**	11.3 ± 1.2 ^{R}	10.5 ± 0.8	8.7 ± 1.0 ^{SR}
	MONO	11.7 ± 0.7 ^{R†}	11.0 ± 0.5	9.9 ± 0.4 ^{*SR}
	5TD	10.9 ± 0.3	10.7 ± 0.3	10.3 ± 0.3 *
MRT	**ACT**	6.2 ± 28.4	25.0 ± 15.2 ^{S}	35.7 ± 12.1 ^{S}
	MONO	28.2 ± 8.1 *	23.1 ± 6.4	17.3 ± 3.7
	5TD	22.6 ± 8.0	21.2 ± 7.1	21.0 ± 5.4

significantly different ($p<0.05$) than: * - ACT, ^{S} – SR, ^{R} – RR, † - 5TD

Table 7: Gain Comparisons Up to 80% Maximal $\dot{V}O_2$

	Model	SR	RR	FR
Gain	**ACT**	10.7 ± 1.1	10.2 ± 0.5	9.2 ± 1.0 [SR]
	MONO	11.0 ± 0.8 [R]	10.3 ± 0.6	9.2 ± 0.5 [SR]
	5TD	10.4 ± 0.4	10.2 ± 0.3	9.8 ± 0.2 [S]

significantly different ($p<0.05$) than: [S] – SR, [R] - RR

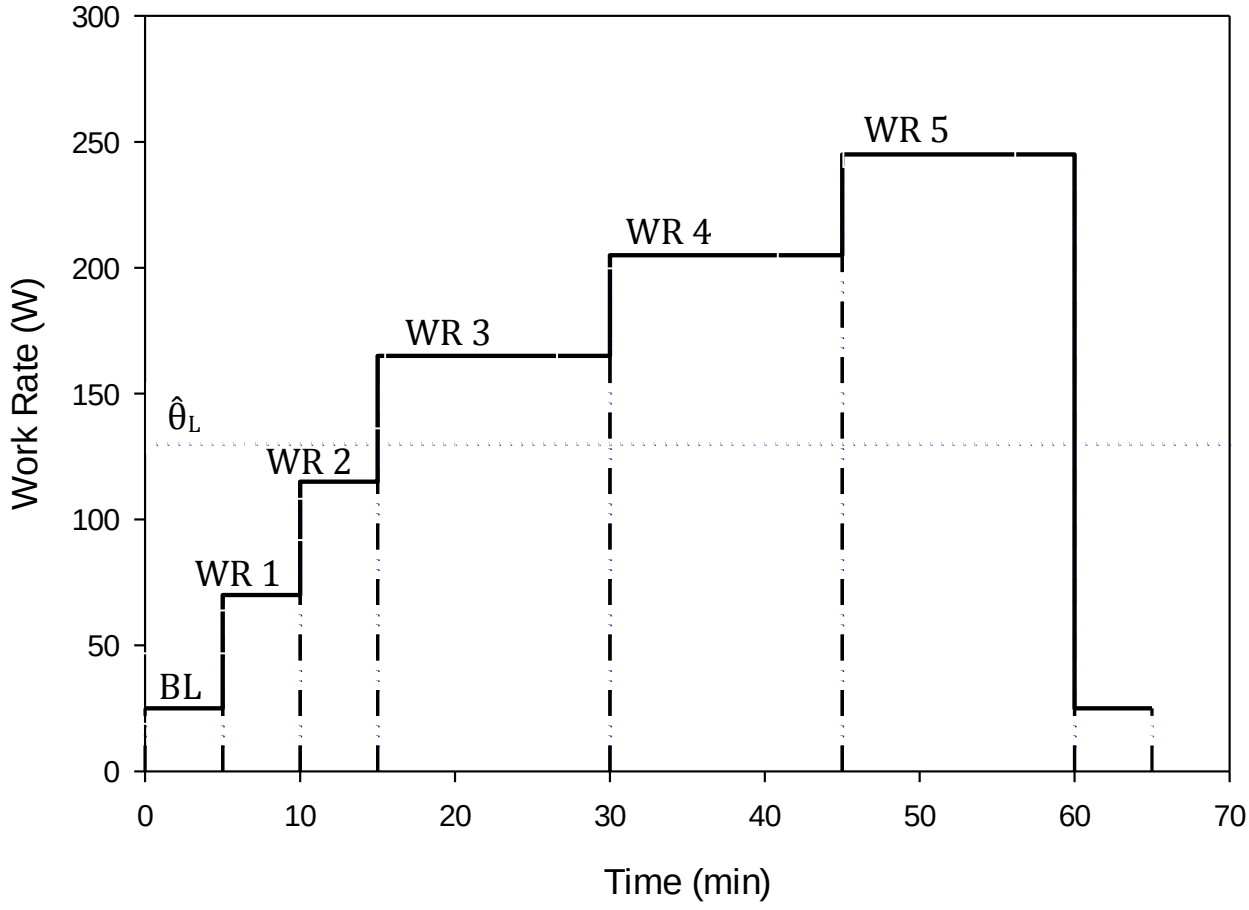
Table 8: Comparison of Segmental Gains

Segment	Model	SR	RR	FR
S1	**ACT**	10.1 ± 0.9	9.7 ± 0.9	9.7 ± 1.7
	MONO	10.3 ± 1.0	9.9 ± 1.0	9.0 ± 0.8
	5TD	9.7 ± 0.5	9.4 ± 0.5	9.1 ± 0.5
S2 (up to 80%μV̇O2)	**ACT**	11.1 ± 2.0 [F]	10.5 ± 1.2 [F]	8.4 ± 1.6
	MONO	11.8 ± 0.7 [1]	10.7 ± 0.7	9.6 ± 0.7
	5TD	11.1 ± 0.5 [1]	10.8 ± 0.4 [1]	10.3 ± 0.4 *

significantly different ($p<0.05$) than: * - ACT ($p<0.05$), [F] – FR, [1] – S1 segment

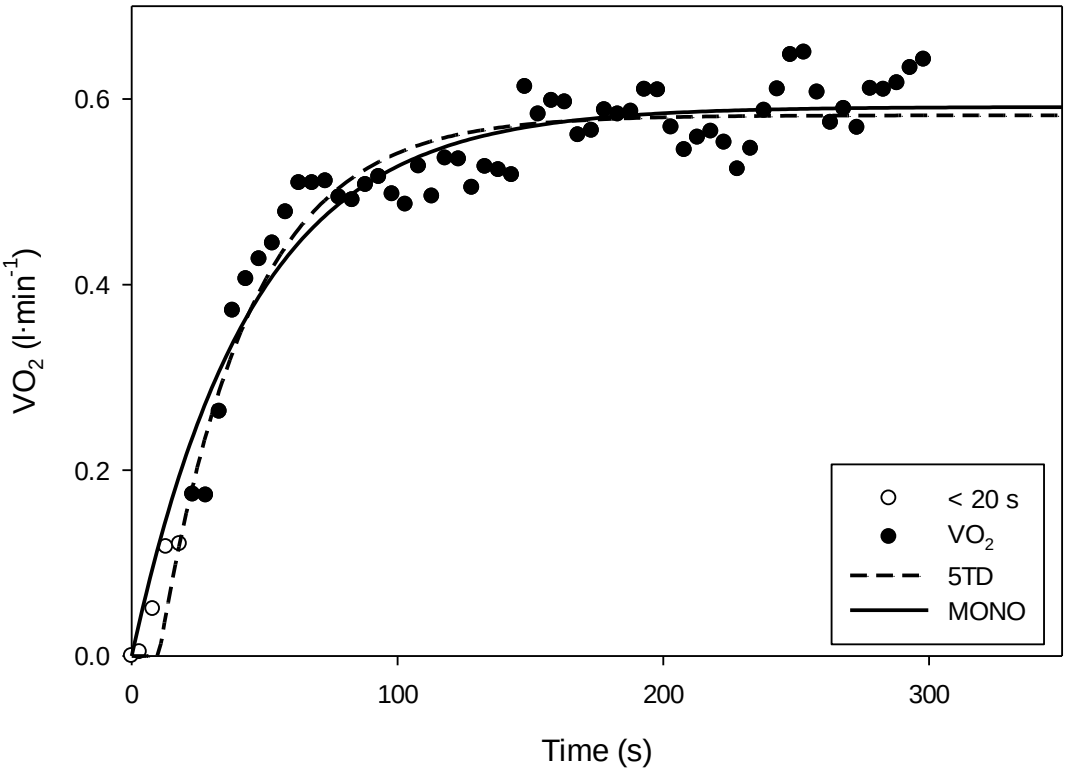
Figures

Figure 1: Extended-Step Incremental Protocol



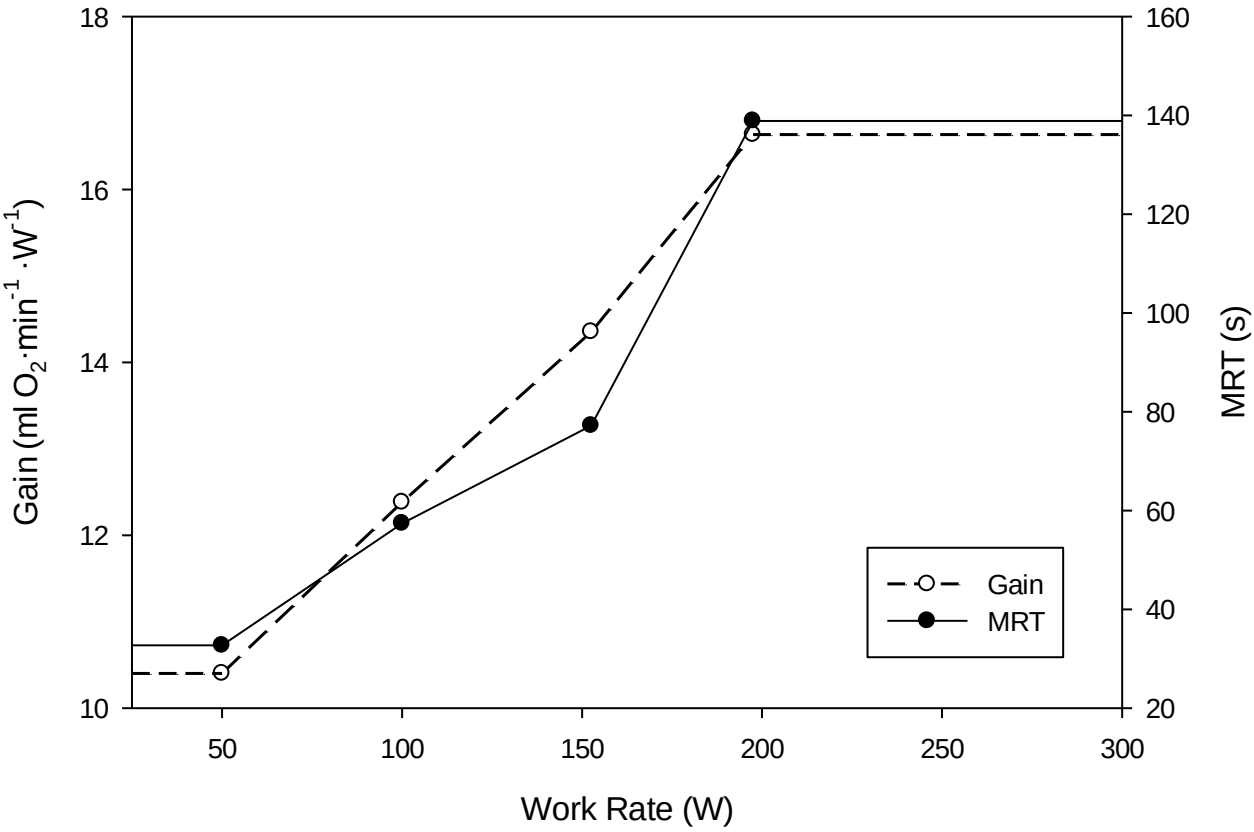
Schematic of extended-step incremental protocol. Below $\hat{\theta}_L$ stages are only 5 min, whereas above $\hat{\theta}_L$ stages are 15 min or until failure. Baseline and recovery WR is 25 W. The remaining WR are determined from the WR associated with the corresponding $\dot{V}O_2$ from the RR trial. WR 1 and 2 are 45 and 90% $\hat{\theta}_L$, while WR 3, 4, and 5 are 20, 40, and 60% of the difference between $\hat{\theta}_L$ and $\mu\dot{V}O_2$.

Figure 2: $\dot{V}O_2$ Fitting Strategies



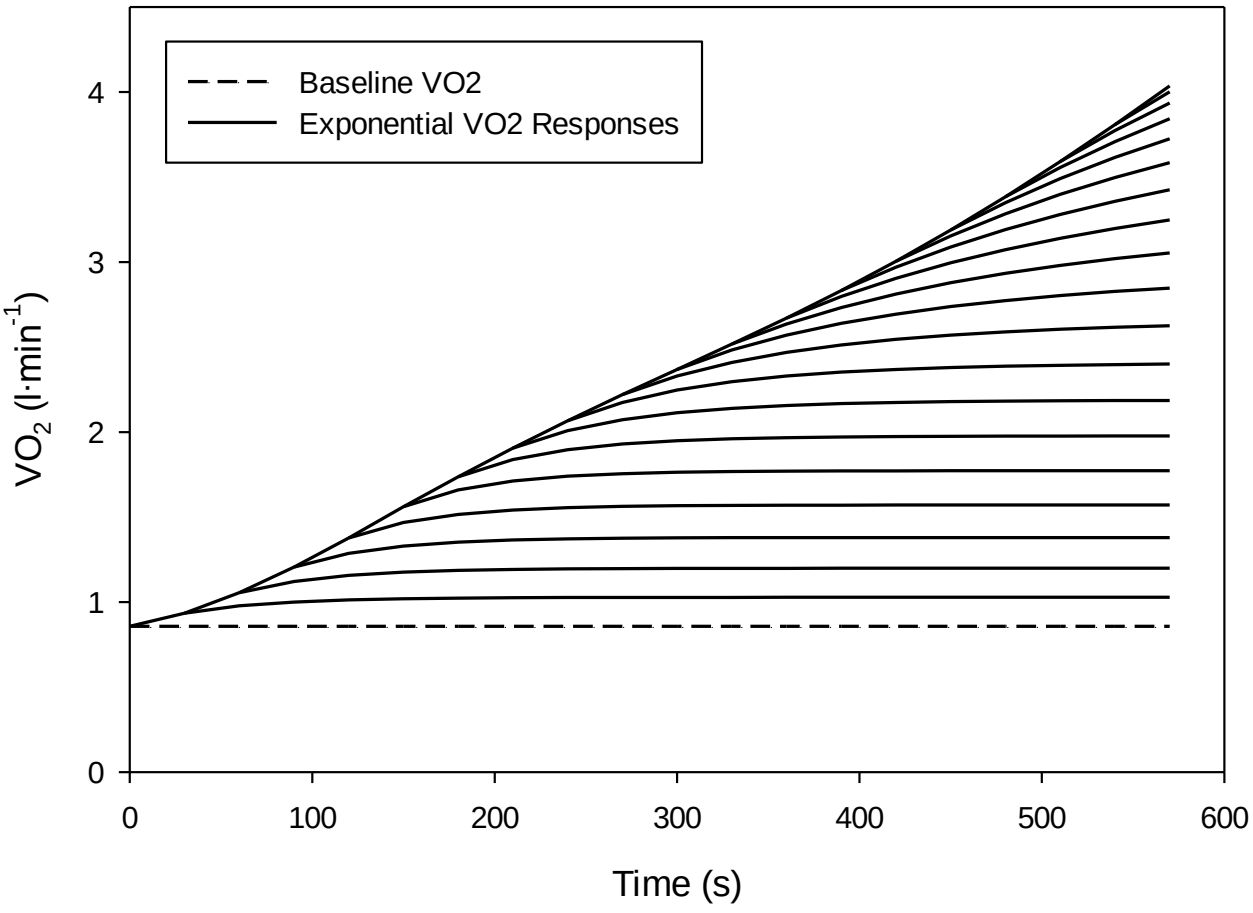
Demonstration of fitting strategies for each stage of the extended-step incremental protocol. MONO (solid line) is fit from time zero to the entire dataset for each stage. 5TD (broken line) allows a time delay and is always fit to only the first 5 minutes of data. Note that the first 20 s of data are always excluded to avoid the cardiodynamic phase of the response.

Figure 3: Parameter Determination



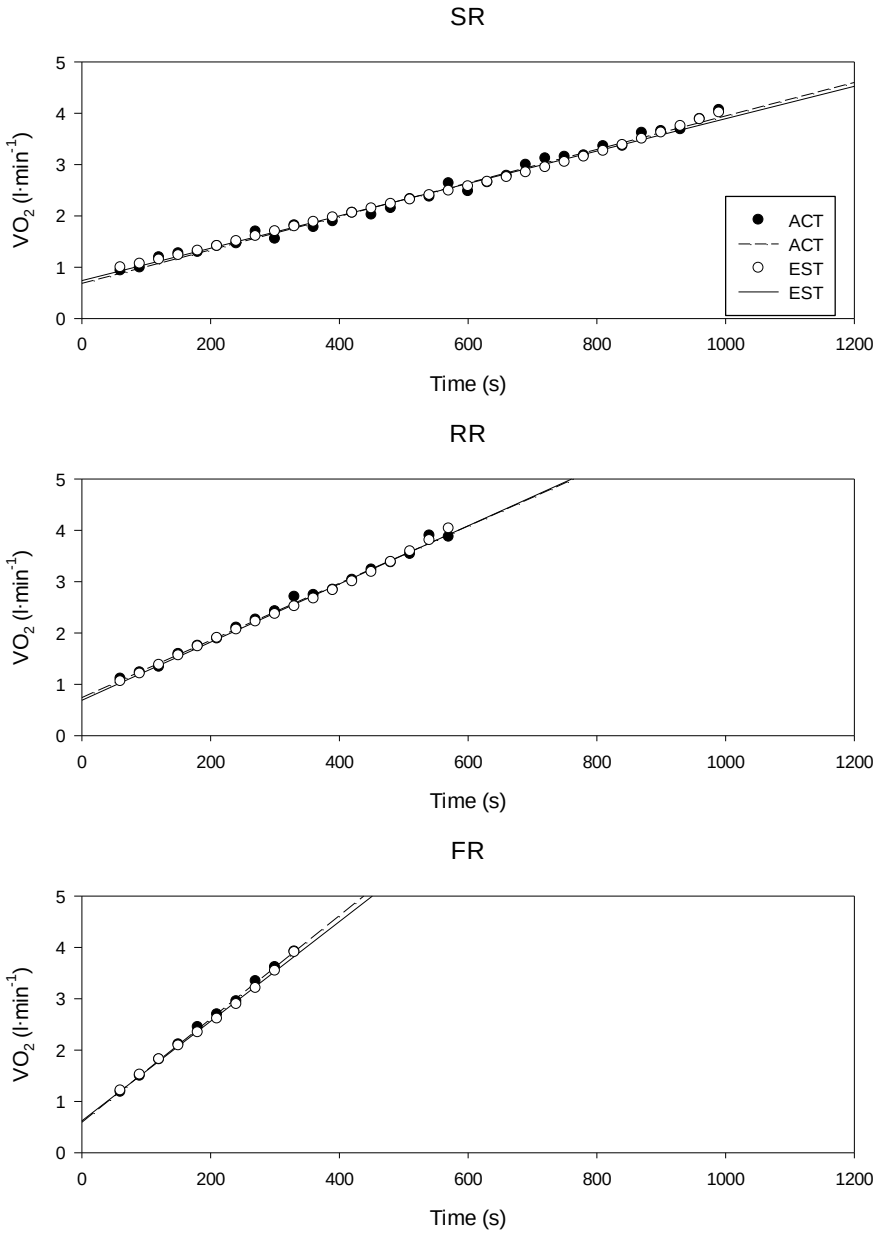
Parameter values determined from the extended-step incremental protocol are graphed according to WR. To provide parameter values for WRs not directly measured, parameter values were interpolated linearly between known values. For WRs below the lowest step, the lowest known parameter value is held constant. For WRs above the highest step, the highest known parameter value is held constant. Despite maximal efforts by the subjects, task failure precluded the measurement of parameter values for the 60%Δ stage.

Figure 4: Integral of 30 s Responses



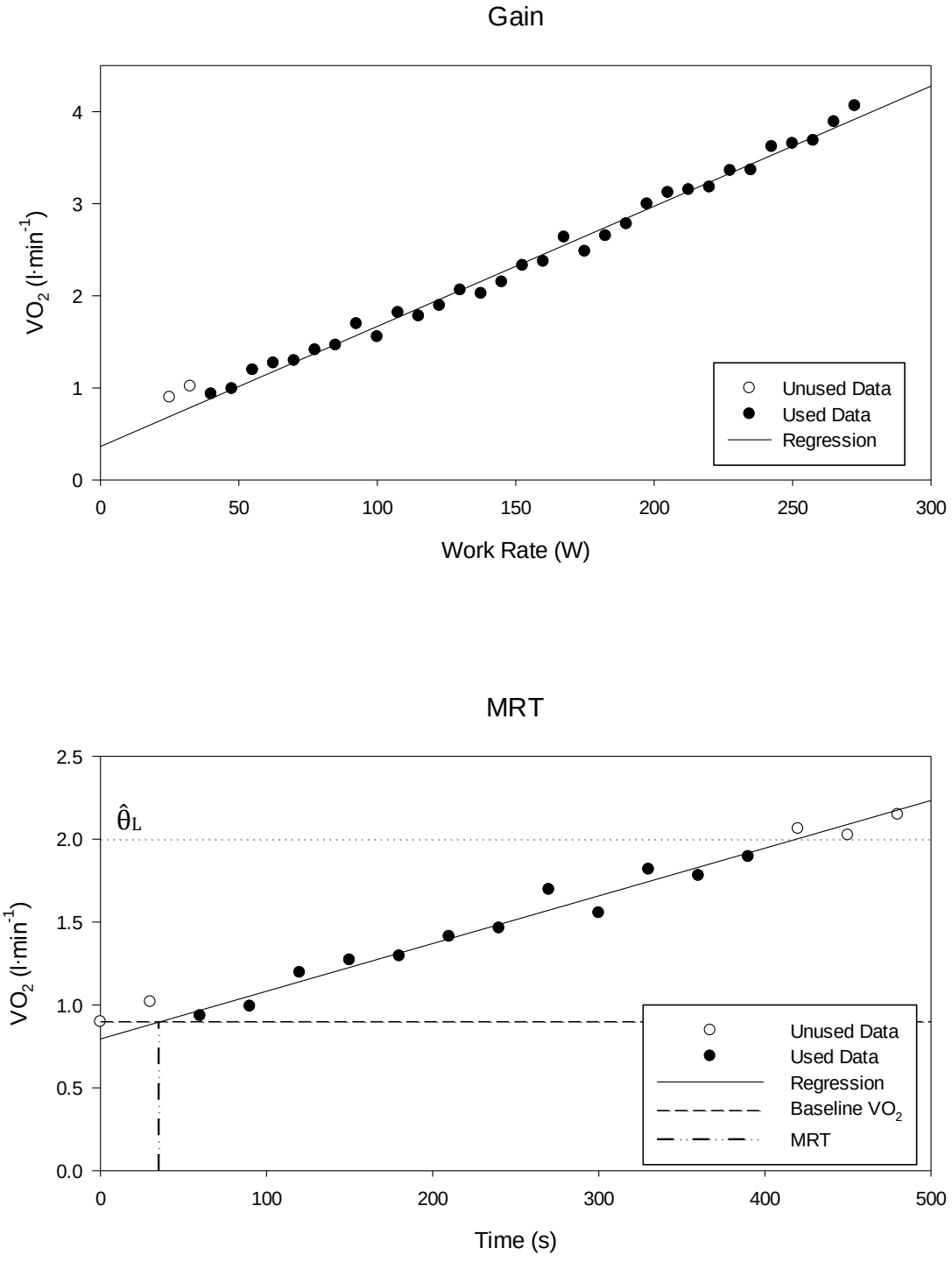
Using parameter values determined from extended-step incremental data, an exponential $\dot{V}O_2$ response is started every 30 s. Every 30 s all active exponential responses are summed and added to the baseline $\dot{V}O_2$. This summed response estimates the ramp response.

Figure 5: Comparison of Model and Actual $\dot{V}O_2$ Responses to Various Ramps



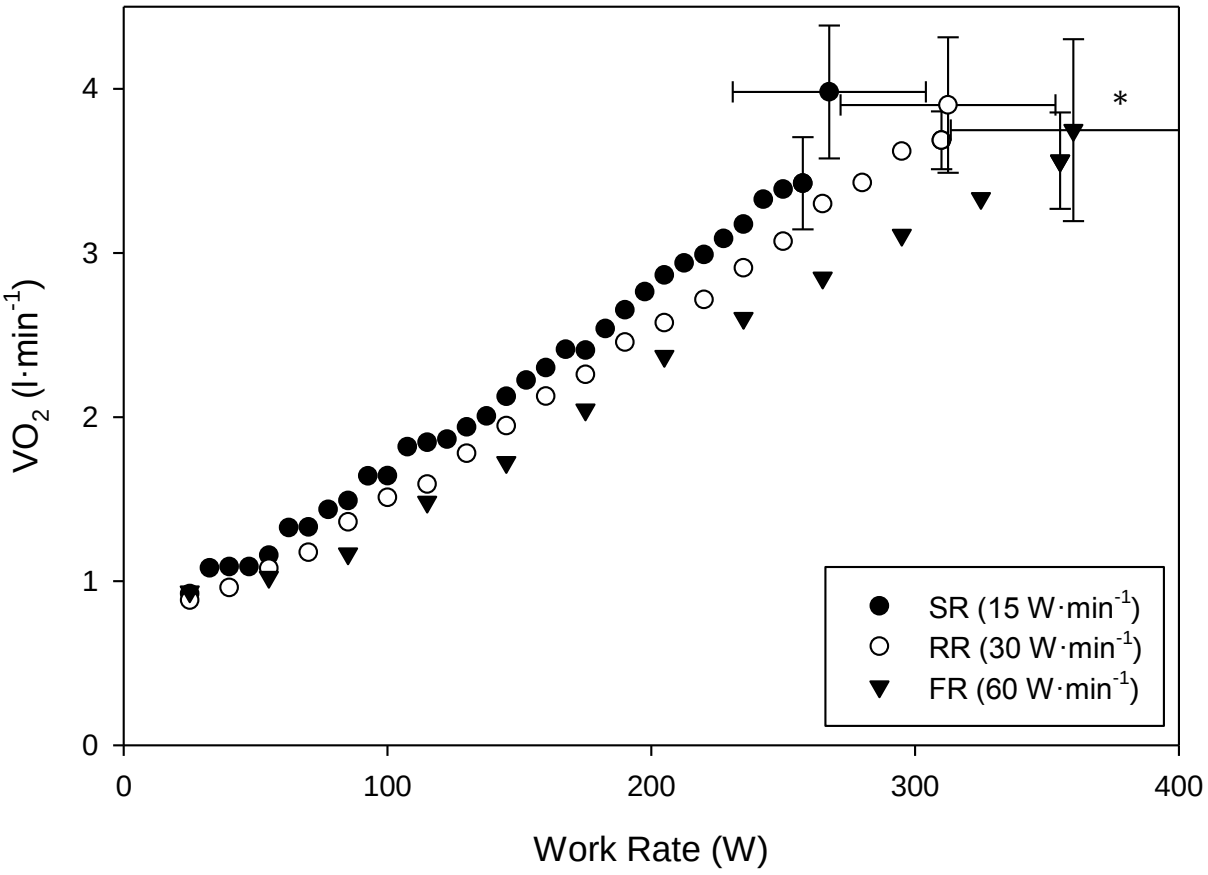
The model estimates (open circles and solid lines) from SR, RR, and FR are fit by linear regression. The resulting Gain and MRT parameter values were then compared to the parameters derived from the same treatment of ACT data (solid circles and broken lines).

Figure 6: Calculation of Parameters from Ramp Responses



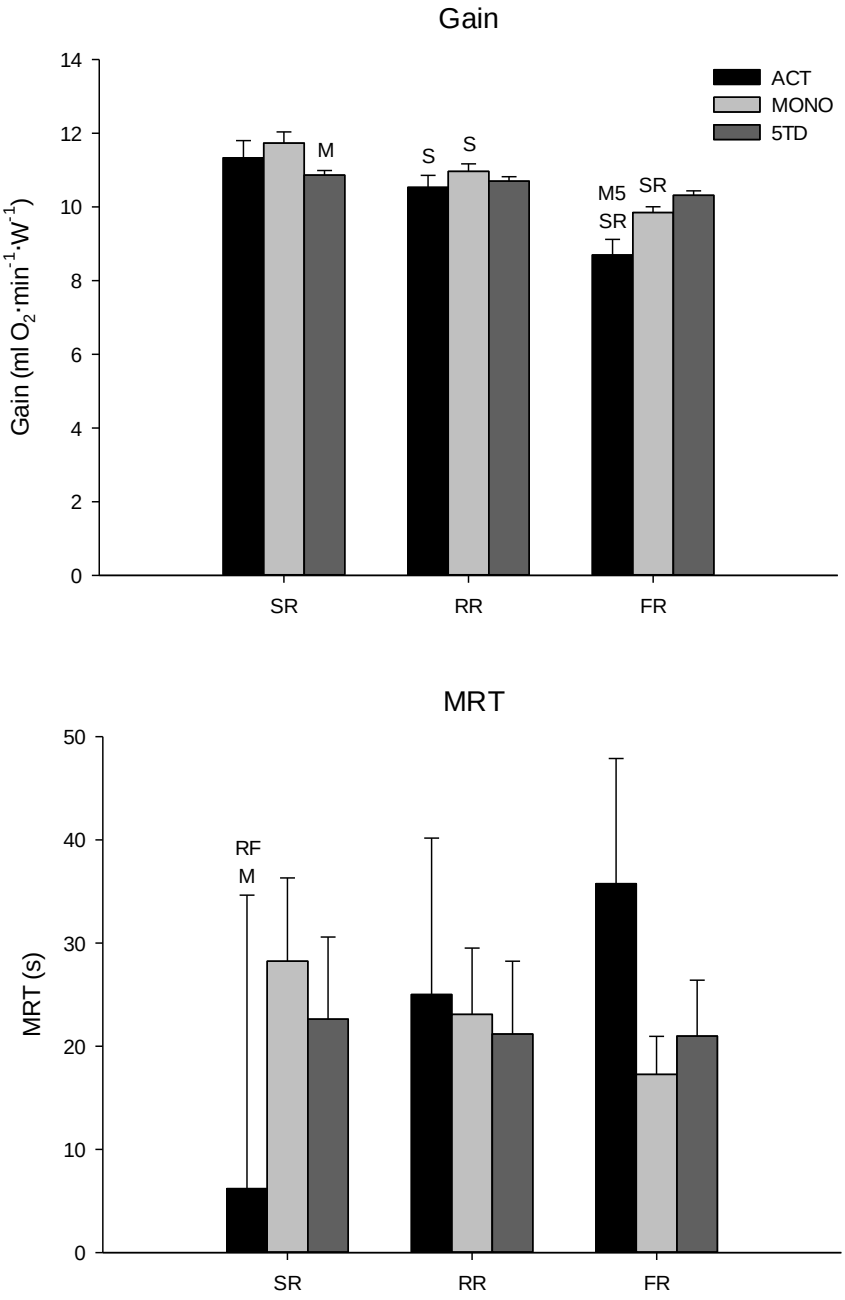
The first 60 s of data are removed to evaluate the "linear" portion of the response. The slope of the linear regression through remaining data is the Gain. For MRT, data above lactate threshold are removed as well. The x-value at the intersection of the linear regression and baseline $\dot{V}O_2$ is the MRT.

Figure 7: Mean Responses to Various Ramp Rates



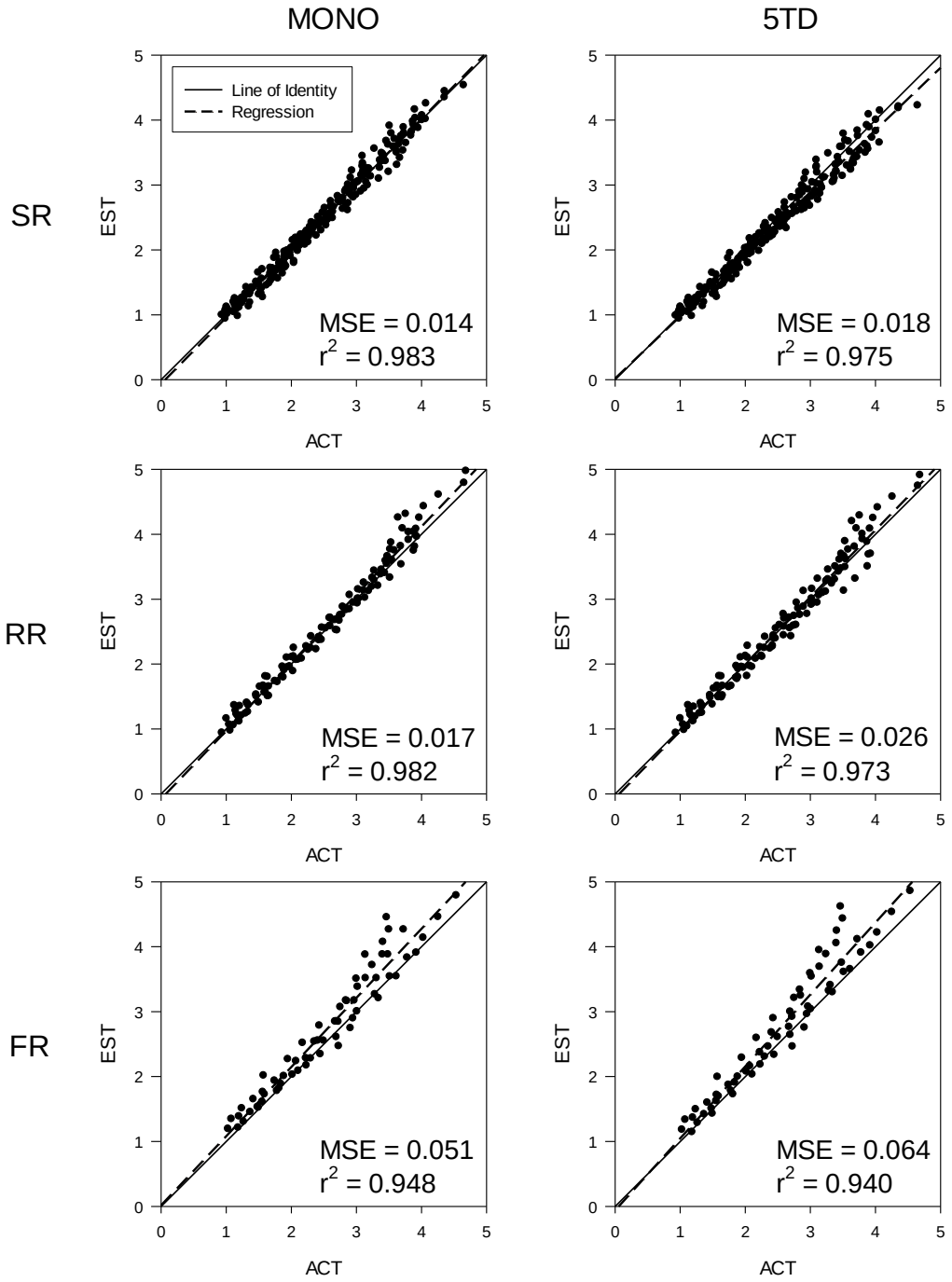
$\dot{V}O_2$ responses to the three ramp rates used. Peak $\dot{V}O_2$ from SR and RR were not significantly different, although the RR ended at a higher WR, demonstrating a true $\mu\dot{V}O_2$. However, the FR condition caused task-failure before $\mu\dot{V}O_2$ could be reached. * - significantly different from SR ($p=0.037$)

Figure 8: Comparisons of Parameters Up to Maximal $\dot{V}O_2$



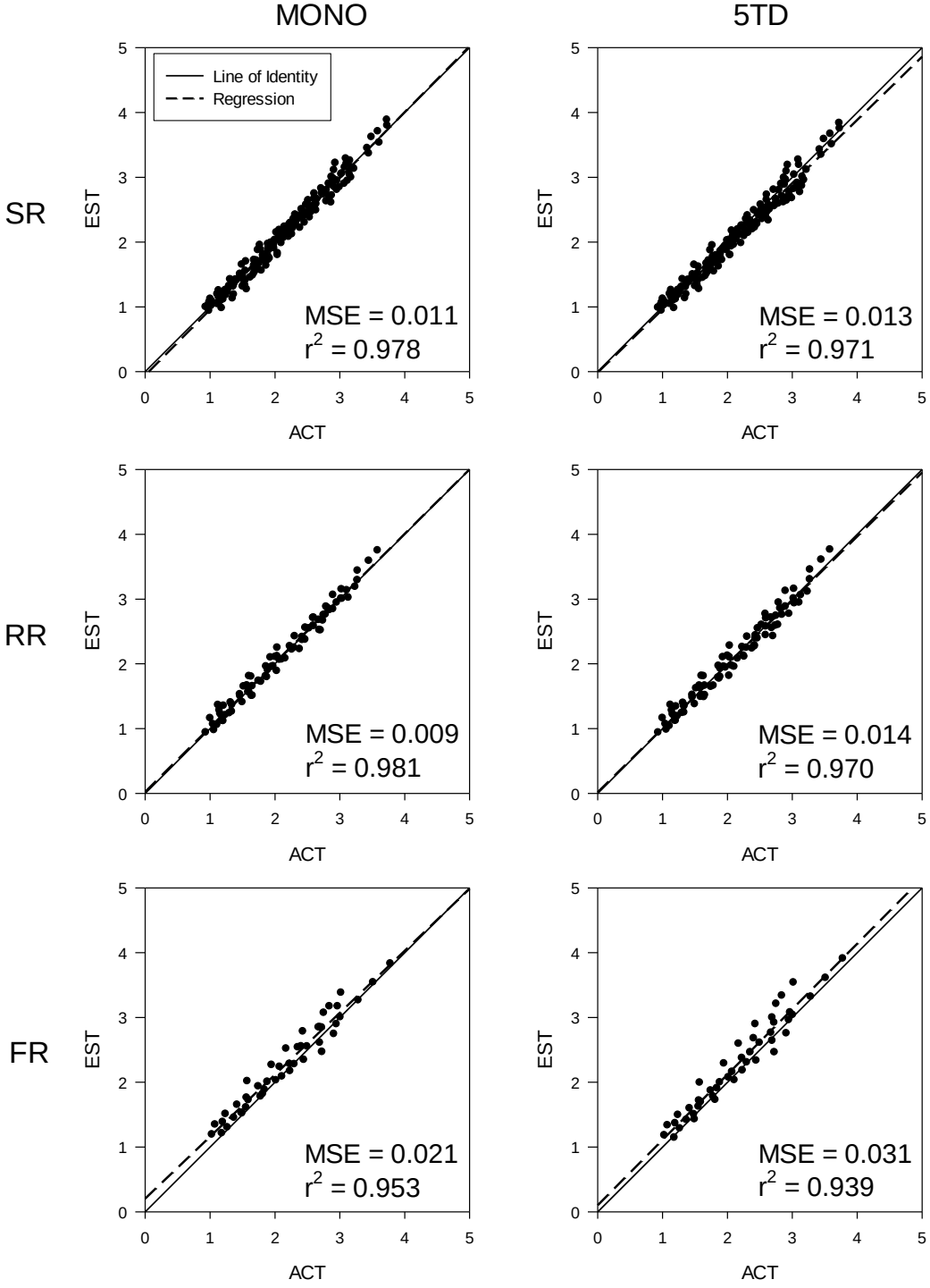
Comparison of average±SD parameter values from both models and the actual response for each of the three ramp rates. Upper graph: Gain was different across all ramp rates for ACT and MONO, but not different for any in 5TD. Both models over-estimated the actual Gain in FR. Lower graph: In ACT, the MRT from SR was shorter than for RR or FR. The MONO model over-estimated the actual MRT in SR. However, note the high variability of MRT values. Significantly different ($p<0.05$) than: ^{S} - SR, ^{R} – RR, ^{F} – FR (across the same model); ^{M} – MONO, ^{5} – 5TD (within the same ramp rate)

Figure 9: Actual Versus Estimated Group $\dot{V}O_2$ Responses Up to Maximal $\dot{V}O_2$



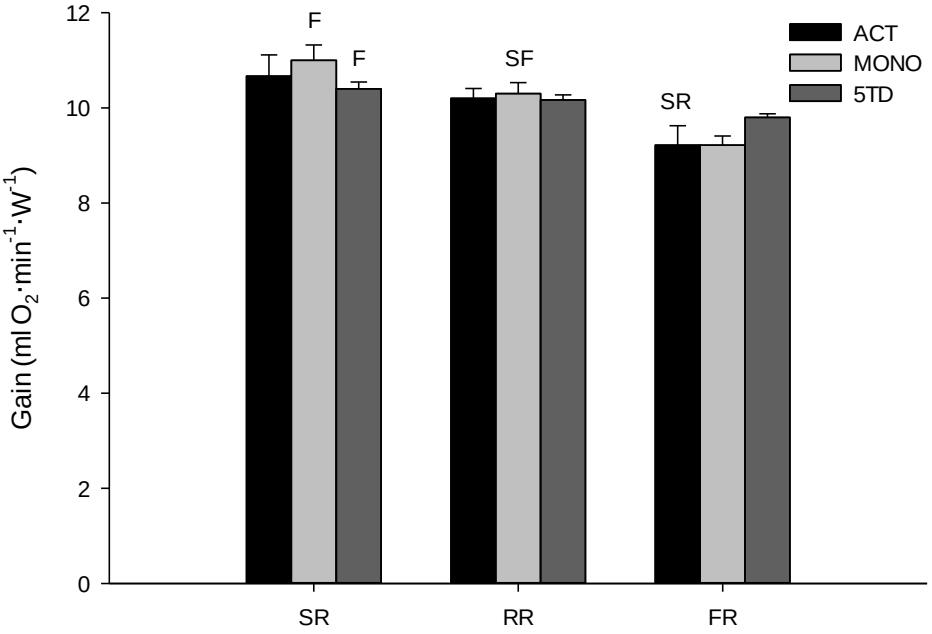
Actual $\dot{V}O_2$ data paired to estimated $\dot{V}O_2$ values up to $\mu\dot{V}O_2$. MONO model on the left, 5TD on the right. SR is top, RR is middle, FR is bottom. Line of identity is solid line. Linear regression is dashed line.

Figure 10: Actual Versus Estimated Group $\dot{V}O_2$ Responses Up to 80% Maximal $\dot{V}O_2$



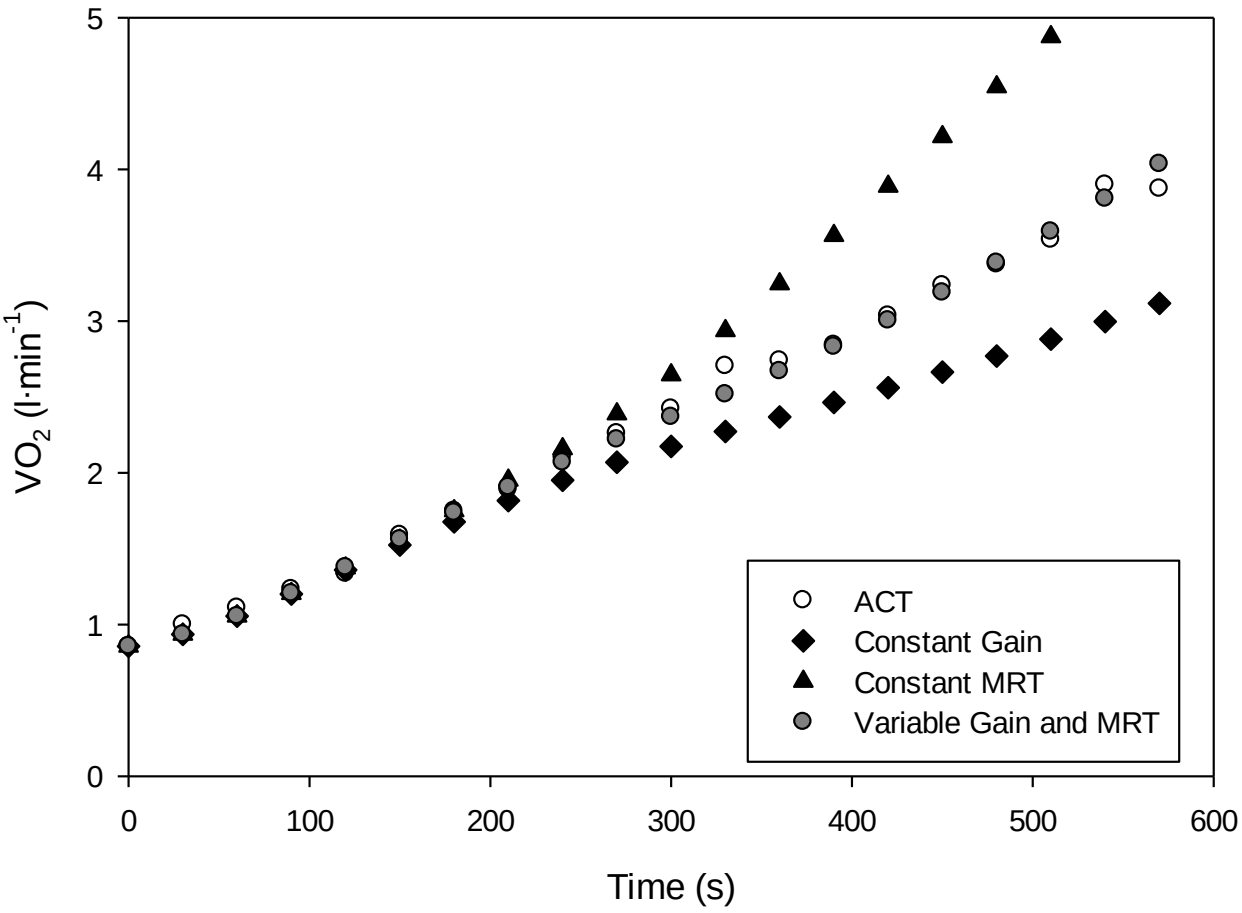
Actual $\dot{V}O_2$ data paired to estimated $\dot{V}O_2$ values up to 80%$\mu\dot{V}O_2$. MONO model on the left, 5TD on the right. SR is top, RR is middle, FR is bottom. Line of identity is solid line. Linear regression is dashed line.

Figure 11: Comparison of Gain Values up to 80% Maximal $\dot{V}O_2$



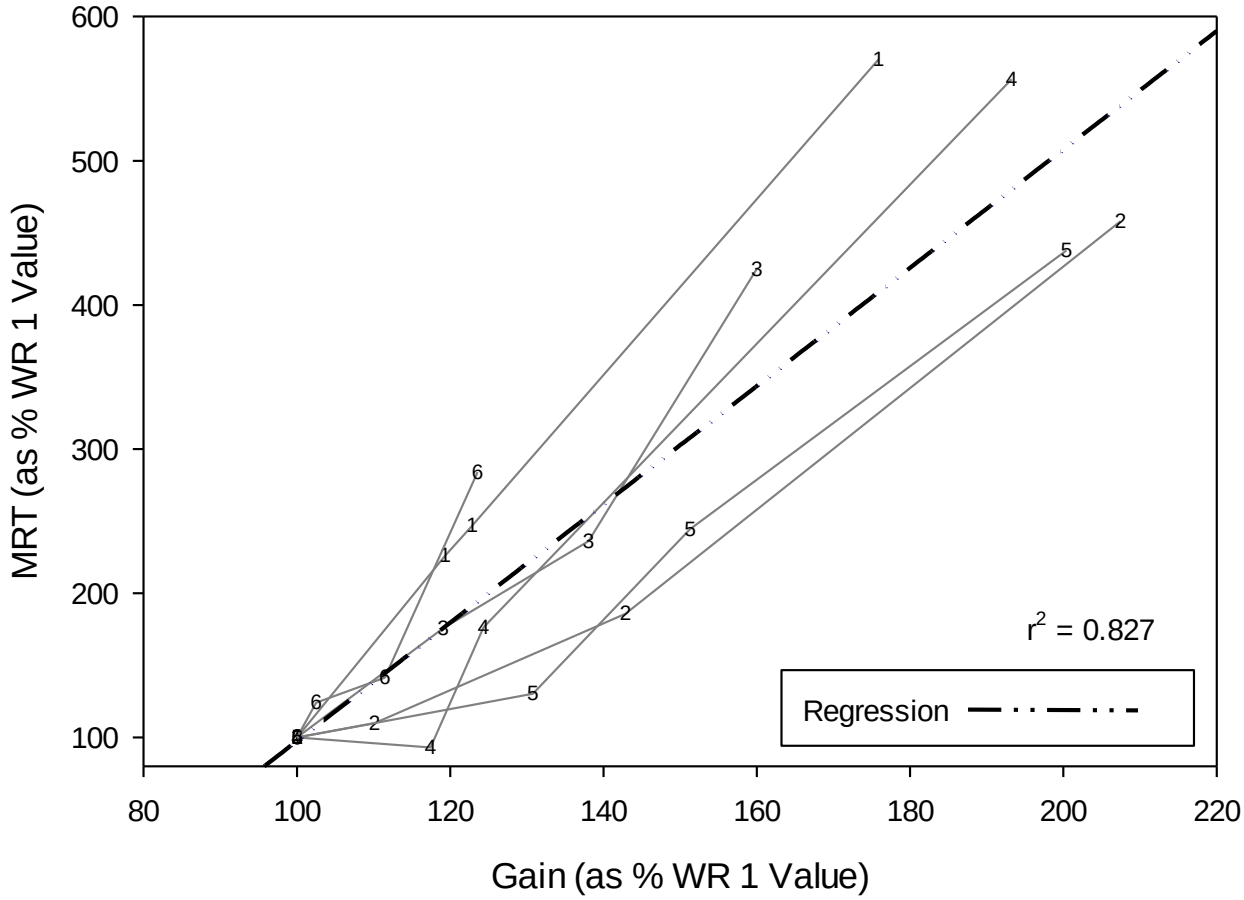
Comparison of model estimates and actual Gains across ramp rates. No differences were found between the model estimates and ACT Gain. However, differences in Gain across ramp rates but within model or ACT data were found. Significantly different ($p<0.05$) than: ^{S} – SR, ^{R} – RR, ^{F} – FR (across the same model)

Figure 12: Effects of Parameter Changes



30 s $\dot{V}O_2$ data from ramp protocol from Actual response (open circles) and MONO model allowing both Gain and MRT to vary (grey circles). The MONO model was then applied where Gain was kept constant (diamonds) or MRT was kept constant (triangles). Notice the opposing effects of an increase in Gain or MRT.

Figure 13: Comparison of Change in Gain and MRT



Comparison of increases in parameter values from extended-step incremental values. Values are normalized as a percent of WR 1 value. Both Gain and MRT increased as WR increased. However, the scaling shows that MRT changes to a much greater extent than Gain.