July 2021

Link Between Muscle and Whole-body Energetic Responses to Exercise

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Link between Muscle and Whole-body Energetic Response to Exercise

A Thesis Presented

by

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Submitted to the Graduate School of the

University of Massachusetts Amherst in partial fulfillment

of the requirements for the degree of

MASTER OF SCIENCE

May 2021

Kinesiology

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ACKNOWLEDGMENTS

While you are reading this thesis, for whatever reason that may be (a form of punishment perhaps), it is my wish that you are aware of the many people who were involved not only in the creation of this document, but everything leading up to this point as well.

First, thank you to my parents, who have provided me with unconditional support and encouragement. Whoever I become and whatever I accomplish is undoubtedly because I was lucky enough to have the two of you raise me. Thank you to my siblings, whose paths I closely watched and followed growing up. Without your merciless teasing and the constant threat of physical attack, I may never have developed any grit at all. Thank you to the Springfield College Football team, and specifically the Roach Patrol. You taught me about character, how to put my head down and work, and to always 'Get up and get another', all of which has been put to use during this thesis. Thank you to all of my friends. You pushed me to relax and have fun, and whatever hair I have left on my head, it is likely due to you. Thank you to the MP Lab, current, past, grad, undergrad, staff, and honorary (Ericber) members. You have put countless hours into helping me, even when it was not your responsibility, this project would not have been completed without you. Thank you to my committee for your guidance, it was quite the task but we made it. Specific thanks to my advisor Jane and mentor Mike. You have both taught me so much about science and research, and have always been patient and looked out for me. Lastly, thank you to the rest of the Kinesiology Department and UMass for providing me with an excellent experience over the past three years, it was a hike, but it was a nice one.

ABSTRACT

LINK BETWEEN MUSCLE AND WHOLE-BODY ENERGETIC RESPONSE TO EXERCISE

MAY 2021

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Substantial evidence exists regarding how skeletal muscles use energy and how this affects muscular performance. What remains unclear is how characteristics of muscle energetics affect whole-body energetics during daily living, and what effects this may have on mobility. The goal of this study was to determine the associations between muscle and whole-body energetics including the relationships between: 1) muscle PCr depletion (ΔPCr) in response to light intensity isotonic contractions and the oxygen deficit at the onset of a 30-min treadmill walk (30MTW), and, 2) muscle oxidative capacity and excess post-exercise oxygen consumption (EPOC; 30MTW), respiratory exchange ratio (RER; 30MTW), and peak oxygen consumption (VO_2 peak) from a graded treadmill test. Eight healthy young (28.4 ± 3.5 years) male participants were studied. Muscle energetics were measured via 31-Phosphorus magnetic resonance spectroscopy (^{31}P-MRS). Muscle ΔPCr was determined as the change in PCr during 2-min of isotonic knee extensor contractions. Muscle oxidative capacity was determined as the rate constant (k_{PCr}) of a PCr recovery following 24-s of maximal isokinetic knee extensor contractions. Whole-body energetic responses to the 30MTW were measured via indirect calorimetry. Oxygen deficit and EPOC were determined as the time constants

of the change in oxygen consumption at the onset and offset of the 30MTW, respectively. Respiratory exchange ratio was determined as the mean RER during minutes 7-30 (RER L23), 25-30 (RER L5), and 29-30 (RER L1). Peak oxygen consumption was the highest 30-s average of oxygen consumption during a graded treadmill test, normalized to total mass and lean mass measured by dual-X-ray absorptiometry. Spearman rank correlation coefficients (r_s) were calculated to evaluate the associations between independent variables (muscle ΔPCr and oxidative capacity) and dependent variables (oxygen deficit, EPOC, RER, and VO_2 peak). Muscle ΔPCr had a positive association ($r_s = 0.46$, $p = 0.30$) with oxygen deficit. Muscle oxidative capacity had a negative association with EPOC ($r_s = -0.64$, $p = 0.14$), RER L23 ($r_s = -0.64$, $p = 0.14$), L5 ($r_s = -0.68$, $p = 0.11$), and L1 ($r_s = -0.74$, $p = 0.07$). Muscle oxidative capacity had a positive association with VO_2 peak per lean mass ($r_s = 0.64$, $p = 0.10$), but not VO_2 peak per total mass ($r_s = 0.14$, $p = 0.75$). These results provide promising preliminary evidence that muscle energetics are associated with whole-body energetic response to daily-living type exercise.

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CHAPTER 1

INTRODUCTION

Presentation of the Problem

Mobility can be defined as the ability to move freely and easily. A decline in mobility-related function (i.e., the capacity to complete mobility tasks) poses a significant threat to the quality of life in multiple segments of the population, including those who are elderly, diseased, or disabled (27, 35, 85). Many physiological systems contribute to mobility, but the production of movement is the primary function of skeletal muscle. In particular, skeletal muscle converts chemical energy into mechanical energy, which enables muscles to contract and create bodily motion. To produce and sustain movement, muscle energy production must increase several orders of magnitude above its resting level. This high energy requirement makes adequate energy production vital to mobility (103). The general study of how organisms derive and use energy is termed bioenergetics. Bioenergetic research, as it relates to movement, is often performed at either the muscle (*in vitro, in situ,* or *in vivo*) or the whole-body level (24, 29, 30, 34, 49, 71, 78, 97, 108). The study proposed here will evaluate both.

Historically, our lab has used ^{31}phosphorus magnetic resonance spectroscopy (^{31}P-MRS) to investigate the effects of aging and physical activity on muscle energetics and fatigue *in vivo*, with the goal of improving mobility and increasing quality of life. We have demonstrated that aging and physical activity are related to altered muscle oxidative capacity (36, 70), glycolytic flux (66, 69), and the accumulation of fatiguing metabolites (66, 69). Although it is clear that muscle energetics are important for mobility, determining the effect of altered muscle energetics on mobility-related function proves

difficult. This is largely because the most effective way to measure muscle energetics, ^{31}P-MRS, is limited to the confines of a magnetic resonance scanner and cannot be used during mobility tasks (Fig. 1). One potential solution lies in the measurement of whole-body energetics, which can be completed during mobility tasks, and is related to indices of mobility-related function (4, 9, 15, 92, 98, 99). Whole-body energetics is largely dependent on muscle energy demand (21, 93, 94, 110), and could potentially act as an intermediate pathway through which muscle energetics affects mobility-related function (Fig. 2). Therefore, the goal of this project is to analyze the relationship between muscle and whole-body energetics; during the completion of and recovery from daily-living type exercise.

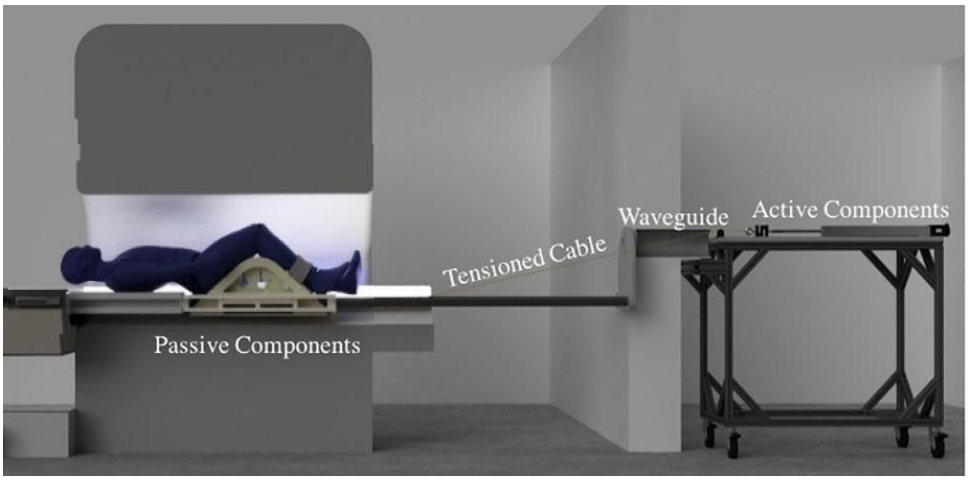


Figure 1. Experimental set up for ^{31}P-MRS analysis of muscle energetics. (50)

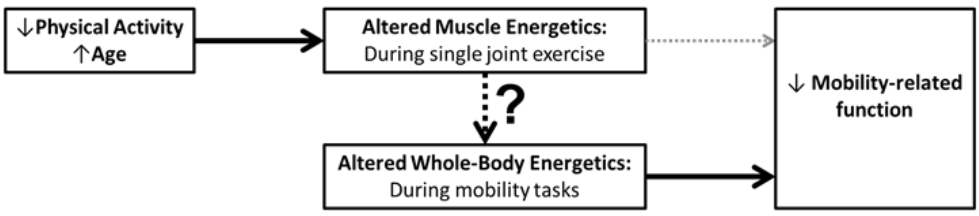


Figure 2. Study focus

Background

ATP: The Primary Energy Source

Energy cannot be created or destroyed, only transferred. In living organisms, chemical energy is transferred into mechanical energy to complete tasks such as contracting muscle. Humans derive this chemical energy from food; the majority comes from carbohydrate and fat sources and a small portion comes from protein (106). These three substances are considered macronutrients and serve as substrates for energy-producing chemical reactions (energy metabolism). The amount that each substrate contributes to energy production is termed substrate use. Through the breakdown of macronutrients, the body generates adenosine triphosphate (ATP), which is a small molecule that can both store and transfer chemical energy. Energy transferred through ATP is used by the body to transmit signals, transport ions, contract muscles, build and breakdown substances, and drive chemical reactions, among other functions.

To release energy from ATP, one of the three phosphates is cleaved off by an enzyme, producing adenosine diphosphate (ADP) and inorganic phosphate (Pi) and releasing energy as a byproduct. Phosphate-cleaving enzymes (ATPases) can serve many functions, and how the released energy is used depends on the enzyme involved. In muscle, for example, myosin acts as an ATPase by splitting ATP and using the energy released to complete a “powerstroke” causing muscle to contract. Without ATP, vital enzymes would not be able to complete their functions and living organisms would quickly perish. Thus, the production of ATP is one of the most important processes of the human body (90).

There are three main pathways through which ATP is generated: the phosphocreatine (PCr) system, glycolysis, and oxidative metabolism (consisting of the

Krebs cycle and oxidative phosphorylation). The first two pathways can generate energy without the use of oxygen and are collectively referred to as non-oxidative metabolism. Conversely, oxidative metabolism can only occur when oxygen is available. Cells rely on different pathways based on their needs, function, structure, and enzymatic makeup. In human cells, non-oxidative metabolism occurs in the cytoplasm and oxidative metabolism occurs in the mitochondria. All three energy pathways contribute to ATP generation at all times, however, the relative contribution of each varies due to differences in rate, duration, and the conditions under which they can provide energy (55). As shown in Fig. 3 the maximal rate of ATP generation and the amount of ATP that can be derived from the body's energy stores differs for each pathway.

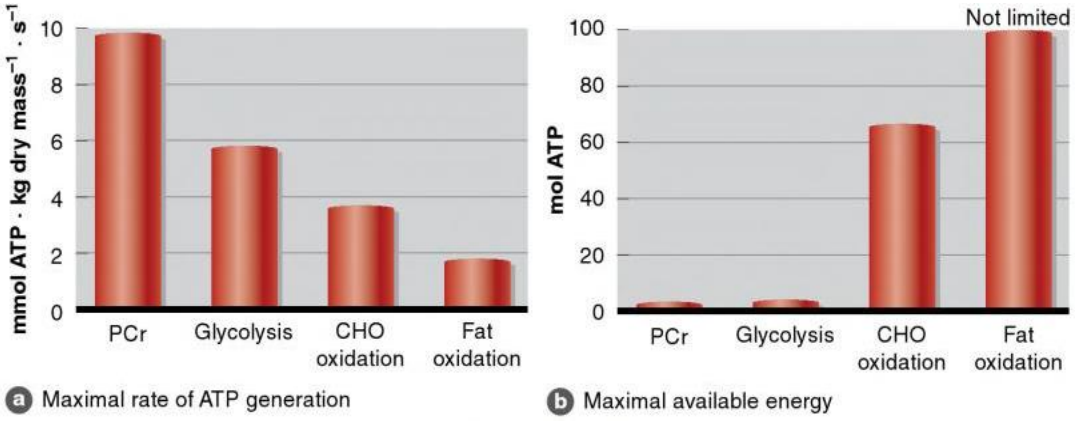


Figure 3. Maximal energy rates and availability from different pathways. (55)

The PCr system consists of a single-step reaction that transfers a phosphate from PCr to ADP, resulting in the synthesis of ATP and creatine (Cr). Cells have limited stores of PCr (~15 s worth), but the simplicity of the reaction allows for very rapid energy production (Fig. 3).

Glycolysis is a slightly more complex process involving ten chemical reactions that break down carbohydrate (glucose or glycogen) into smaller intermediate molecules

eventually producing pyruvate, NADH (an energy shuttle), and ATP. Only the ATP can be used directly for energy, while the pyruvate and NADH can be sent into the mitochondria for use in oxidative metabolism. For this reason, glycolysis is a non-oxidative source of ATP). The amount of energy that can be produced by glycolysis from carbohydrate stores is more than that from the PCr system (Fig. 3) but is still only sufficient for short-duration exercise (~1 min). The rate of glycolytic ATP generation is slower than the PCr system but faster than oxidative metabolism (Fig. 3).

Oxidative metabolism is made up of two multi-step processes; the Krebs cycle and oxidative phosphorylation. The products of either glycolysis or beta-oxidation (the breakdown of fat) enter the Krebs cycle and oxidative phosphorylation where they are further metabolized, eventually producing ATP, carbon dioxide (CO_2), and water. Oxidative metabolism generates ATP at a slower rate than the PCr system or glycolysis; however, it can use carbohydrate, fat, or protein as substrates. For this reason, the duration that ATP can be produced oxidatively from the body's substrate stores is on the order of days. Oxidative metabolism also generates the largest amount of ATP per substrate consumed- roughly 15-52 times that of glycolysis depending on the substrate used- making it the most efficient in terms of energy per gram of fuel (55).

^{31}P-MRS: A Window into Muscle ATP Production

In skeletal muscle, the rate that PCr, glycolysis and oxidative metabolism produce ATP can be observed using ^{31}P-MRS. This technique is a non-invasive method that uses radio waves in the presence of a static magnetic field to determine the concentration of ^{31}P-containing chemical compounds within the body. Typical ^{31}P-MRS measurements include quantitation of: ATP flux through each pathway, oxidative capacity (i.e.,

maximal rate), metabolic economy, and intracellular acidification in response to muscle contractions. A muscle's ability to generate force and power over time is dependent on adequate energy production, from sustainable pathways, for the entirety of the exercise.

Analysis of energetics has shown that at the onset of exercise (muscle contractions) muscle first relies on the PCr system to provide immediate energy. Muscle stores of PCr decline at a rate relative to the energy demand and would quickly deplete without the additional contribution from the other two systems. Glycolysis will assist first because the rate of energy production can be increased rapidly to meet energy demands. At low-moderate intensity, after there has been enough time for its rate to increase, energy reliance will then shift mainly to oxidative metabolism. At higher intensities, glycolysis will continue to provide the majority of energy, because oxidative metabolism cannot produce ATP at a sufficient rate (47, 102, 103). However, high rates of glycolysis are also associated with increased muscle fatigue (decrease in force or power production) and are therefore likely to shorten the duration that contractions can be performed (108). After contractions cease, PCr stores that were depleted at the onset of exercise will be replenished by ATP generated through oxidative metabolism. A muscle's oxidative capacity can be estimated by measuring the rate of PCr recovery after a PCr-depleting bout of exercise (57). This is a measure of the muscle's maximal ability to generate energy through oxidative metabolism and is dependent on mitochondrial content, quality, enzyme content, as well as muscle capillary density, and oxygen (O_2) perfusion. After exercise, other metabolites that accumulated during the contractions (H^+, ADP, and Pi) will also return to baseline levels; hence this period is referred to as recovery (81, 87).

Indirect Calorimetry: A Window into Whole-body Energetics

Indirect calorimetry is a respirometry technique that estimates energy expenditure via measurement of O_2 and CO_2 expired from the lungs. This gas exchange is representative of the O_2 consumption and CO_2 production of the entire body because all blood is eventually circulated to the lungs to allow gas exchange with air from the environment. In contrast to the muscle level, analysis of whole-body energetics through the use of indirect calorimetry allows for the evaluation of oxidative energy production only. Currently, there are no direct methods to determine the contribution of the PCr or glycolytic pathways to whole-body energy expenditure. Common variables assessed using indirect calorimetry include: the energetic cost of a task, oxygen uptake kinetics at exercise onset ("on" kinetics), oxygen uptake kinetics at the end of an exercise ("off" kinetics), and substrate use throughout exercise (grams oxidized per substrate). Similar to the measurement of muscle oxidative capacity, graded exercise tests can be used to determine the maximal rate of oxidative metabolism at the whole-body level (VO_2 peak). The same traits associated with high oxidative capacity in muscle are also associated with smaller and shorter O_2 deficits (on kinetics) and excess post-exercise oxygen consumptions (EPOCs; off kinetics) and increased use of fats during higher-intensity exercise (90).

At the beginning of a given exercise bout, energy demand increases instantaneously to a level dependent on the activity. Oxidative energy production, however, cannot increase instantaneously and will slowly rise to meet energy demand. The difference between the actual O_2 consumption and the amount required to meet demand is termed the O_2 deficit (Fig. 4). While in a state of O_2 deficit, the non-oxidative

pathways (PCr and glycolysis) provide the remainder of energy needed to perform the task.

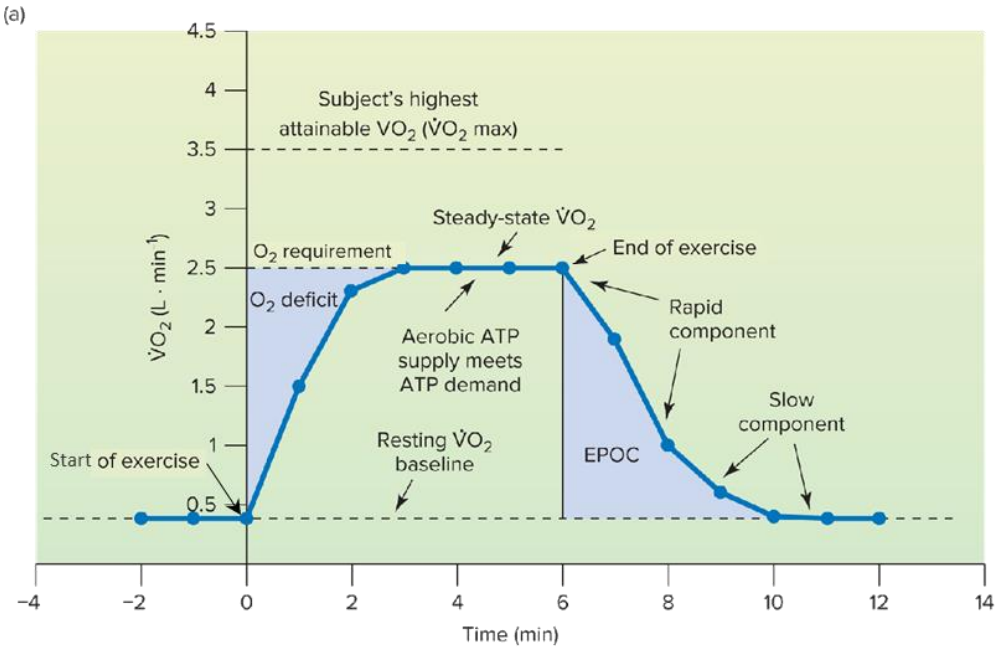


Figure 4. Oxygen consumption response to moderate-intensity exercise. (32)

At low-moderate exercise intensities, oxidative energy production will eventually meet demand and O_2 consumption will plateau. This is called steady-state, during this time the energy systems have reached equilibrium, and oxidative metabolism is assumed to be the main provider of energy (Fig. 4) (40). Once a steady-state is reached, the rate of carbohydrate and fat use can be estimated, as can energy cost of the activity (kcals). At high exercise intensities, oxidative energy production is never able to fully meet demand, and oxygen consumption will not plateau. This is not considered steady-state and the relative contribution of each pathway is unclear.

Post-exercise recovery is the process of the body returning to homeostasis. During recovery from exercise, oxygen consumption (and therefore energy expenditure) remains elevated above resting levels for a period of time even though the direct energy demand

of the exercise has ceased. This period is called the EPOC and can be broken into a fast (initial rapid decrease in O_2 consumption) and a slow (prolonged low-level elevation of O_2 consumption) component (Fig. 4). The excess O_2 consumed during the fast component is believed to contribute to the replenishment of hemoglobin and myoglobin oxygen stores (within seconds) and the oxidative regeneration of PCr to resting levels (within minutes). The excess O_2 consumed during the slow component is believed to be required due to the residual elevation in ventilation and heart rate, lactate's oxidation or conversion to glucose, glycogen re-synthesis, an increased rate of metabolism due to elevated temperature and catecholamine levels, re-establishment of ionic equilibrium, and potentially an increased protein turnover (38, 40).

There are two primary modes of indirect calorimetry; measurement of gas exchange by a metabolic cart or by a room calorimeter (also referred to as a metabolic chamber). Metabolic carts can allow for breath-by-breath analysis of respiration and therefore have a very fine temporal resolution. This technique requires the participant to wear a mask or a mouthpiece connected to a stationary cart or a backpack, which can be cumbersome, and uncomfortable. This makes them unsuitable for detecting small, long-duration changes in metabolism, and are typically only used for short-term analyses (<3 hr). Room calorimeters have a lower temporal resolution but do not require the use of masks or bulky equipment. In a room calorimeter, the participant is able to eat meals, exercise, and sleep while gas exchange is measured continuously. This allows for long-term (multi-day) metabolic analyses in an environment that more closely resembles free-living.

Connecting Muscle to Whole-body Energetics

While the energetics of a single muscle can be observed through ^{31}P-MRS, movement requires the energetic contributions of multiple muscles and physiological systems. Together, the energy requirements of muscular, cardiovascular, neural, and respiratory components of the body contribute to the cost of physical activity energy expenditure. However, muscle makes up 20-30% of energy expenditure at rest and up to 90% during exercise, and therefore, should have a large impact on metabolic variables observed during whole-body energy expenditure (110). The vastus lateralis (VL), a large muscle located on the anterior-lateral portion of the thigh, is one of the more common muscles studied using ^{31}P-MRS. The VL helps to extend the leg at the knee and plays an important role in ambulation during both the swing and support phase of gait (22, 76). It is activated during gait in a similar manner to the vasti medialis and intermedius, which contribute to gait increasingly as walking speed increases and make up the majority of the quadriceps muscles (76, 86). Due to the VL's significant role in ambulation, its energetic characteristics are likely to affect whole-body energy expenditure during mobility tasks.

Oxygen Deficit

At the onset of exercise, during the O_2 deficit, non-oxidative sources must compensate for the difference between energy demand and oxidative energy supply. While it is not currently possible to quantify the non-oxidative contribution at the whole-body level, it is possible to estimate non-oxidative ATP production in a single muscle. If an individual's VL has a high reliance on non-oxidative pathways and is slow to increase oxidative energy production, then it is likely to coincide with a greater oxygen deficit during whole-body exercise that relies heavily on the VL and like muscles.

Maximal Oxygen Consumption

The maximal rate of oxidative energy production can be measured at both the single muscle and whole-body levels as oxidative capacity and VO_2 max, respectively. Maximal oxygen consumption is approached during forms of exercise that require significant work from a large amount of muscle mass. Therefore, it is likely that the oxidative capacity of large muscles such as the VL would have a considerable impact on an individual's VO_2 max, which has been demonstrated previously (111).

Excess Post-exercise Oxygen Consumption

During recovery, extra energy (above resting) is required to return the body to homeostasis; this energy is produced via oxidative metabolism. This process can be observed at the whole-body level through the measurement of EPOC, a significant portion of which allows for the re-synthesis of muscle PCr. Therefore, a high muscle oxidative capacity may enable faster recovery from exercise and a shorter EPOC (63).

Substrate Use

Although ^{31}P-MRS can be used to measure the rate of oxidative energy production it cannot be used to determine substrate use (fat vs. carbohydrate use). However, substrate use can be estimated with indirect calorimetry using the respiratory exchange ratio (RER). Studies of substrate use during exercise have shown that at low intensities the body relies more on fat as an oxidative fuel source, but shifts to carbohydrates use as intensity increases (51). This occurs because carbohydrates can be used by oxidative pathways at a faster rate than fat. Studies have also shown that aerobically trained individuals will continue to rely on fat at higher relative and absolute intensities than their untrained counterparts (4). The same adaptations that occur with aerobic training, an increase in mitochondrial and capillary density, are also responsible

for increases in muscle oxidative capacity. Individuals with a greater oxidative capacity may then be more reliant on fat at any given intensity.

The Gap in Knowledge and Proposed Solution

Researchers have suggested that both single-muscle and whole-body energetics affect free-living daily function through changes in fatigue, fatigability, mobility, and physical activity (4, 9, 23, 24, 30, 92, 97-99, 108). While connections between muscle and whole-body energetic characteristics may appear relatively straightforward, few studies have attempted to evaluate the connection between the two in a comprehensive manner that is applicable to free living. One study by Rossiter et al. (93) measured muscle and whole-body energetics simultaneously during rhythmic alternating knee extensions. A direct association was shown between energy consumption measured at the muscle and whole-body level. However, the isolated non-ambulatory exercise used in the Rossiter et al. study is significantly different from normal activities of daily living. Another study by Hunter et al., in 2006, attempted to relate muscle metabolic function to free-living energy expenditure and physical activity. This study used a combination of room calorimetry and doubly-labeled water for estimation of free-living energy expenditure and activity levels. However, the precise physical activities, their time course, and the characteristics of O_2 deficit, substrate use, and EPOC could not be analyzed, as only the sum of two weeks of energy expenditure was recorded (48). To the best of the author's knowledge, a study has yet to be performed that evaluates the connection between high-resolution, whole-body energetics during free-living activities and muscle energetic characteristics during contractions of comparable intensity.

To fully understand the impact of alterations in muscle energetics on mobility-related function, their relation to whole-body energetics during mobility activities must be determined. For this reason, this study will consist of a comprehensive analysis of muscle and whole-body energetic responses to exercise, and then evaluate potential links between them. Completion of this project will advance the field by providing evidence that whole-body energetics can be used as a reliable intermediary point between muscle energetics and mobility-related function.

Specific Aims

With the above stated, the aims of the proposed research are:

Aim 1. *Evaluate the relationships between muscle ΔPCr in response to light intensity (6% MVIC) isotonic contractions and the O_2 deficit at the onset of a standardized treadmill walk.*

Hypothesis 1. *There will be a positive association between muscle ΔPCr and O_2 deficit because reliance on non-oxidative energy sources (ΔPCr) is and indicator that the ATP need not being met through oxidative metabolism. Therefore, those who rely more on PCr likely do so because it takes longer for them to increase oxidative energy production to meet demand at exercise onset (O_2 deficit).*

Aim 2. *Evaluate the relationships between muscle oxidative capacity and; EPOC and RER in response to a treadmill walk, and VO_2 peak from a graded maximal exercise test.*

Hypothesis 2.1. *There will be a negative association between muscle oxidative capacity and EPOC because a greater ability to produce energy oxidatively will result in less depletion of PCr and creation of lactate at the start of exercise, and, faster repletion of PCr and oxidation of lactate following exercise, both of which should result in a shorter EPOC time constant.*

Hypothesis 2.2. *There will be a negative association between muscle oxidative capacity and RER because a greater ability to produce energy oxidatively would decrease the relative intensity of a standardized exercise, and, is related to an increased ability to use fat as a fuel source, both of which are related to a greater reliance on fat and therefore a lower RER.*

Hypothesis 2.3. *There will be a positive association between muscle oxidative capacity and VO_2 peak because they are indicators of the maximal ability of the muscle's*

and the whole-body to consume oxygen, and muscle consumes the majority of the body's oxygen during maximal exercise.

CHAPTER 2

LITERATURE REVIEW

The purpose of this thesis is to connect two common forms of bioenergetic analysis in a way that will be useful in future research in the realm of aging, physical inactivity, and mobility-related function. Because the primary goal is to provide strong evidence of the relationship between energetic characteristics measured at the muscle and the whole-body levels, it is necessary that the techniques used, as well as their physiological significance, are understood. For this reason, this literature review will start with a relatively in-depth review of the methods and measurements that are the focus of this study. This review will be followed by a more succinct review of the literature regarding this study's motivation and justification.

Introduction of Measurements

^{31}Phophorus Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a method that that takes advantage of sub-atomic nuclear properties to determine the chemical composition of a given sample without the need to physically disrupt it or interfere with its function (i.e., noninvasively). Discussed here is ^{31}Phosphorus-MRS (^{31}P-MRS) as it is used in human bioenergetic research to determine the concentration of ^{31}P containing chemical compounds within the body. Analysis of these compounds allows for the observation of energy production and consumption in the body. There are many metabolically important ^{31}P containing chemicals in the body, but the ones most commonly measured are inorganic phosphate (Pi), phosphocreatine (PCr), and adenosine triphosphate (ATP). During human ^{31}P-MRS, a participant is put inside a magnetic resonance scanner where they are exposed to a

strong static magnetic field, and radio wave signals are sent into the body. The energy from these radio waves will be absorbed by ^{31}P atoms, which will then re-emit signals that can be detected by the scanner. These signals are emitted at specific frequencies, called resonant frequencies. Different frequency signals will be emitted based upon the characteristics of the molecule that the ^{31}P atom is bound to. The amount of signal at a certain frequency is based on the amount of a particular chemical in the sample, and it is from these signals that chemical concentrations are derived.

The output of this technique is a graph with frequency on the x-axis and amplitude on the y-axis. Peaks at different frequencies represent the ^{31}P signal from specific molecules and the amplitude of each peak represents the relative concentration of that molecule. This graph is known as a spectrum and an example can be seen in Fig. 5. In this spectra obtained from skeletal muscle peaks for Pi, PCr, and ATP are easily observable. There are three peaks for the ATP signal because there are three ^{31}P atoms in ATP, each of which resonates at a slightly different frequency due to its position and surroundings in the molecule (Fig. 6).

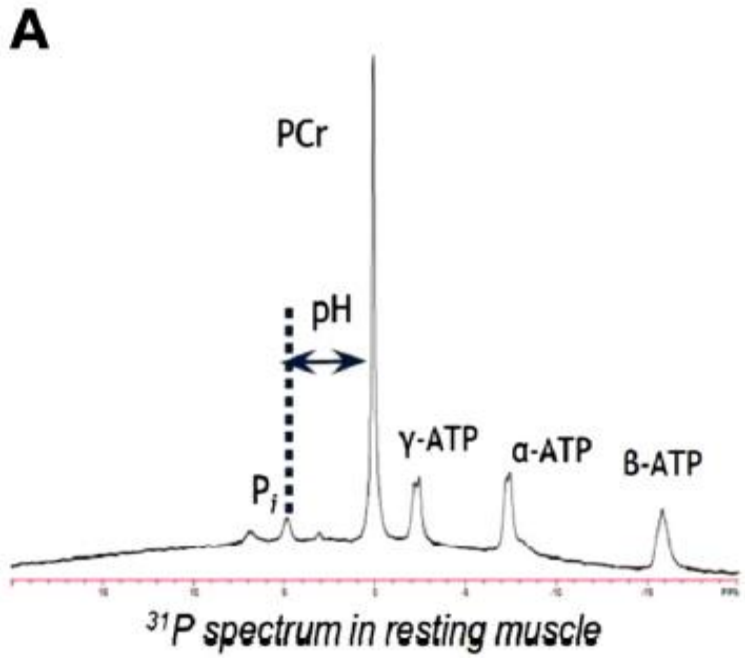


Figure 5. ^{31}P-MRS spectrum from resting muscle. (57)

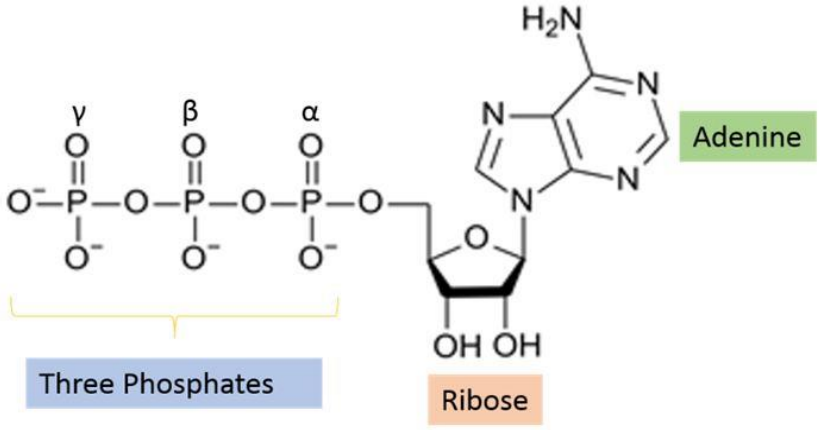


Figure 6. Labeled structure of ATP (Wikimedia Commons)

The use of ^{31}P-MRS to investigate phosphorus-containing chemicals began in 1960 when Cohn and Hughes successfully observed multiple ^{31}P peaks in solutions of both ADP and ATP. In the same study, they also demonstrated an important characteristic of MRS known as a chemical shift. A chemical shift is a change in the resonance frequency of an atom due to a change in the chemical environment, which in this case was altered pH. As seen in Fig. 7, when exposed to extremely low pH (3.60), the alpha (ά) and gamma (γ) ^{31}P peaks in ATP shift to both resonate at a similar frequency

and appear to merge into one signal. Measurement of particular chemical shifts in ^{31}P-MRS signals became of great importance in the years to follow (25).

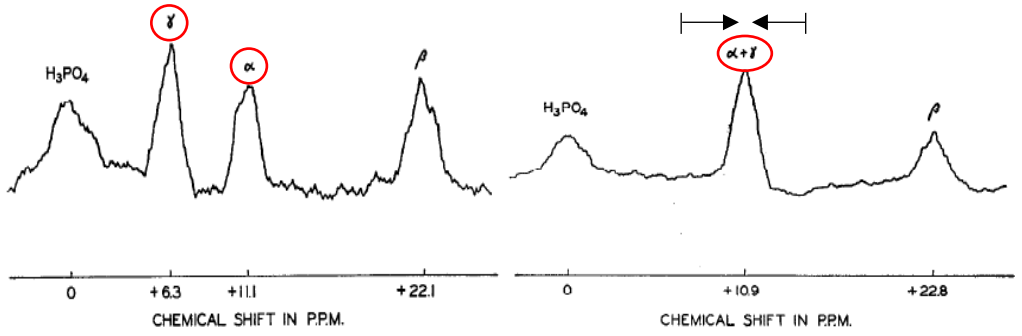


FIG. 3. The phosphorus magnetic resonance absorption spectrum of ATP at pH 8.75; all conditions are the same as Fig. 2.

FIG. 2. The phosphorus magnetic resonance absorption spectrum of the tetramethylammonium salt of ATP (0.09 M) at pH 3.60, μ = 1.0, with 85% phosphoric acid as reference.

Figure 7. The chemical shift in ATP Adapted from. (25)

In the 1970s scientists began using ^{31}P-MRS to investigate biological tissue (77). By the mid 70's Burt and colleagues (among others) brought the technique to the study of excised intact skeletal muscle, where they were able to resolve clear peaks for Pi, PCr, and the three phosphates of ATP, as well as several other metabolites. Since these experiments were conducted in live intact muscle, it became possible to observe how the concentration of metabolites changed over time, greatly increasing the efficacy of using this technique to investigate energetics (see Fig. 8).

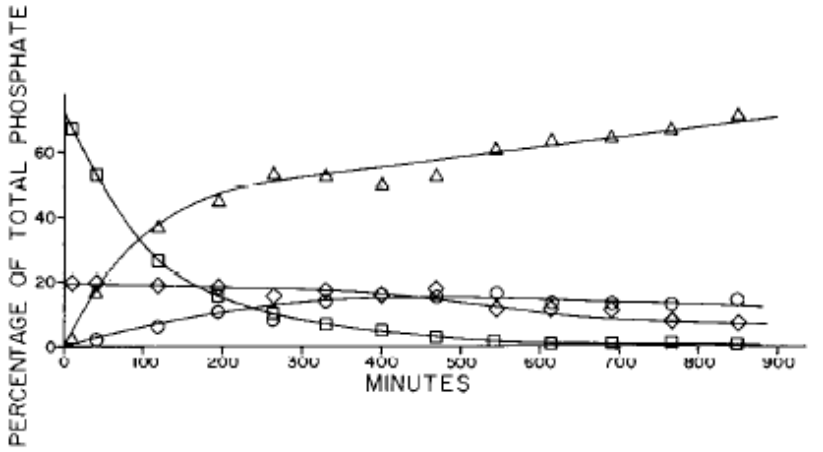


FIG. 2. Time course for the change in relative concentrations of phosphate compounds of Northern frog leg muscle at 28°. □——□, P-creatine; Δ——Δ, P_i; □——□, ATP; ○——○, sugar phosphates. The muscles were placed into the NMR tube and readings were taken at time intervals as indicated on the abscissa.

Figure 8. Change in concentrations of phosphorus metabolites as detected by ^{31}P-MRS. (16)

Through several titration experiments, it was also observed that Pi had a significant chemical shift across a physiologically relevant pH range (7.5 – 6.0 pH), but PCr and ATP did not (Fig. 9). This was an important discovery because it showed that the chemical shift of Pi could be used to accurately estimate the pH of intact muscle without the need for invasive measurements. The concept of which is as follows; because a change in pH across a normal physiological range causes a consistent and repeatable change in the resonance frequency of Pi, that resonance frequency can then be used to back-calculate pH (16).

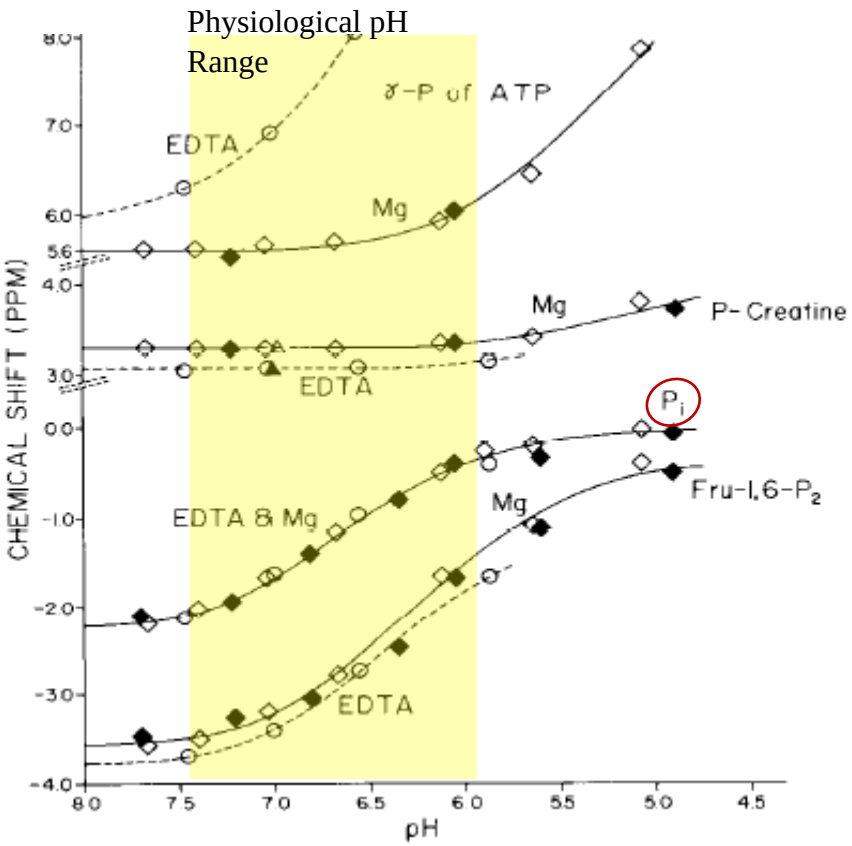


Figure 9. The chemical shift of various relevant phosphate compounds as a function of pH Adapted from (16)

In 1978 Dawson, Gadian and Wilkie pioneered techniques that allowed them to investigate the energetics of muscle fatigue (i.e., a decline in force production) in contracting muscle. By attaching a strain gauge to a sample of excised frog muscle inside a spectrometer (an MRS device) and stimulating it with electrodes, they completed one of the first ^{31}P-MRS analyses of live contracting muscle. From this, the energetic cost of force development was calculated and it was found that muscle fatigue was proportional to H^+ and ADP accumulation, but not to decreases in ATP or PCr. This suggested that muscle fatigue was likely due not to a lack of available energy, but rather inhibition caused by the buildup of metabolites. It was also observed that as less force was produced less ATP was used, suggesting that the production of force has a fixed cost and that the

economy of ATP hydrolysis is relatively stable. These findings demonstrated the potential power of ^{31}P-MRS and helped set the stage for years of muscle fatigue research to follow (28). A large breakthrough was made two years later by Ackerman et al. when they created an instrument called a 'surface coil' which when used in conjunction with standard MRS equipment greatly enhanced spatial resolution. Until this point, it was not possible to select a region of interest when scanning a sample, meaning that to study a specific tissue or organ it would first have to be removed from the organism. Utilizing this new tool Ackerman et al. became the first team to obtain ^{31}P-MRS spectra in animal muscle and brain *in vivo*. This breakthrough was of great importance because it opened the door for the use of MRS techniques in human muscle without the need for any invasive procedures (e.g., biopsies) (1). In the years since methods have continued to improve and MRS has become one of the most important tools used in the study of human bioenergetics.

Work with MRS has allowed scientists, in the words of Doris Taylor, to "define those features of muscle metabolism that are relatively invariant in healthy subjects" to enable the "diagnosis and understanding of different disorders of the locomotor system" (102). Some of these important features include the following. At the beginning of submaximal exercise PCr concentrations in muscle decline proportionally to work rate, but will reach a steady-state with a similar time constant (Fig. 10 B.) (6, 81). The rate of glycolysis will increase to assist in ATP production during contraction but not does contribute significantly to ATP or PCr synthesis during recovery (91, 102). Intracellular pH will decrease more with increasing work intensities and in relation to an increased rate of glycolysis (6, 17, 103). The creatine kinase (CK) reaction is maintained near-

equilibrium during submaximal contractions, which allows for the calculation of ADP concentrations (6, 103). Oxidative energy production is the major source of energy during light steady-state exercise and is solely responsible for the re-synthesis of PCr after exercise (Fig. 10 A.) (91, 102).

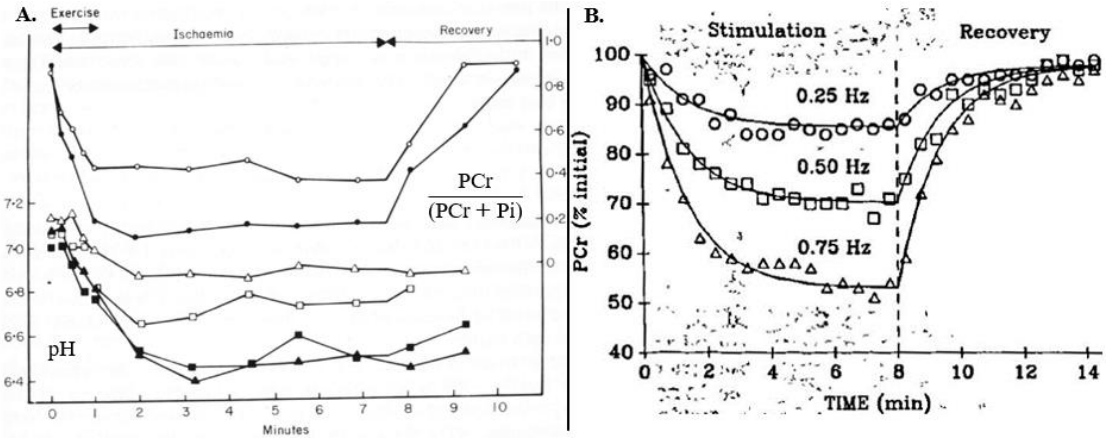


Figure 10. Muscle energetics responses to exercise, A) followed by ischemia, B) in response to various work rates. A) The 4 lower curves represent the intracellular pH of four participants. The upper top curve represents the PCr changes in participants 1-3 (open circle) and 4 (closed circle). B) Each curve represents the PCr response to repetitive muscle stimulation at increasing intensity representing 0.25, 0.50, and 0.75 Hz (A: (34), B: (38)).

With the knowledge of these and other basic features of skeletal muscle metabolism researchers have developed several measures that vary across subjects and can be used as indicators of energetic function. The two measures that are important to this study's aims are muscle oxidative capacity and non-oxidative ATP flux and are discussed further here.

Oxidative Capacity

Muscle oxidative capacity is a measure of the maximal rate of oxidative ATP production by the mitochondria. For this reason, muscle oxidative capacity can serve as a measure of mitochondrial function. Indeed, a higher oxidative capacity is associated with endurance training and physical activity and is likely a result of increased mitochondrial

content and oxidative enzyme activity (70). During exercise, PCr acts as a cellular buffer to transiently maintain ATP concentrations until demand can be met by glycolysis or oxidative metabolism. To complete this PCr donates a phosphate to ADP producing ATP and free creatine. In times of low energy demand, free creatine is then used to resynthesize PCr with the donation of a phosphate from oxidatively generated ATP, (Fig. 11).

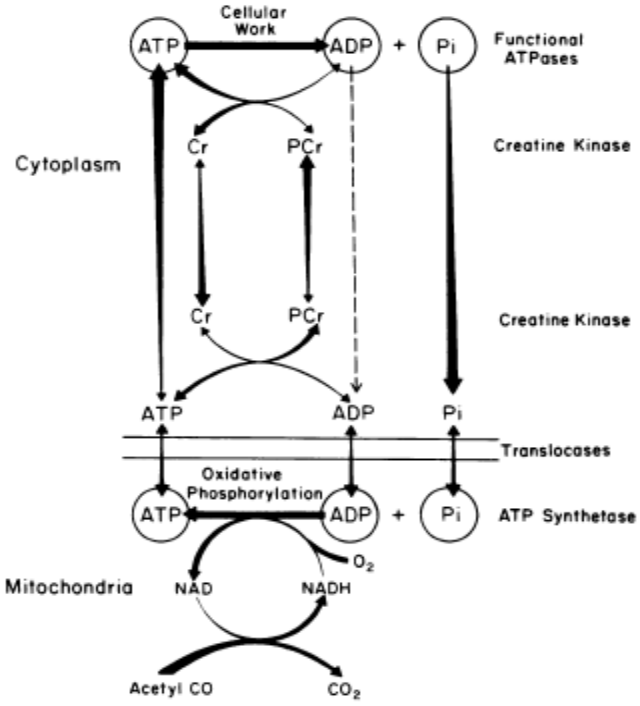


FIG. 1. Illustration of the feedback interactions of the cytosolic and mitochondrial compartments of muscle cell.

Figure 11. Mechanisms of phosphate transfer in the cell. (20)

Oxidative capacity is estimated using PCr recovery rate after a bout of contractions that decrease its concentration by ~50%, without altering pH or ATP levels. This is based on the observation that PCr is replenished after exercise almost exclusively by the oxidative pathway and follows a monoexponential trajectory (81, 91, 102). The use of this ^{31}P-MRS measurement of mitochondrial function has been validated against in

vitro respiration measures and when applied appropriately is both accurate and precise (67, 72).

Non-Oxidative ATP Flux

As previously stated, any time that oxidative energy production cannot meet demand the non-oxidative energy pathways will generate the remainder of ATP needed. This is particularly important during the onset of exercise due to the slow response time of oxidative metabolism, as seen in see Fig. 12 (54).

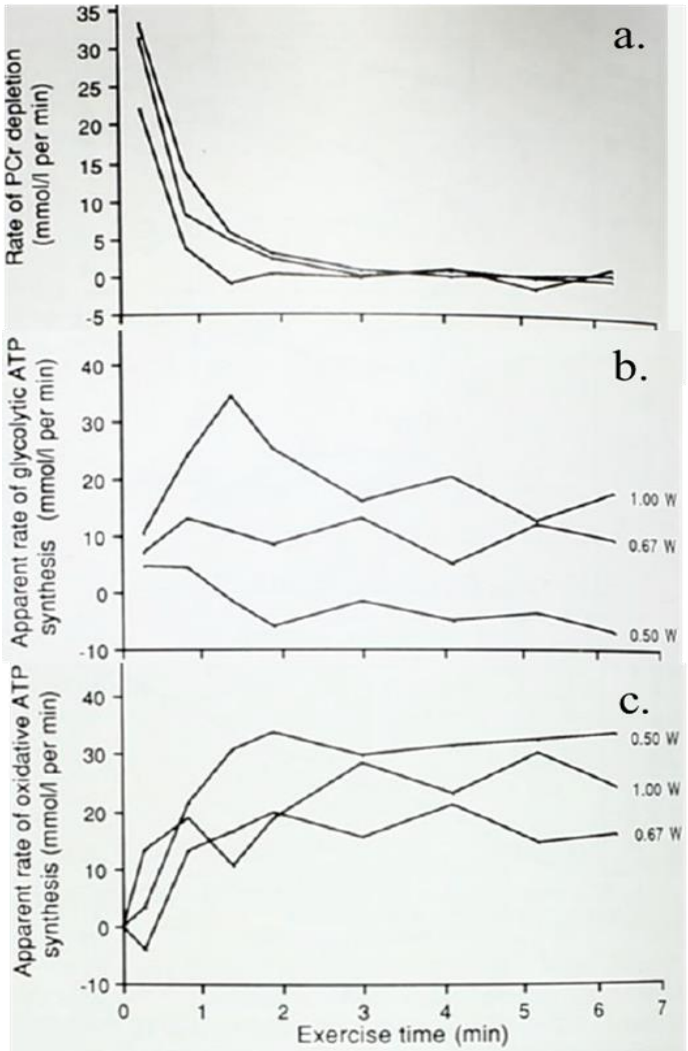


Figure 12. Time course for the rates of PCr depletion a), glycolysis b), and oxidative metabolism c), in skeletal muscle at three different workloads. (54)

This flux of ATP is proportional to the intensity of the exercise and the time constant for the rise in oxidative metabolism (54). Non-oxidative ATP flux is contributed to by both the PCr and glycolytic pathways. The rate of non-oxidative metabolism is important as higher rates are associated with greater fatigue and slower recovery (28, 103). The generation of ATP from PCr can be measured directly from the change in concentration of PCr. Glycolytic ATP generation is estimated using its stoichiometric relation to proton (H^+) production. Proton production is measured using the chemical shift as discussed earlier (16, 28, 102). A portion of the protons generated from glycolysis is either excreted from the cell or buffered by intracellular chemicals. This means that the pH of the muscle does not fully reflect the proton generation and an additional calculation must be used to account for the proton buffering capacity of the cell (28). Many different methods exist for these calculations so they are not included here.

Indirect Calorimetry

Humans derive energy through a series of reactions that release stored energy from the chemical bonds in the food they consume. During this process, whether it be oxidative on non-oxidative, heat is produced. Direct calorimetry is the measurement of this heat production, which is proportional to the summed energy expenditure from oxidative and non-oxidative metabolism. Due to this, direct calorimetry can be used in biological research to measure the energy expenditure of various organisms and has been applied to the study of human energetics since the 18th century. The basis for a direct calorimeter is simple. It is a device (usually a sealed chamber) that measures the change in temperature of its contents. However, the implementation of these devices is far more complex. Direct calorimeters that can be used for human research must be rather large

and are expensive, low in temporal resolution, and have limitations that make them undesirable for the study of exercise. An alternative technique for human metabolic analysis is indirect calorimetry. Indirect calorimetry is a method that uses measurements of O_2 gas consumption and CO_2 gas production to estimate energy expenditure (Fig. 13).

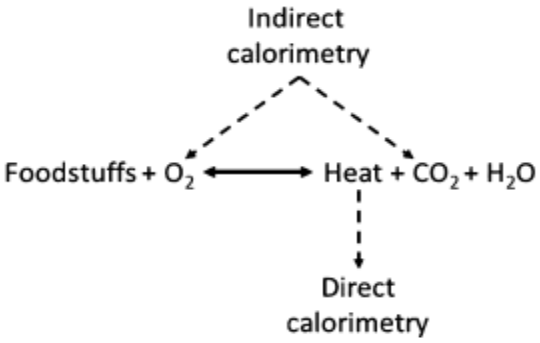


Fig. 1 Indirect versus direct calorimetry. Instead of measuring the heat produced as a result of biological reactions (direct calorimetry), the rate of oxygen (O_2) consumption and carbon dioxide (CO_2) production during biological oxidations at rest or during physical work is used to estimate heat production (indirect calorimetry)

Figure 13. The relationship between direct and indirect calorimetry. (56)

As mentioned earlier, all human energy is originally derived from food and the breakdown of this food releases energy and heat. An important aspect of this breakdown (catabolism) is that when energy is derived oxidatively, a specific amount of O_2 is consumed and CO_2 is produced, depending on the substrate that is involved (particularly carbohydrate or fat). The amount of energy expended as well as the food source that it comes from can then be calculated using measurements of gas exchange by observing the sum and the ratio of O_2 consumed to CO_2 produced (Fig. 14).

Carbohydrate- $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

Fat- $C_{16}H_{32}\ O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16\ H_2O$

Figure 14. Stoichiometry of carbohydrate and fat catabolism. Adapted from (51).

Compared to direct calorimetry this method is less expensive, has better time resolution, and can be used to estimate energy expenditure during exercise as well as the amount of carbohydrate and fat used (i.e., substrate use). Indirect calorimetry equipment measures the gas exchange between an organism's lungs and the environment and may come in the form of masks, ventilated hoods, and whole-room chambers (Fig. 15). A major drawback of indirect calorimetry is that it only measures energy expenditure related to oxidative metabolism, although the size of the O_2 deficit can be used to estimate relative the contribution of non-oxidative metabolism. Despite this, it is currently the most popular calorimetric technique used to analyze human energy metabolism (56).

Figure 15. Methods of indirect calorimetry. From left to right Hans-Rudolph mask, ventilated hood, whole-room chamber.

Indirect calorimetry was developed during the early 1800s and was validated against direct calorimeter in animal studies. While the basis of indirect calorimetry is slightly more complex than direct calorimetry, most information gained from this technique comes from three outputs: volume of O_2 consumed (VO_2), the volume of CO_2 produced (VCO_2), and the ratio between the two (RER). Just before the start of the 20th century, the Atwater-Rosa calorimeter became the first device to simultaneously measure

direct and indirect calorimetry. Studies from this chamber verified that indirect calorimeter measures in humans were proportional to direct calorimeter measures, and were, therefore, an appropriate technique for studying human energetics (7). In the years to follow indirect calorimeters became popular research instruments and many improvements were made to the devices and techniques used to analyze their data (83). In particular, two important equations were developed in the early 1900s that have almost ubiquitous use in the field.

The first is called the Haldane Transform, which was developed by John Haldane and Joseph Graham in 1912. This equation allows for the estimation of inspired volume (*VI*) using the measured expired volume (*VE*) and the concentrations of exchanged gases. During indirect calorimetry, gas concentrations are measured as fractions of the total air sampled during inspiration (fraction inspired '*FI*') and expiration (fraction expired '*FE*'). The composition of ambient air is typically ~78% nitrogen, ~21% oxygen, and ~.04% carbon dioxide (values which are sometimes substituted for direct measurement of inspired air). Since gas concentration measurements are fractions they are meaningless until multiplied by the absolute volume of air exchanged (i.e., *VI* and *VE*). This makes the volume measurements very important in the calculations of VO_2 and VCO_2, as can be seen in equations 2 and 3.

$$VO2 = VI \cdot FIO2 - VE \cdot FEO2$$

Eq. 1

$$VCO2 = VI \cdot FICO2 - VE \cdot FECO2$$

Eq. 2

However, *VI* can be difficult to measure experimentally. For this reason, the Haldane Transform can be used to estimate *VI*, based on the assumption that no nitrogen exchange occurs in the lungs (a topic that is outside the scope of this review). The Haldane Transform is as follows for the calculation of VO_2, but can be used likewise for the calculation of VCO_2.

$$HT:\ VO2 = VE * kH * FIO2 - VE * FEO2 \quad \text{Eq. 3}$$

$$kH = \frac{(1 - FEO2 - FECO2)}{(1 - FIO2 - FICO2)} \quad \text{Eq. 4}$$

As can be seen in Eq. 3, *VI* has been replaced by *VE* multiplied by the correction factor *kH*. The correction factor represents the ratio of volume inspired to volume expired. The Haldane Transform is widely used in the field of research and has been shown to be an accurate means of calculation when used in normal atmospheric conditions ($FIO_2 \cong 21\%$) (65, 83).

The second important equation is related to the calculation of energy expenditure. This equation was first described by J.B. de V. Weir in his 1949 article in the Journal of Physiology (106). It has since come to be known as the Weir formula. One of the desirable attributes of indirect calorimetry is that it allows the calculation of metabolic rate (energy expended per unit time), both total and by individual substrate contribution. Weir had recognized that it was common in metabolic research to calculate energy expenditure (in kcals), without either measuring or correcting for protein metabolism. For this reason, he set out to demonstrate that protein metabolism could easily be accounted for using several simple methods. Previous works had determined the kcals produced per liter of O_2 consumed in the metabolism of carbohydrate, protein, and fat. Using these

Weir developed a formula where protein metabolism could be estimated from urinary nitrogen;

$$Total\ kcals = (3.941 \cdot VO_2) + (1.106 \cdot VCO_2) - (2.17 \cdot grams\ of\ urinary\ nitrogen)$$

Eq. 5

a formula where protein metabolism could be assigned a constant contribution *p;*

$$Total\ kcals = ((3.941 \cdot VO_2) + (1.106 \cdot VCO_2)) / (1 + .082p)$$

Eq. 6

and a formula where protein metabolism can be ignored with less than .2% error, so long as protein contribution is between 10-14%;

$$Total\ kcals = ((3.9 \cdot VO_2) + (1.1 \cdot VCO_2))$$

Eq. 7

These equations remain a popular means of calculating energy expenditure today and are directly integrated into many indirect calorimetry systems (106). The use of Eq. 7 will be appropriate in this study as the conditions are unlikely to induce a large use of protein for energy production.

Since 1949 numerous studies have been completed using indirect calorimetry. Today it may be the most widely used tool in the world of exercise science. Indirect calorimetry is a common tool in other fields as well, such as nutrition, pediatric care, and animal research. Depending on the purpose of the experiment many different metrics may be derived from this one measurement technique. Discussed here are important papers regarding the main outcomes of this study: VO_2 max, O_2 deficit, EPOC, and substrate use.

VO_2 Max

The maximal rate that an individual can consume O_2, which is dependent on intake, transport, and use, is termed VO_2 max. The origins of this measurement lie with the observations of Hill and Lupton in 1923 when they described the tendency of O_2 consumption to increase linearly with workload until a certain point where it would plateau despite an increase in workload (46). Consumption of O_2 is reliant on the pulmonary, cardiovascular, and muscular systems and maximal consumption is, therefore, an indicator of the function of these combined systems (34). Specifically, the lungs are responsible for bringing O_2 into the body, which then needs to be transferred to the blood where it is bound to hemoglobin. Once O_2 is in the blood, the heart must circulate that blood to the working tissue, where the O_2 can be transferred again into the cells. Finally, the mitochondria in the cells must be able to uptake and use the O_2, to ensure that a concentration gradient (which drives O_2 transfer) is maintained. The test itself is usually performed on a cycle ergometer or a treadmill, and the work rate is increased gradually until the max is reached. Although it is a subject of debate, VO_2 max is generally considered to be limited by O_2 delivery ($SV \cdot HR$) and not O_2 utilization (avO_2 diff). Physical activity and exercise training can cause physiological adaptations (Fig. 16) that improve VO2 max, which is important as this may decrease mortality risk and improve sports performance (74, 78)

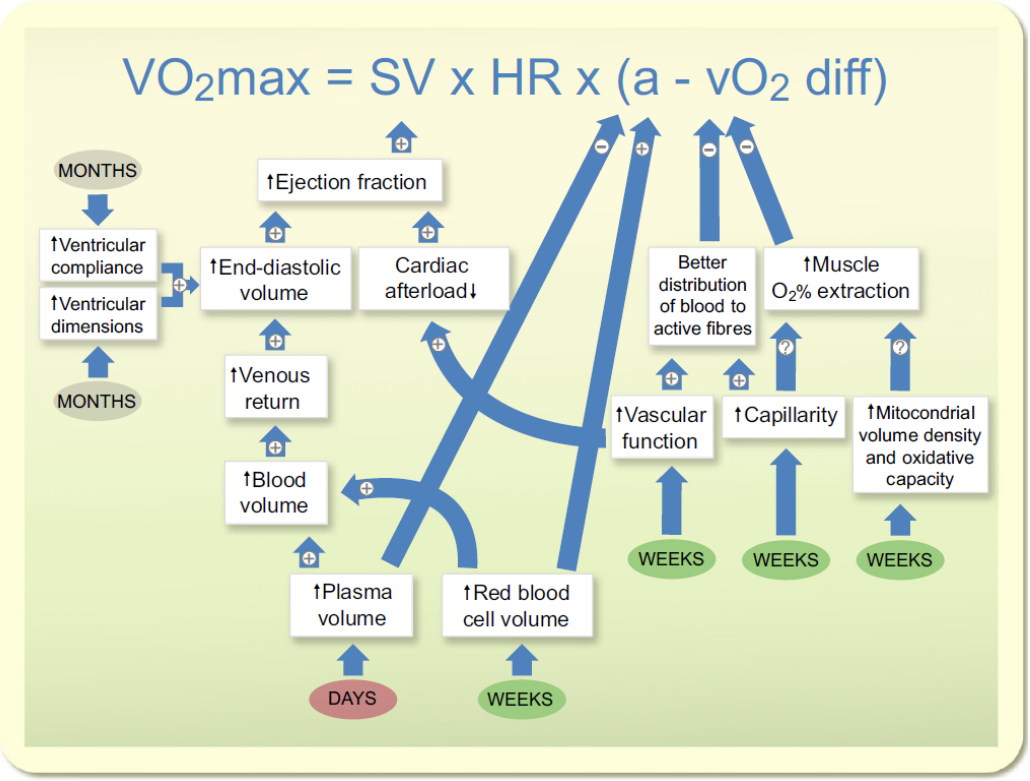


Figure 16. Physiological adaptations that lead to an improvement in VO_2 max. (78)

O_2 Deficit

At the onset of exercise, energy demand increases immediately to a level dependent on the activity intensity. The pulmonary, circulatory, and metabolic processes required for oxidative energy production cannot increase instantaneously and will slowly rise to meet energy demand. The difference between the actual O_2 consumption and the amount required to meet energy demand is termed O_2 deficit (Fig. 17) (40). At low-moderate exercise intensities, oxidative energy production will eventually meet demand and O_2 consumption will plateau. During a constant work rate exercise, the level of this plateau can be used to estimate the energy demand for the entirety of exercise (red line in Fig. 17). While in a state of O_2 deficit the non-oxidative pathways (PCr and glycolysis) provide the remainder of energy needed. This has led various researchers to suggest that

measures of O_2 deficit could be used to estimate an individual's "anaerobic capacity" (8, 79). Deficit increases linearly with exercise intensity, meaning there is greater non-oxidative reliance at higher work-rates. Larger O_2 deficits are associated with lower exercise tolerance and earlier onset of fatigue (29). Exercise training, however, can reduce the O_2 deficit for a given workload and potentially increase exercise tolerance (84). Physiological adaptations that lead to a decreased O_2 deficit are identical to those that improve VO_2 max, including those affecting O_2 delivery (i.e., cardiovascular adaptations) and utilization (i.e., increased mitochondrial size and content, etc) (90).

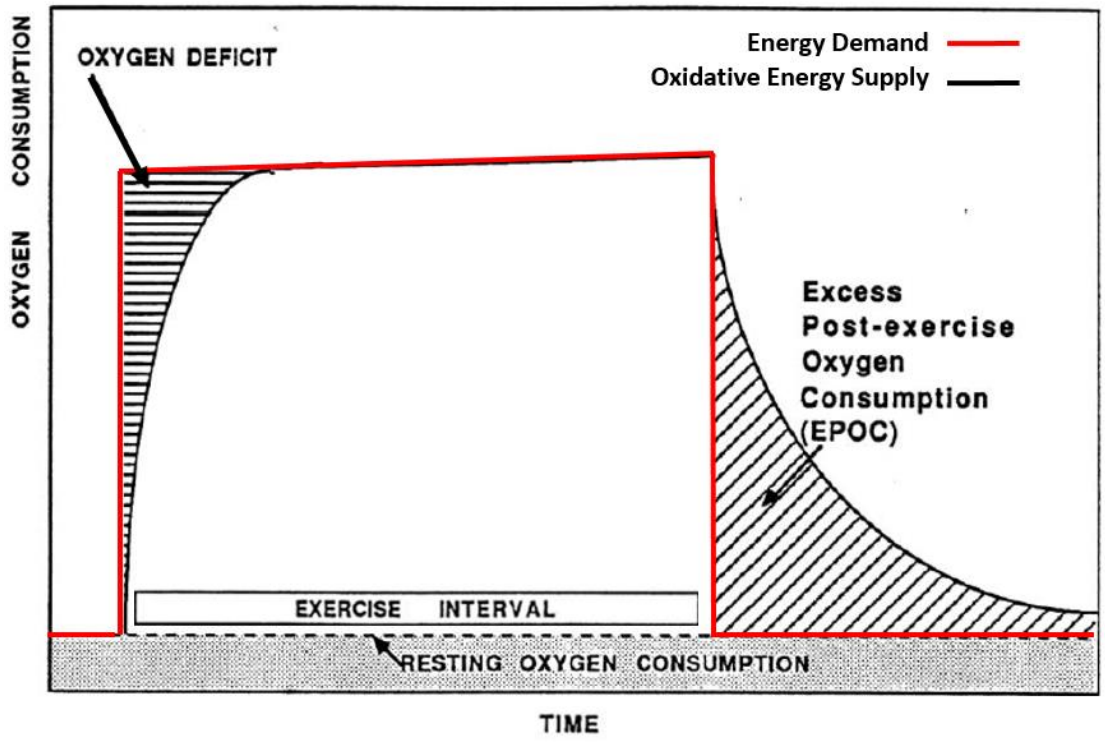


Figure 17. Oxygen consumption in response to exercise over time. Adapted from (62).

Excess Post-Exercise Oxygen Consumption

After the cessation of exercise, oxygen consumption remains elevated above resting levels despite the apparent immediate decrease in energy demand (Fig. 17). This

is termed excess post-exercise oxygen consumption (EPOC), and its magnitude is proportional to both the duration and intensity of the exercise (40). Post-exercise oxygen consumption follows an exponential curve as it recovers to baseline resting values. This recovery curve can be broken into two components: an initial rapid decrease in O_2 consumption (fast component) and a prolonged gradual decline in O_2 consumption (slow component) (Fig. 18). The excess O_2 consumed during the fast component is believed to contribute to the replenishment of hemoglobin and myoglobin oxygen stores (within seconds) and the oxidative regeneration of PCr to resting levels (within minutes). The excess O_2 consumed during the slow component is believed to be due to the residual elevation in ventilation and heart rate, lactate's oxidation or conversion to glucose, glycogen synthesis, an increased rate of metabolism due to elevated temperature and catecholamine levels, re-establishment of ionic equilibrium, and potentially an increased protein turnover (38, 40). The total duration of EPOC can be anywhere from a few minutes to multiple hours. A study by Knab and colleagues showed an elevated metabolic rate as long as 14 hr in response to a 45 min cycling bout (60). In a study examining the effect of training status, trained participants were shown to have EPOCs that were shorter in duration but similar in magnitude (area under the curve) when compared to untrained participants exercising at the same relative intensity. This study also suggested the cardiovascular and muscular adaptations that occur with exercise training and affect oxygen delivery and utilization were responsible for the faster recovery rates due to an increased efficiency of metabolic regulation (100). These adaptations, again, are the same as those that improve VO2 max, and O2 deficit.

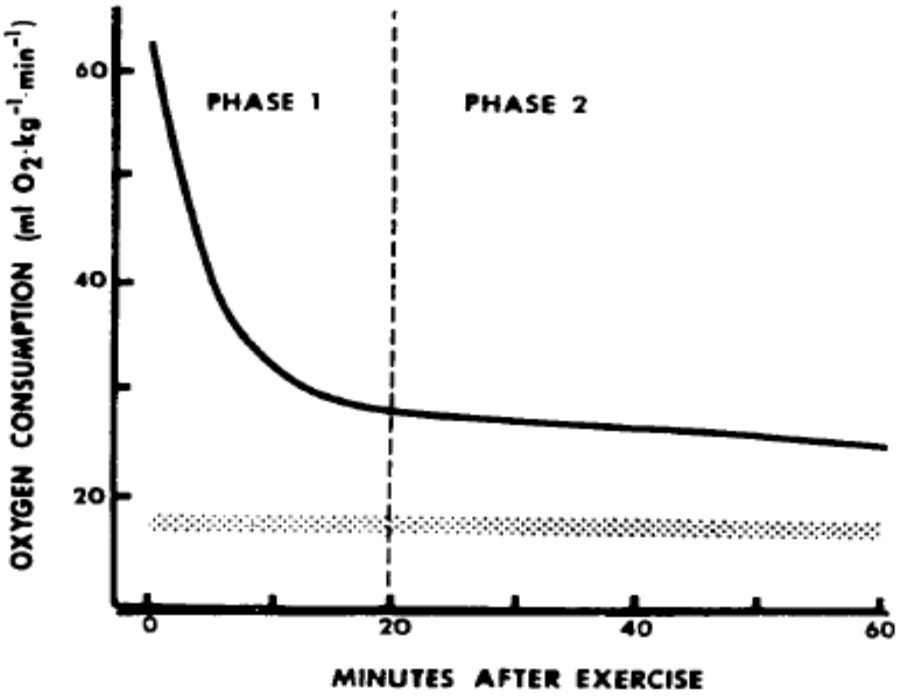


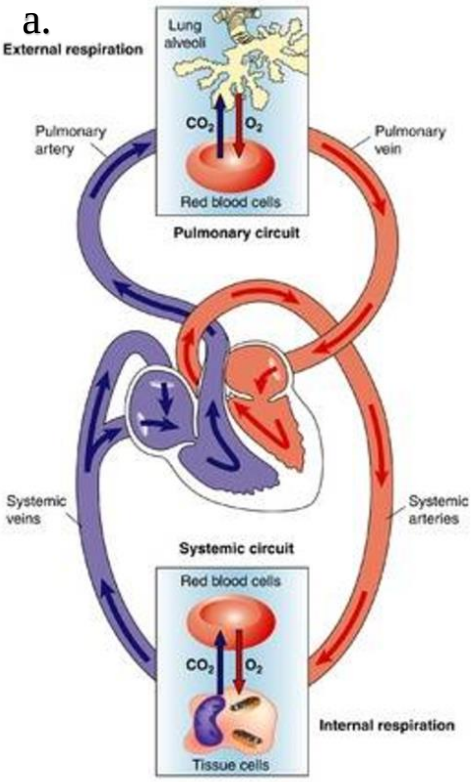
Figure 18. Visualization of EPOC fast component (Phase 1) and slow component (Phase 2). (38)

Substrate Use

The amount of carbohydrate, fat, or protein oxidized is termed substrate use (because protein oxidation is minimal in most cases it will be ignored here). Carbohydrates and fat are each oxidized in tissue with a unique ratio of CO_2 consumption to O_2 production, which is called the respiratory quotient (RQ) (Fig. 14). The ratio of O_2 production and CO_2 consumption of an entire cell is, therefore, the average of the RQ for all of the substrates oxidized, and can be used to determine the relative proportion of substrate use. Because the O_2 and CO_2 exchange at the cells is mirrored at the lungs, pulmonary gas exchange can be used to estimate RQ (Fig. 19a). This is done through the measurement of the ratio of VCO_2 to VO_2, or the respiratory exchange ratio (RER), and is representative of the substrate use for the whole-body. The value of RER generally ranges from ~.7 to ~1, and each increment is associated with a percentage contribution

from carbohydrate and fat (Fig. 19b). Different calculations based on the setting in which RER is measured (rest vs exercise), can then be used to determine the absolute amount of substrate oxidized (51). The use of RER has been validated through a carbon isotope exchange technique, and was shown to be a valid measure at steady-state intensities up to 75% of VO_2 max, but not valid during higher intensities or work-to-rest transitions (51).

a.



b.

Non-protein respiratory quotient.	Calories per liter of O_2.[1]		Calories per liter of CO_2.[2]		Proportion of calories from—	
	Number.	Logarithm.	Number.	Logarithm.	Carbohydrate.[3]	Fat.[3]
					Per cent	*Per cent*
0.70	4.686	0.67080	6.694	0.82569	0.0	100.0
.71	4.690	67117	6.606	81994	1.4	98.6
.72	4.702	67228	6.531	81498	4.8	95.2
.73	4.714	67339	6.458	81010	8.2	91.8
.74	4.727	67459	6.388	80536	11.6	88.4
.75	4.739	67569	6.319	80065	15.0	85.0
.76	4.752	67688	6.253	79609	18.4	81.6
.77	4.764	67797	6.187	79148	21.8	78.2
.78	4.776	67906	6.123	78696	25.2	74.8
.79	4.789	68024	6.062	78262	28.6	71.4
.80	4.801	68133	6.001	77822	32.0	68.0
.81	4.813	68242	5.942	77393	35.4	64.6
.82	4.825	68350	5.884	76967	38.8	61.2
.83	4.838	68467	5.829	76559	42.2	57.8
.84	4.850	68574	5.774	76148	45.6	54.4
.85	4.863	68690	5.721	75747	49.0	51.0
.86	4.875	68797	5.669	75351	52.4	47.6
.87	4.887	68904	5.617	74950	55.8	44.2
.88	4.900	69020	5.568	74570	59.2	40.8
.89	4.912	69126	5.519	74186	62.6	37.4
.90	4.924	69232	5.471	73807	66.0	34.0
.91	4.936	69338	5.424	73432	69.4	30.6
.92	4.948	69443	5.378	73062	72.8	27.2
.93	4.960	69548	5.333	72697	76.2	23.8
.94	4.973	69662	5.290	72346	79.6	20.4
.95	4.985	69767	5.247	71991	83.0	17.0
.96	4.997	69871	5.205	71642	86.4	13.6
.97	5.010	69984	5.165	71307	89.8	10.2
.98	5.022	70088	5.124	70961	93.2	6.8
.99	5.034	70191	5.085	70629	96.6	3.4
1.00	5.047	70303	5.047	70303	100.0	0.0

[1] For the factors here given see Zuntz and Schumburg, Physiologie des Marsches, Berlin, 1901, p. 361. The logarithms, however, correspond to the numbers of calories.
[2] Benedict and Talbot, Carnegie Inst. Wash. Pub. No. 201, 1914, p. 29.
[3] Williams, Riche and Lusk, Journ. Biol. Chem., 1912, **12**, p. 357.

Figure 19. a.) Gas exchange at the cells and lungs; b.) Caloric values of O_2 and CO_2 for non-protein RQ and proportions of substrate used (a (90), b (64))

Substrate use at a given intensity is variable across the population but is relatively invariant in an individual (51). One way to characterize substrate use in an individual is to find their crossover point, which is the intensity at which carbohydrate oxidation exceeds fat oxidation (Fig. 20). Training has been shown to increase fat use across relative intensities and shift the crossover point to the right (13, 31).

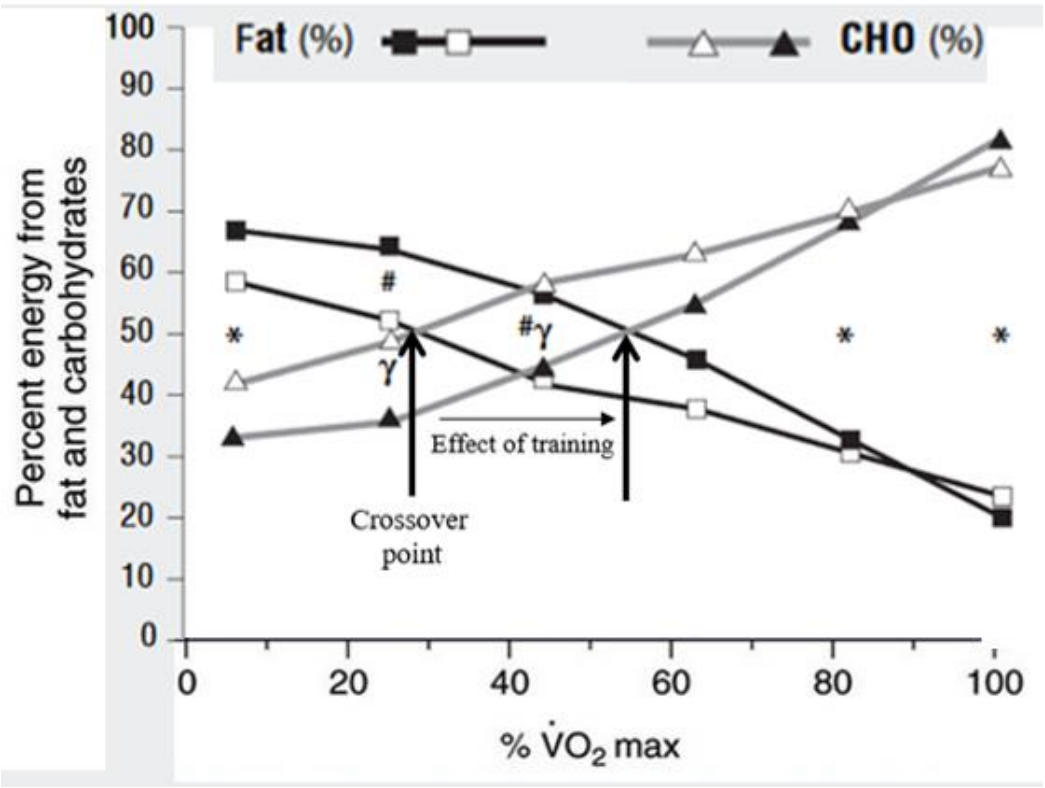


Figure 20. Crossover point and the effect of training. Adapted from (13)**.**

Measurement Comparison

As stated at the start of this review, the goal of this thesis is to provide strong evidence of the relationship between measured characteristics at the muscle and the whole-body level. Now that the measures have been described it is important to note the similarities and differences between the two methods. This is perhaps most efficiently done through the use of a table (Table 1).

Table 1. Comparison of ^{31}P-MRS and Indirect Calorimetry Measures

	31P-MRS	Indirect Calorimetry
Measures	Muscle	Whole-body
Oxidative Energy	Yes	Yes
Steady State Consumption	Yes	Yes
Maximal Consumption	Yes	Yes
Substrate Utilization	No	Yes
O2 Deficit	No	Yes
EPOC	No	Yes
Non-Oxidative Energy	Yes	No
PCr Hydrolysis	Yes	No
Glycolysis	Yes	No
pH	Yes	No
Other Metabolites	Yes	No

It is also important to note that because both levels measure characteristics of energy expenditure and reflect the acute energy demands placed on the body, they will likely be affected similarly by physiological adaptations brought on by chronic energy demand. For instance, during prolonged walking or running, energy demand will increase and stress both the muscles involved (such as the VL) and other systems in the body (such as the cardiovascular system). Stress which over time leads to adaptation. This notion will be expanded upon in the next section, which reviews the literature directly related to this project.

Problem to be addressed and proposed solution

Problem to be Addressed

Aging and physical inactivity leads to a decline in mobility-related function, which includes a decrease in walking speed, postural control, and the ability to transition from sitting to standing or walking (30, 35). Mobility limitations are associated with

increased falls and healthcare use and expenditures, and a decreased quality of life in older populations (27, 35, 85). Performance of mobility test such as the 'timed up and go' can predict all-cause mortality (12). Aging and physical inactivity are also associated with a decline in muscle oxidative capacity, specifically in the knee extensor muscles, which is likely detrimental to mobility-related function (Fig. 21) (2, 23, 24, 30, 36, 97, 108). However, even modest improvements in mobility-related function have been suggested to increase physical, emotional, and social well-being in older adults (12, 95). For these reasons, our lab has long researched age and physical activity-related changes in muscle energetics, to support the combat of age-related declines in mobility and physical activity (18, 36, 66, 96, 105).

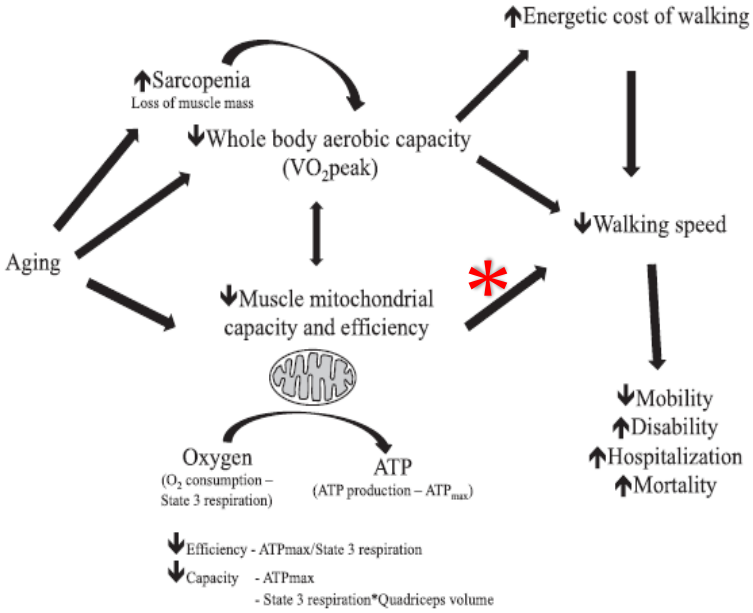


Figure 1. Concept map illustrating age-related changes in muscle physiology and how they contribute to reduced walking speed in older adults. This study examined the relationships between muscle mitochondrial capacity/efficiency, aerobic capacity, and walking speed in older adults. VO_2 peak = maximal oxygen consumption during maximal dynamic exercise. This is an index of whole-body aerobic capacity.

Figure 21. The mechanistic pathway connecting aging, muscle energetics, mobility, and health outcomes. (* indicates the relationship of interest here). Adapted from (24).

As discussed above, the vital role and large energy requirements of muscle during ambulatory tasks make muscle energetic function inherently important for mobility.

Correspondingly, researchers have sought to determine the effect of decreased energetic function on mobility performance (23, 24, 97, 108). In 2013, Coen et al. (24) were the first to investigate the association between of three different oxidative energy measures (*in vitro* mitochondrial respiratory capacity, *in vivo* muscle oxidative capacity, and VO_2 peak) on preferred walking speed over 400 in older adults. They found that all three energetic measures were significantly associated with preferred walking speed, such that individuals with a lower energetic capacity tended to walk slower. Similarly, in 2016 Choi et al. (23) found that muscle oxidative capacity was positively associated with preferred walking speed during a 1.5 min test, as well as maximal walking speed over 6 and 400 m, but not preferred walking speed over 6 m. This led them to suggest that muscle bioenergetics may be a limiting factor for walking performance in tasks of greater duration and intensity (23). Given the duration of a 6 m walk (< 20 s) it would seem unlikely that muscle energetics would be a limiting factor in the absence of serious impairment. Santanasto et al. demonstrated a positive relationship between muscle oxidative capacity and walking speed as well, although the association fell apart for older adults with lower physical function (mean SPPB = 7.8 ± 1.2) (97). In cases of impaired physical function, other comorbidities such as muscle weakness, neurological disease, or previous injury may have a larger impact than decrement in energetic function. In an attempt to identify the mechanism by which muscle oxidative capacity affects walking, Zane et al. (108) found that this interaction is partially mediated by muscle strength for short-duration activities but not for long-duration activities. This finding suggests that although during short-duration activities the effect of decreased muscle oxidative

capacity may be confounded by decreases in muscle strength, the same does not appear to be true for long-duration activities.

While the above studies provide robust evidence of the relationship between muscle energetics and mobility in a large sample of older adults (total n=551), they are largely limited to the measurement of one *in vivo* energetic variable and a relatively low number of mobility outcomes. Also, the mechanism by which muscle energetics impacts mobility remains unclear, and it seems likely that other characteristics of muscle energetics, such as non-oxidative ATP flux, may be of importance as well. A more comprehensive framework of the relationship between muscle energetics and mobility-related function, such as the one proposed in Fig. 22, would be beneficial for understanding and combating aging and physical inactivity-related issues. One major obstacle to this is the restriction of ^{31}P-MRS measurement to the bore of a magnetic resonance scanner. If *in vivo* muscle energetics could be assessed during mobility tasks, it would be much easier to uncover a potential energetic mechanism by which performance in those task may be impaired. While technology is far from allowing this type of analysis, measurement of whole-body energetics may provide an alternative mechanistic avenue. Although a correlation between muscle and whole-body energetic characteristics cannot prove causality, it would show that the changes in whole-body energetics (that have been associated with decreases mobility-related function), can be partially explained through decrement in muscle energetic function. Such a relationship would suggest that improving muscle energetic capacity - perhaps through isolated single joint exercises - could be an effective strategy for improving mobility-related function.

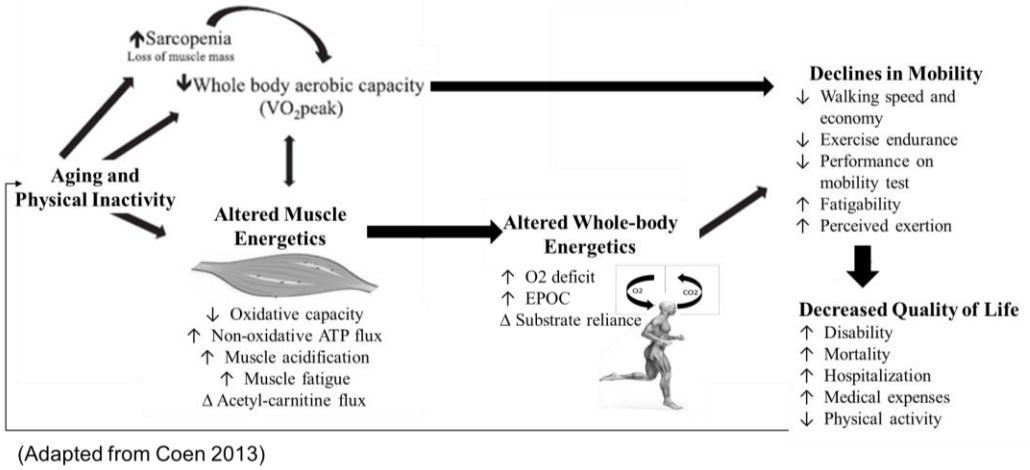


Figure 22. The proposed framework for physiological interactions that lead to decreases in mobility-related function. Adapted from (24).

Proposed Solution

Characteristics of whole-body energetics have long been linked to exercise performance (26, 52, 104), and more recently measures of mobility-related function (4, 9, 15, 98, 99). Indirect calorimetry measurements of whole-body energetics have also been linked to those of *in vivo* muscle energetics (2, 63, 111) and their relationship has even been evaluated simultaneously on multiple occasions (19, 21, 73, 82, 93, 94), albeit only in highly constrained settings. Based on these observations whole-body energetics could potentially act as an intermediary point between muscle energetics and mobility-related function (Fig. 22).

The Advantages of Whole-body Energetic Measures

Due to its relatively low cost and accessibility, non-invasive nature, and physiological relevance, indirect calorimetry has become a popular means of bioenergetic study. Indirect calorimetry measures have long been performed on athletes where they have been used as indices of performance or fitness (33), or a tool for training (101).

These approaches have also been applied in the world of medicine in the areas of diabetes (13), cardiorespiratory fitness (75), pediatric care (83), nutrition (45), and aging (61, 80, 88). From such studies we know that in older adults the energy cost of daily activities and exercise are relatively higher (61, 107), exercise capacity is lower (107), and VO_2 recovery kinetics are slowed (88).

Recently, a growing number of studies have used indirect calorimetry to evaluate the effects of aging components of mobility-related function. Many of these studies have focused on the impact of the increased energetic cost of walking and decreased aerobic capacity (VO_2 max) observed in older adults (9, 92, 98, 99). Their findings suggest that the slowing of gait speed that occurs with walking may be a compensatory action for the increased energetic cost, such that older adults walk slower to maintain an energy expenditure during transport similar to that of young healthy individuals (9, 92, 98, 99). An increased energetic cost of walking has also been shown to be associated with higher rates of perceived fatigability and rating of perceived exertion cross-sectionally (9, 92), and more recently longitudinally (98). It has also been proposed that the difference between walking oxygen consumption and maximal oxygen consumption, representing the "aerobic reserve", has important consequences for mobility, such that older adults that with a smaller aerobic reserve may be more limited during mobility task. Likewise, older adults that require a higher percentage of their peak oxygen consumption to walk have been shown to have reduced preferred gait speed and increased fatigability (92, 98).

Other metrics, such as the kinetics of O_2 uptake have received less attention, but may be equally as important. In 2003, Alexander et al. (4) suggested that, although aerobic capacity is often used as an indicator of function in older adults that submaximal

measures (O_2 kinetics) may be a more practical measure of aerobic function because it does not require maximal effort testing; which can be confounded by participant motivation or other pathological limitations. In testing this, it was shown that when compared to unimpaired older adults (VO_2 peak >18 $ml \cdot kg^{-1} \cdot min^{-1}$), impaired older adults (VO_2 peak <18 $ml \cdot kg^{-1} \cdot min^{-1}$) had over twice the time constant for O_2 deficit and trended towards ($p=.09$) a 43% higher time constant for EPOC (Fig. 23 A-B.). It was also demonstrated that O_2 kinetics were as strong or stronger predictors of functional performance during a variety of mobility-related tasks (Fig. 23 C.) (4), and low-frequency fatigue (104).

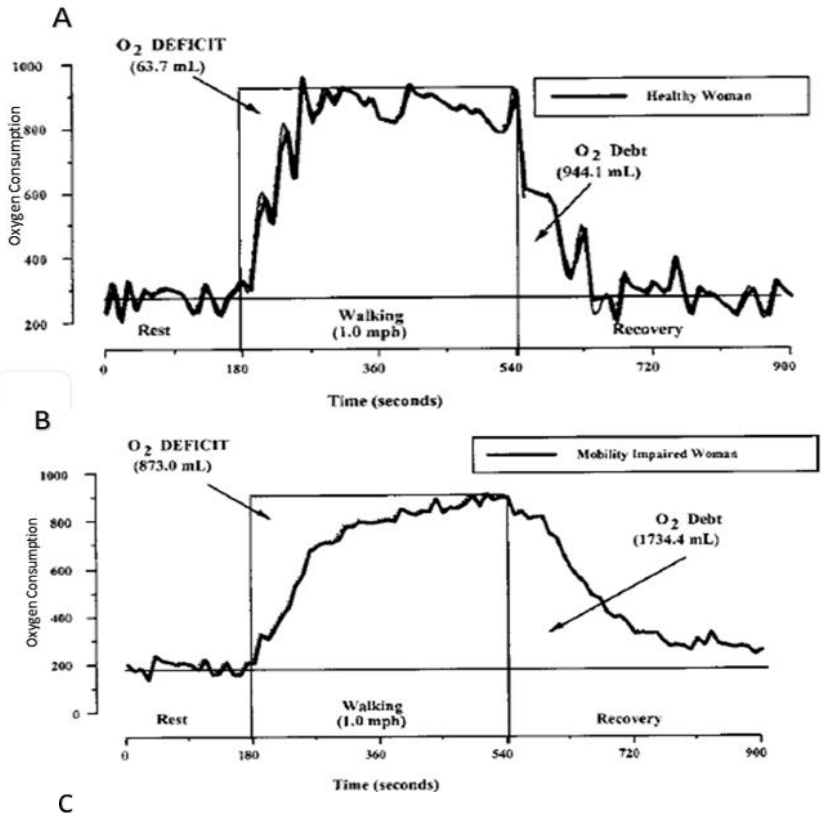


Table 4. Relationships Between VO_2, Oxygen Uptake Kinetics, and Functional Performance in Unimpaired and Impaired Older Adults

Task	Peak VO_2	$tc_{deficit}$	tc_{EPOC}
Unimpaired Old			
Peak VO_2		0.62**	0.29
GUG	0.48*	0.58*	0.06
GUG × 3	0.55*	0.60**	0.13
Bag carry	0.29	0.22	0.59**
6-Minute walk	0.45*	0.31	0.15
Impaired Old			
Peak VO_2		0.11	0.49*
GUG	0.21	0.10	0.42
GUG × 3	0.41	0.02	0.33
Bag carry	0.35	0.07	0.53*
6-Minute walk	0.62**	0.18	0.64**

Notes: $**p < .01$, $*p < .05$. VO_2 = oxygen uptake; GUG = get-up-and-go test; tc = time constant; EPOC = excess postexercise oxygen consumption.

Figure 23. A-B) Oxygen deficit and EPOC in unimpaired and impaired older women, respectively, C) Pearson's correlations between aerobic measurements and mobility test performance. (4)

A limitation of all of these studies has been the measurement of aerobic metrics during brief periods of walking or running in a laboratory setting. For example,

Alexander et al. measured O_2 kinetics in response to a very slow (1 mph) 6 min treadmill walk, with only 3 min of collection pre and post, and the other studies measured only the total cost of walking and VO_2 peak or max. Although walking is a major component of mobility, the whole-body energetic responses during the mobility task themselves could be beneficial for assessing mobility-related functions during daily living. Also, longer periods of measurement may be useful as metrics such as EPOC have been shown to last as long as 14 hr in response to more intense exercise. In 2013, Buchowski et al. (15) measured energy expenditure in response to eight different physical tasks, for three hours in a whole room calorimeter. This was one of few studies to examine the effects of energy expenditure during a task on perceived fatigability and performance fatigability (measured through accelerometry), and they found both were significantly related to total energy expenditure during the task that required around 3.5 METs (15). However, they only considered the amplitude of energy expenditure, and based on Alexander's research it is possible that further analysis of O_2 kinetic measurements may have uncovered associations between energetics and fatigability during lower-intensity tasks as well. This is an approach that our lab could potentially use in the future.

Together these studies demonstrate the efficacy of indirect calorimetric measurements for the assessment of mobility-related function. It should be clear that linking these lines of energetic research to *in vivo* muscle energetic research could be greatly beneficial to both sides and may provide a mechanistic pathway by which altered muscle energetics impact mobility-related function. As will be explained in the following section, much of the groundwork to establish this connection has already been completed.

While inferences based on this work could currently be made, it is the aim of this study to validate these assumptions.

The Relationship between Muscle and Whole-body Energetics

Basic physiology and logic dictate that muscle energetics are related to whole-body energetics as follows; 1) indirect calorimetry measures the sum of energy expenditure (O_2 consumption and CO_2 production) from all of the body's cells, 2) muscle accounts for ~20-30% of whole-body energy expenditure during rest, and up to ~90% during exercise depending on intensity (110), 3) the way muscles use energy (muscle energetics) should, therefore, have an impact on whole-body energetics with an amplitude corresponding to the activity being performed. The precise interactions of this relationship, however, are not implicit and have thus been subject to investigation. Chilibeck et al. in 1998 and Rossiter et al. in 2002 (21, 93), both approached this problem by simultaneous measurement of phosphorous and O_2 kinetics (i.e., rates of change). Both groups used isolated exercise (plantar flexion and knee extension) inside a magnetic resonance scanner and found similar time constants and kinetics between measurements. Rossiter et al. also found that during intense exercise the VO_2 slow component was reflected in PCr consumption (Fig. 24). Other research groups have used this connection to investigate topics such as energy cost, energy efficiency, and quantification of aerobic and anaerobic contributions (19, 73, 82).

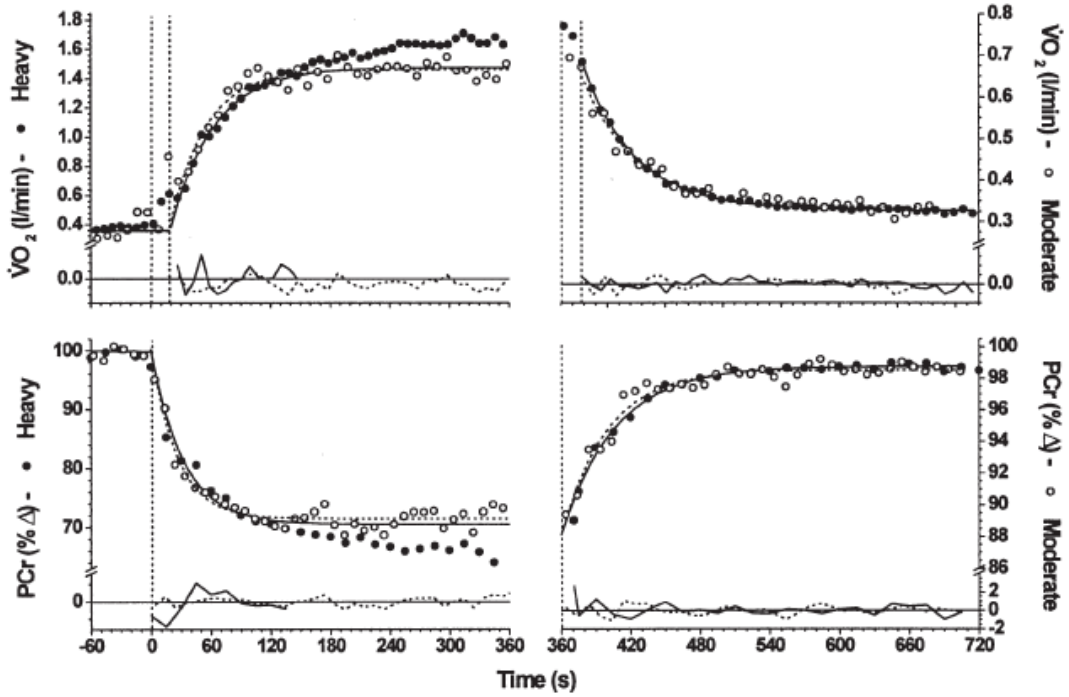


Figure 24. The simultaneous response of VO_2 and PCr kinetics to different intensity constant rate exercise. The graphs on the left are from the onset of exercise and the graphs on the right are from the cessation. Filled circles represent heavy exercise and open circles represent moderate (93).

These studies are limited to exercise in the confines of a magnetic resonance scanner. Thus, other researchers have used non-simultaneous measures to investigate the importance of muscle energetics in relation to more practical whole-body measures. For example, Zwaard et al. (111), showed that although VO_2 max is likely limited by O_2 delivery and not mitochondrial respiration, that muscle fiber oxidative capacity (*in vitro*) is highly correlated (r^2 = .81-.89) with VO_2 max in diseased (CHF), healthy, and endurance-trained participants. Similar observations found that muscle mitochondrial capacity in the gastrocnemius assessed by near-infrared spectroscopy was associated with VO_2 peak (R^2 = .57) and EPOC duration (R^2 = .34) (63), and that age-related decline in muscle oxidative capacity in the vastus lateralis measured by ^{31}P-MRS correlated with a decline in VO_2 peak (R^2 = .541) as well (3).

While these measures add a level of practicality to interpreting the whole-body effects of alterations in muscle energetics, they do not provide enough support for the comprehensive analysis of how such alterations might affect mobility-related function in everyday life that is the goal of this lab. For this reason, this study attempted to elucidate a strong relationship, if one exists, between dynamic measures of both muscle and whole-body energetic characteristics.

Summary

In summary, 1) the methods used to evaluate muscle energetics and whole-body energetics are valid and have strong physiological bases; 2) there is reason to believe that characteristics of both are inherently connected, especially in regards to exercise; 3) characteristics observed at both levels have been previously associated with indices of mobility-related function, but more thoroughly and directly at the whole-body level; 4) analysis of kinetic attributes at both muscle and whole-body levels and not just total or maximal energy consumption could further the understanding of this connection, as well as the relationship to mobility-related function; and 5) whole-body energetics could potentially act as an intermediary point between muscle energetics and mobility-related function, which with further research could advance the field by providing a mechanism that can be used to evaluate and combat decline in mobility-related function.

CHAPTER 3

METHODS

Study Overview

This thesis study was completed on a subset of data from a larger multi-lab project. As such, portions of the larger project that are not directly relevant to this thesis are not included here but can be provided upon request. The purpose of this project was to evaluate the connection between muscle (vastus lateralis) and whole-body energetics. Muscle energetics were analyzed using MRS in response to two separate knee-extensor contraction protocols and whole-body energetics were analyzed using indirect calorimetry in response to a treadmill walk designed to mimic what an individual might encounter during a typical day.

All participants completed 4 total visits at the Institute for Applied Life Sciences inside either the Center for Human Health and Performance (CH^2P) or the Human Magnetic Resonance Center (*h*MRC). The first visit included consenting (informed consent can be found in *Appendix A*), anthropometric measures, a dual-energy X-ray absorptiometry (DXA) scan, and a 30-min treadmill walk (30MTW) monitored by a metabolic cart. The following three visits consisted of two stays in a whole-room calorimeter (24-h visits), and one 3-h visit (MR Visit). The 24-h visits consisted of an overnight stay including three meals, structured activities of daily living (ADL's), and the 30MTW, all interspersed with free-time. The MR visit included magnetic resonance spectroscopy (MRS) measures, both at rest and during knee extension exercise, followed by a graded maximal oxygen consumption treadmill test (VO_2 peak test). For all visits, participants were asked to abstain from alcohol, tobacco, and exercise for the previous 24

hours, and caffeine the morning of. All visits were completed within 30 days. An overview of the visits and measurements is shown in Fig. 25.

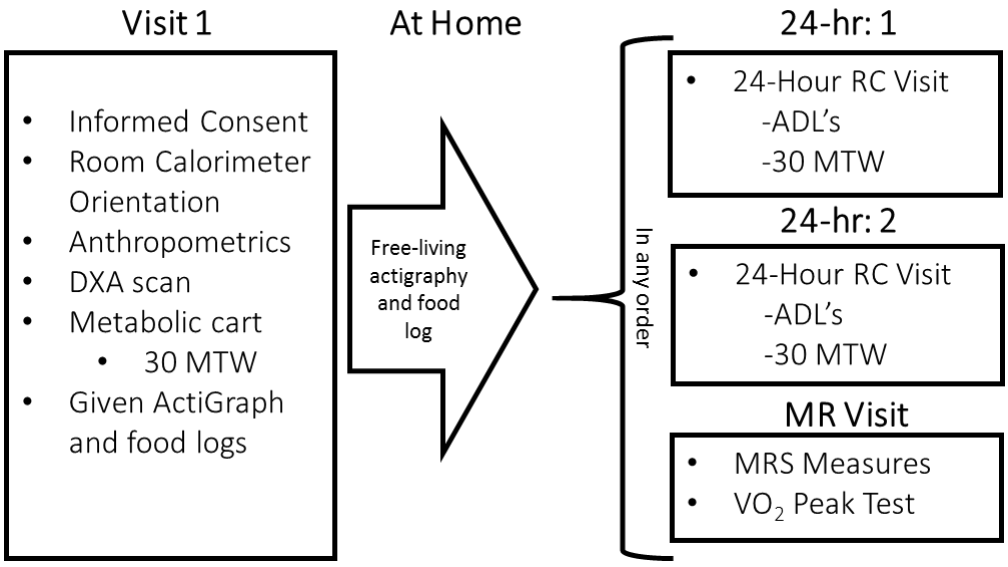


Figure 25. Study Visit Overview. DXA, dual-x-ray absorptiometry; 30MTW, 30-min treadmill walk; RC, room calorimeter; MR, magnetic resonance; MRS, magnetic resonance spectroscopy; VO_2, volume of oxygen consumed.

Participants

This study has been approved by the University of Massachusetts Amherst institutional review board. Eight young (25-40 yrs.), healthy males were recruited for this study. All participants were relatively weight stable (Δ weight $< \pm$ 5lbs for two months prior) and ranged from sedentary to recreationally active. Participants were free of any musculoskeletal injury, and acute or chronic illness that may affect their neuromuscular or metabolic function. Additionally, participants were screened for medications that have the potential to impact study results (screening form can be found in *Appendix B*). Dietary restrictions and preferences were also be recorded to ensure the safety of and adherence to the provided meals.

Meals

For all visits, participants were fed a standardized 400 kcal meal bar (PRO Bar, Park City, UT) at 11:30 am for lunch (for the MR visit the bar was given to the participant prior). The macronutrient distribution was 43g of carbohydrate, 22g of fat, and 11g of protein, and was chosen to be both satiating and relatively low calorie. The key measurements of each visit (indirect calorimetry and MRS) occured roughly 2.5-3.5 hrs after lunch (commencing at ~2:00 pm). This standard meal and timing helped to control for the thermic effect of food on energy expenditure as well as other meal-based stimuli and diurnal variations, however, some inter-participant variation likely remained.

Descriptive Data

Physical Activity Monitoring

Following Visit 1, participants were asked to wear a GT3x+ accelerometer (ActiGraph, Pensacola, FL) on their dominant hip for 7 days, and fill out an accompanying physical activity log to classify their habitual, free-living physical activity. This is a standard practice in our lab, and these data were used to identify free-living physical activity levels and determine their potential effect on the results. The participant was instructed to wear the accelerometer on their right hip. A minimum of 4 days with 10 hours of wear time per day was required to be included in the analysis. ActiLife v6.13 software (ActiGraph, Pensacola, FL) was used to calculate average daily activity counts as well as minutes spent in moderate-to-vigorous physical activity.

During each 24-hour visit participants tracked their step count using the screen on a GT9X accelerometer (ActiGraph, Pensacola, FL) and were asked to reach a step count goal of 1200 steps before 12:00 pm in an attempt to avoid complete inactivity.

Anthropometrics and DXA Scan

Height, weight, and femur length (greater trochanter to lateral condyle) were measured and recorded for all participants. All measures were completed twice and averaged. Next, a full-body DXA scan was obtained by a certified technician on a Lunar iDXA (GE Lunar, Madison, Wisconsin, USA), to quantify body composition. These data were used for normalization and anthropometric comparison of participants, as well as for choosing the correct height settings on the magnet ergometer. Primary outcomes of these measurements included weight, body mass index (BMI), lean body mass (LBM), and fat mass.

Whole-body Metabolic Measures

Treadmill Walk

The 30MTW protocol (37, 43) was performed at 2:00 pm during visit 1, and both 24-h visits. The treadmill speed (incline = 0%) was increased over the first 30 s until 1.3 $m \cdot s^{-1}$ is reached. This speed was set to be achievable by most populations and was based on the average overground walking speed (1.37 $m \cdot s^{-1}$) completed by 17 older women in a previous study in our lab (37). At minutes 7, 17, and 27, grade was increased to 3% for 1 min to provide a slight challenge and to simulate a hill that the participant might encounter in everyday life. After 1 min, the grade was returned to level. Immediately before the 30MTW, participant gas exchange rates was measured during 20 min of quiet sitting followed by 10 min of quiet standing during which time a television show was played. Following the walk, the participant were required to remain seated and passive for a 1-hr recovery period at visit 1 and a 2-hr recovery period at the 24-h visits, during which time a television show was played.

During the first visit, metabolic response to the 30MTW were monitored with a Parvo Medics' TrueOne® 2400 (Salt Lake City, UT) metabolic cart, which was calibrated according to manufacturer recommendations prior to the participant's arrival. Data was sampled breath by breath and recorded in ~5-s averages. During post-processing, data was inspected for errors and smoothed using a 3-sample (~15 s) centered moving average.

During the 24-h visits, metabolic response to the 30MTW was monitored using a custom-built full room calorimeter (MEI Research, Enida, MN) as part of a longer 24 hr stay. The calorimeter (4.1 by 3.3 m) is configured for long-duration stays during which individuals can receive meals or medications through pass-through ports. It houses a toilet, sink, bed, desk, TV, treadmill (Valliant 2, Lode, Groningen Netherlands), and a two-camera observational video system. The calorimeter was calibrated using zero (0% CO_2, 21% O_2) and span (1% CO_2 and 20% O_2) gases before the participants arrive. A walkie-talkie was provided for communication while inside the calorimeter. Data was sampled every 15s and post-processed using a 6-min centered derivative.

These data were used to determine the energetic response to and recovery from a light intensity free-living task. Data from the first visit allowed for oxygen kinetic analyses of higher temporal resolution than can be recorded from the room calorimeter. Data from the 24-h visits allowed for analysis of a longer recovery period than would be comfortable with the metabolic cart.

VO2 Peak Measures.

Following the MR measures, the participant was removed from the scanner and given time to use the restroom, then was taken upstairs to the CH^2P for measurement of

peak oxygen consumption (VO_2 peak). Graded exercise tests have long been used to determine the maximal amount of oxygen that can be consumed for aerobic (oxidative) energy production, often referred to as VO_2 max. In this study, we chose to use the term VO_2 peak as opposed to VO_2 max due to the inherent difficulties in determining whether or not an individual truly reaches their physiological limit. Our use of the term peak denotes that we accept that our values may not represent true maximal values, but simply the peak values observed on that day. Participants completed a self-paced ~5-min warm-up on a treadmill. A TrueOne® 2400 metabolic cart was used to measure gas exchange and respiration rate during the VO_2 peak test. Data was sampled breath by breath and recorded in ~5-s averages. The test followed the BSU/Bruce Ramp treadmill protocol as described by Kaminsky and Whaley (53). This protocol is designed to be well suited for a wide range of populations through its use of a gradual ramp in intensity allowing for long walking periods and small transitions between stages. The test begins with a .76 $m \cdot s^{-1}$ flat walk and gradually increases in speed or incline. Heart rate, oxygen consumption, and RPE were monitored throughout the test. Termination criteria consisted of any one of the following, volitional fatigue, a plateau in oxygen uptake at several consecutive workloads, safety concerns, or equipment malfunction. During post-processing, data was inspected for errors and smoothed using a 6-sample (~30 s) centered moving average. The highest 30 s average was taken as the VO_2 peak, which was the primary outcome of this measurement. A table detailing the test stages can be found in *Appendix C*.

Muscle Bioenergetics

Magnetic Resonance Measures

Upon arriving at the *h*MRC, participants reviewed the MR Safety Form with the MR Technician, and all metal objects were removed. They were escorted into the scanner room and positioned on their back on the bed of the 3 Tesla whole-body MR system (Siemens, Erlangen, Germany). Their dominant leg was be strapped into the custom-built leg exercise apparatus, which is similar to a knee extension machine one might use at a gym. The leg was strapped to the apparatus just above the ankle and above the knee using Velcro straps. Inelastic straps were secured over the participant's hips to prevent unwanted movement during the muscle contractions. A circular 10-cm, dual-tuned (phosphorus and proton) coil was secured over the belly of the vastus lateralis muscle using an elastic bandage and Velcro straps. After the participants were secured to the leg-exercise apparatus, their thigh was positioned in the isocenter of the scanner and the magnet was shimmed. After the magnet was shimmed to an acceptable level of homogeneity, resting proton spectra was obtained for the measurement of cytosolic acetyl-carnitine. These measurements will be used in the analysis of the muscle energetic response to exercise in a tangential study. A visual outline of the magnetic resonance procedures can be seen in Fig. 26.

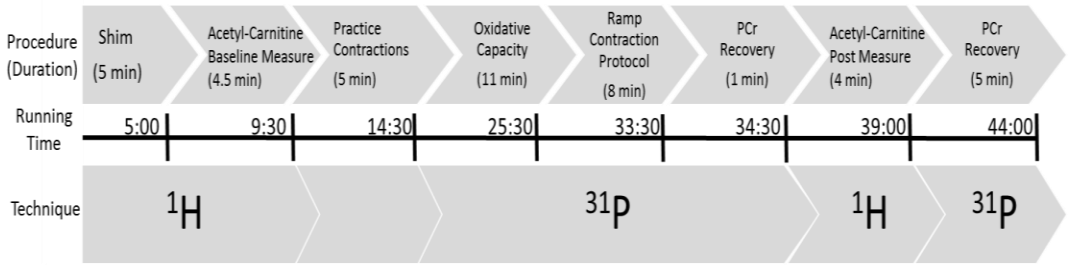


Figure 26: Magnetic Resonance Spectroscopy Protocol

Muscle Power Measures

Participants completed 2 practice submaximal isometric contractions, followed by 3 maximal voluntary isometric contractions (MVIC), each lasting 3-4 s, to establish the workload for the ramp contraction protocol. One minute of rest was given between each set of contractions. Participants were then be familiarized with isokinetic contractions (1 contraction every 2 s at 120° s^{-1}) by performing 2 sets of 3 contractions. During all knee extensor protocols, participants had visual feedback showing the torque they produced as well as their target torque and the timing of contractions.

Oxidative Capacity

The oxidative capacity protocol consisted of 90 s of rest, 24 s of maximal isokinetic contractions (1 contraction every 2 s at 120° s^{-1}), and 10 minutes of recovery. Phosphorus spectra was collected at 2 s resolution for the entire protocol. During post-processing, the first 15 spectra (30 s) were discarded to avoid saturation issues. The remaining resting spectra were then averaged together into one 60 s spectrum. During the 24 s of exercise and the first 20 s of recovery spectra were averaged to 4-s resolution, the following 280 s of recovery to 8-s, and the last 300 s to 30-s. These data were used to determine the maximal aerobic energy production in the muscle. The primary outcome for this measurement was muscle oxidative capacity (measured as a rate constant, $k_{PCr,}$ $s^{-1)}$.

Ramp Contraction Protocol

Following the oxidative capacity measures, participants performed a ramp contraction protocol consisting of 90 s of rest, four 2-min stages of isotonic knee extensions (1 contraction performed every 2 s), and 10 min of recovery. Resistance for the stages were set at 6, 9, 12, and 15% of MVIC and occured in that order. Phosphorus

spectra were collected at 2 s resolution for the first 12 min of the protocol. Two and a half minutes into recovery, phosphorus scans ceased and ~5.5 min of proton shimming and scanning was performed (for purposes outside the scope of this project), following which two and a half more minutes of phosphorus were collect at 2 s resolution. In this way, we collected a full ~10 min of phosphorus recovery, with a gap in the middle that can be accounted for through a monoexponential fit. During post-processing, the first 15 spectra (30 s) were discarded to avoid saturation issues. The remaining resting spectra were then averaged together into one 60 s spectrum. During the 480 s of exercise spectra were averaged to 10-s resolution, the first 20 s of recovery to 4-s, the following 128 s to 8-s, and the last 150 s collected after the proton scanning to 30-s with the first 15 spectra discarded. These data were used to evaluate the energetic characteristics of contracting muscle at various intensities. Primary outcomes included the amplitude of ΔPCr from the first intensity stage (PCr mM).

Kinetic Analysis

Kinetic analysis of both oxygen consumption and PCr responses was performed using the MATLAB 2020a curve fitting toolbox (The MathWorks Inc., Natick, Massachusetts). Oxygen deficit was fit from the onset of the 30MTW to just before the first challenge period at 7 min using the following monoexponential equation.

$$VO_2(t) = (\text{mean } VO_2 \text{ Last Minute of Standing}) + a \cdot (1 - e^{(-(t-d)/tc)})$$

Eq. 8

The EPOC was fit from the time the participant has sat down at the end of the 30MTW through 1 hour of recovery using this similar monoexponential equation.

$$VO_2(t) = (\text{mean } VO_2 \text{ Last Minute of 30MTW}) - a \cdot (1 - e^{(-(t-d)/tc)})$$

Eq. 9

The variable *a* represents the amplitude of change in oxygen consumption, *t* represents time, *d* represents the time delay between the change in physical work and the oxygen response, and *tc* is the time constant representing the amount of time in seconds it takes for the amplitude to change by 63%. All variables were constrained to a minimum value of 0.

The PCr recovery data was fit from the end of the 24-s oxidative capacity protocol to 10 minutes of recovery using the following monoexponential equation.

$$PCr(t) = Y0 + a \cdot (1 - e^{(-k \cdot t)})$$

Eq. 10

The variable *Y0* is the estimated PCr concentration during the last 4 s of exercise, *a* represents the amplitude of change in PCr, *t* represents time, and *k* is the rate constant. All variables were constrained to a minimum value of 0.

The PCr data from the first stage of the ramp was fit from the onset of contractions to the beginning of the second stage (2 min) using the same equation, except the variable *Y0* is now the estimated PCr concentration at rest and is subtracted from.

$$PCr(t) = Y0 - a \cdot (1 - e^{(-k \cdot t)})$$

Eq. 11

Statistics

Data was analyzed as a single group. Descriptive data is reported as mean values with standard deviation and ranges. Due to the low number of participants correlations were assessed with the non-parametric Spearman's rank correlation coefficient test (r_s) in

MATLAB 2020a (The MathWorks Inc., Natick, Massachusetts) using the "corrplot" function.

Aim 1: *Evaluate the relationships between ΔPCr in muscle and the O_2 deficit during the 30MTW.*

To test Aim 1, a Spearman's rank correlation test was performed to determine the association between the independent variable (ΔPCr in the VL during the knee extensor contraction protocol; amplitude (*a*) mM) and the dependent variable (O_2 deficit from the 30MTW, $k_{deficit}$).

Aim 2: *Evaluate the relationships between muscle oxidative capacity and: EPOC and RER in response to activities of daily living, and VO_2 peak from a graded exercise test.*

To test Aim 2, several Spearman's rank correlation tests were performed to determine the association between the independent variable (muscle oxidative capacity from the isokinetic protocol; $k_{PCr,}$ s^{-1}) and the dependent variables (EPOC and substrate use from the 30MTW; k_{EPOC} and RER, and VO_2 peak relative to total and lean body mass from the graded treadmill test; O_2 $mL \cdot kg^{-1} \cdot min^{-1}$).

CHAPTER 4

RESULTS

Participant Characteristics

Male participants aged 25-40 years who were not actively training for or participating in organized sports were recruited for this study. Eight participants were enrolled and completed all four visits. Upon enrollment, each participant was assigned an arbitrary number between 1 and 11 to serve as a de-identified participant code (denoted here as pt#). All participants self-reported being weight stable (< ±5lbs for 2 months), and free from chronic disease and injury. Group data provided in Table 2.

Table 2. Participant characteristics

Age (years)	28.4 ± 3.5	(25, 35)
Height (cm)	179.6 ± 9.3	(164, 189)
Weight (kg)	88.7 ± 15.5	(65.5, 105.3)
Lean Body Mass (kg)	64.9 ± 11.6	(47.4, 84.8)
BMI ($kg \cdot m^{-2}$)	27.2 ± 2.7	(22.6, 30.2)
Body Fat (%)	20.3 ± 7.1	(13.8, 33)
MVPA ($min \cdot d^{-1}$)	33 ± 13.8	(6, 48)
PA ($counts \cdot d^{-1000}$)	400 ± 115	(244, 600)
MVIC (Nm)	283 ± 67	(172, 370)

Data are mean ± SD and (range); BMI, body mass index; MVIC, maximal voluntary isometric contraction; MVPA, moderate- to vigorous-intensity physical activity; PA, physical activity

Whole-body Metabolic Responses to 30MTW

Room Calorimeter Measurements

Due to the low temporal resolution of the whole-room calorimeter data, a standard analysis of oxygen kinetics was not feasible. Further evaluation will be required to determine if meaningful kinetic measures can be extracted from these data. For this reason, we report whole-body metabolic data collected using the metabolic cart.

Treadmill Walk

All participants completed the 30MTW protocol at the prescribed speed of 1.3 $m \cdot s^{-1}$. During seated rest (pre and post-walk), all participants watched documentary-style television shows. The first 4 participants were allowed *ad libitum* access to their phones. Phone use was not allowed following the 4th participant (pt7) due to unexpected increases in VO_2 observed while that participant was holding the phone high in front of their face for an extended period of time. Additionally, metabolic cart data for one participant (pt4) was excluded from analysis due to unusually large oscillations in the VO_2 signal that made it unsuitable for analysis. It has yet to be determined whether this problem was due to an equipment artifact or physiologic phenomenon.

Metabolic Variables during the 30MTW

Analysis of the metabolic cart data from the 30MTW walk protocol showed that oxygen consumption increased from sitting to walking by ~3-4 fold for all participants and steady-state was reached before the first challenge period 7 min into the walk. Oxygen consumption averages from minutes: 10-19 of sitting pre-walk (VO_2 Sitting), 5-9 of standing pre-walk (VO_2 Standing), and 7-30 of walking (VO_2 Walking) were taken to be representative of those periods while avoiding influence from the transitions between them. Representative VO_2 and RER responses are shown in Fig. 27. Mean treadmill walk metabolic variables are presented in Table 3.

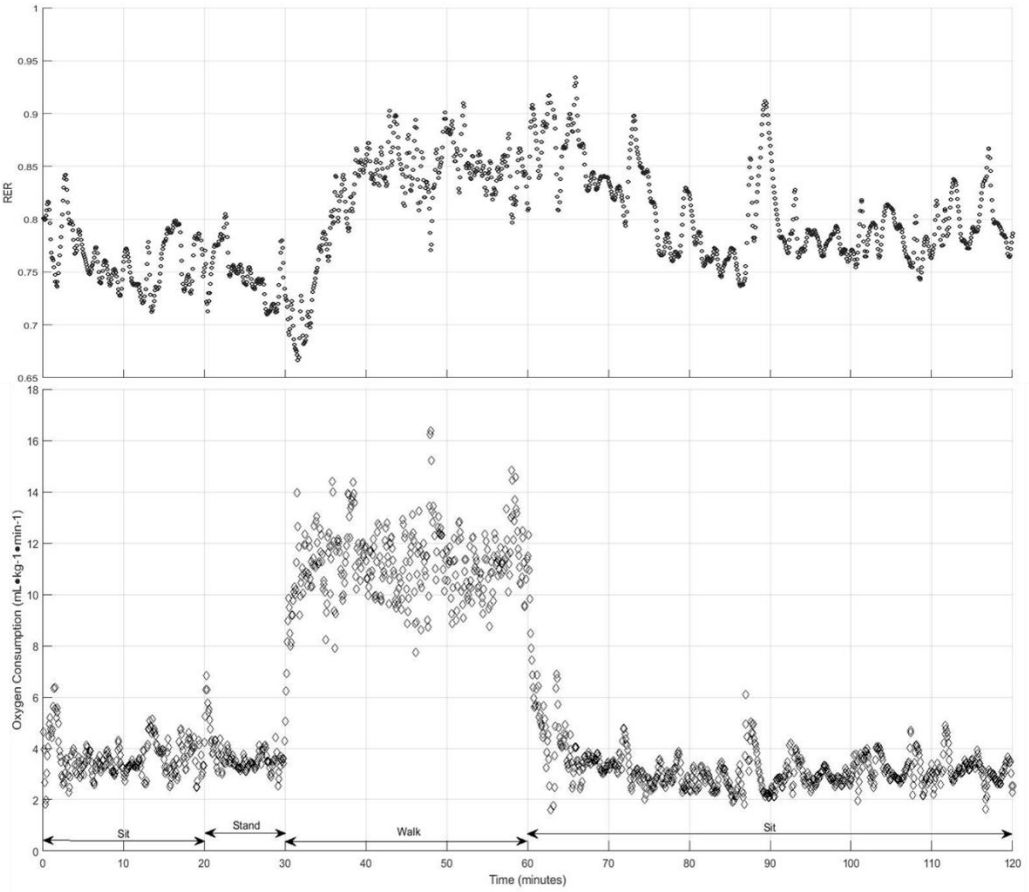


Figure 27: Representative plot of metabolic data from 30MTW. Data are from a single participant (pt9) during the 30-min treadmill walk; VO_2, volume of oxygen consumption; RER, respiratory exchange ratio.

Table 3. Treadmill walk metabolic variables

Participant	VO_2 Sitting ($mL \cdot kg^{-1} \cdot min^{-1}$)	VO_2 Standing ($mL \cdot kg^{-1} \cdot min^{-1}$)	VO_2 Walking ($mL \cdot kg^{-1} \cdot min^{-1}$)
pt1	3.4	3.6	13.7
pt2	4.3	5.4	12.5
pt4	-	-	-
pt6	3.1	3.5	11.1
pt7	1.9	2.7	10.2
pt9	3.7	3.5	10.8
pt10	3.1	3.3	12.9
pt11	3.4	3.8	12.9
Mean ± SD	3.2 ± 0.7	3.7 ± 0.8	12 ± 1.3

VO_2, volume of oxygen consumption

Respiratory Exchange Ratio

The RER response to the 30MTW, was calculated as averages across different time periods. Representing RER as an average, as opposed to picking a single time point (minimum, maximum, or final), limits the impact of noise in the data. As shown in Fig. 27, RER during the 30MTW took longer to reach a steady than VO_2, a finding consistent across participants. For this reason, averages were calculated for the entire oxygen consumption steady state (minutes 7-30; RER L23) and the last minute (minute 29-30; RER L1) of the 30MTW. Additionally, an average of the last 5 min was calculated (minutes 25-30; RER L5), to account for fluctuations that may occur during the last minute due to the final challenge period (minute 27-28) or noise that if not sufficiently filtered by the small sample of RER L1 (12 data points). The RERs from these three time periods (L23, L5 and L1) did not statistically differ by means of one way ANOVA (p= 0.47) or Kruskal-Wallis test (p = 0.43). Individual RER data can be found in Fig. 28 and Table 4.

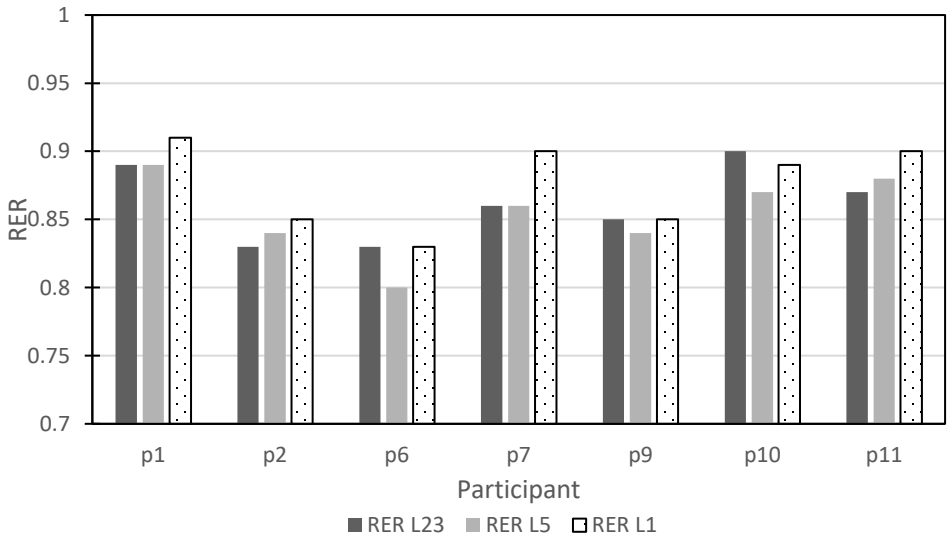


Figure 28. Individual RER values from the 30MTW. 30MTW, 30-min treadmill walk; RER, respiratory exchange ratio; L23, average of the last 23 min of walk; L5 average of the last 5 min of walk; L1 average of the last 1 min of walk.

Table 4. Individual RER values from the 30MTW

Participant	RER L23	RER L5	RER L1
pt1	0.89	0.89	0.91
pt2	0.83	0.84	0.85
pt4	-	-	-
pt6	0.83	0.80	0.83
pt7	0.86	0.86	0.90
pt9	0.85	0.84	0.85
pt10	0.90	0.87	0.89
pt11	0.87	0.88	0.90
Mean ± SD	0.86 ± 0.03	0.85 ± 0.03	0.87 ± 0.03

30MTW, 30-min treadmill walk; RER, respiratory exchange ratio; L23, average of the last 23 min of walk; L5 average of the last 5 min of walk; L1 average of the last 1 min of walk; * denotes significantly different means

On- and Off- Kinetics for Metabolic Variables

To determine the time constants for the oxygen deficit (on-kinetics) and EPOC (off-kinetics), monoexponential equations (Eq. 8 and 9) were fit to each participant's oxygen consumption data during the 30MTW. For two participants (pt1 and pt2) the time at which the walk ended and the time at which the participants began seated rest was not recorded. In these two cases, EPOC was defined as starting from the last VO_2 value at or above the steady-state mean, and the *mean VO_2 Last Minute of 30MTW* was calculated as the average of the minute before that same point. In addition, the minimum time delay (d) for these two participants was constrained to -5 s (as opposed to 0) to allow the fit to account for a possible error in identifying the end of the 30MTW of up to one data point. The fit of the EPOC data for participant 7 was adjusted for the effect of phone use mentioned above (see *Appendix D* for details). Figure 29 shows representative fits of the VO2 data illustrating the oxygen deficit and EPOC analyses. The time constants from these fits can be found in Fig. 30, as well as Tables 5 and 6.

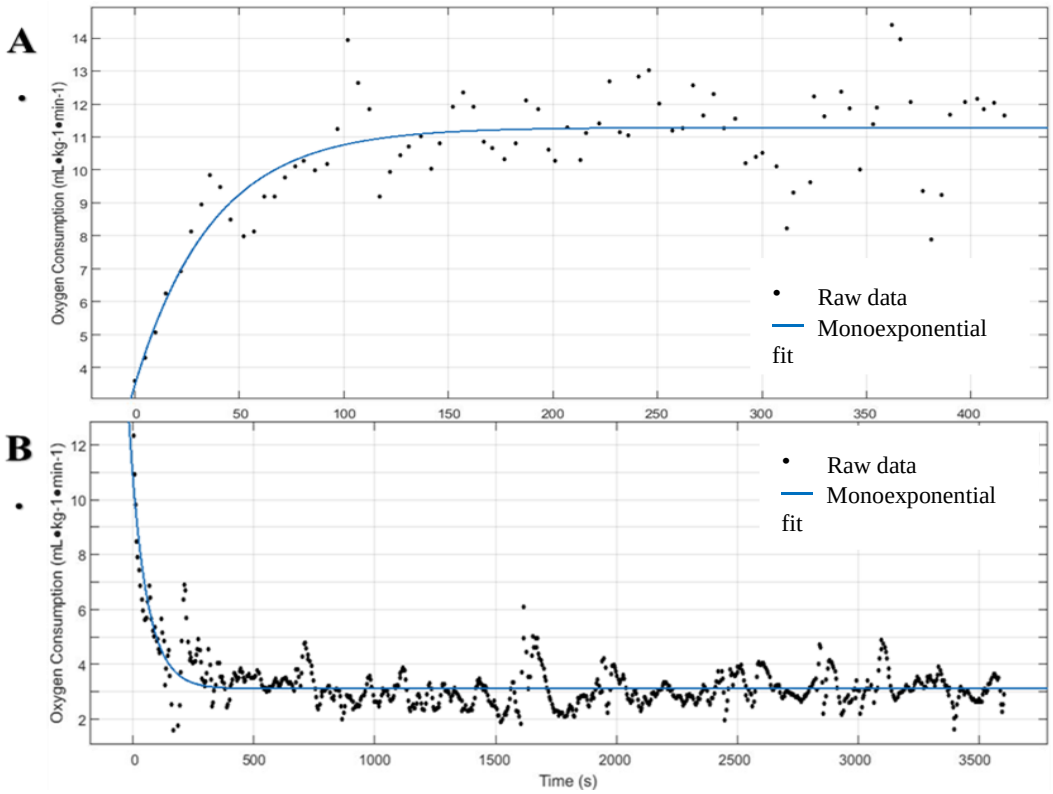


Figure 29. Representative fits of data showing (A) oxygen deficit and (B) EPOC. Data are from a single participant (pt9). Time constants here are 37 s for oxygen deficit and 69 s for EPOC. VO_2, volume of oxygen consumption.

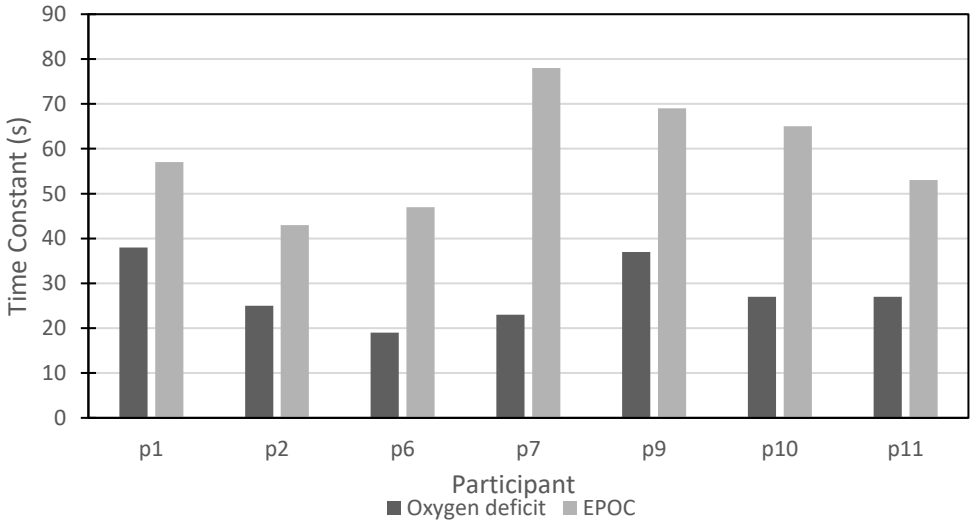


Figure 30. Individual oxygen deficit and EPOC time constants from the 30MTW. EPOC, excess post-exercise oxygen consumption; 30MTW, 30-min treadmill walk.

Table 5. Oxygen deficit variables

Participant	Time constant (s)	(95% CB)	r^2	Amplitude ($mL \cdot kg^{-1} \cdot min^{-1}$)	Time delay (s)
pt1	38	(29, 47)	0.78	10.2	1.0
pt2	25	(5, 45)	0.24	6.7	1.2
pt4	-	-	-	-	-
pt6	19	(11, 26)	0.37	7.4	0
pt7	23	(17, 30)	0.67	6.9	0
pt9	37	(28, 46)	0.64	7.8	0
pt10	27	(19, 35)	0.61	8.8	0
pt11	27	(19, 35)	0.70	7.8	1.3
Mean ± SD	28 ± 7		0.57 ± 0.20	7.9 ± 1.2	0.5 ± 0.6

SD, standard deviation; 95% CB, 95% confidence bounds for the time constant

Table 6. EPOC variables

Participant	Time constant (s)	(95% CB)	r^2	Amplitude ($mL \cdot kg^{-1} \cdot min^{-1}$)	Time delay (s)
pt1	57	(53, 61)	0.63	10.3	-5
pt2	43	(37, 49)	0.27	8.6	-5
pt4	-	-	-	-	-
pt6	47	(36, 58)	0.40	7.9	0
pt7	78	(68, 87)	0.53	8.6	0
pt9	69	(62, 76)	0.56	7.8	0
pt10	65	(61, 70)	0.65	9.1	0
pt11	53	(50, 56)	0.74	9.6	0
Mean ± SD	59 ± 13		0.54 ± 0.16	8.8 ± 0.9	-1.4 ± 2.4

EPOC, excess post-exercise oxygen consumption; SD, standard deviation; 95% CB, 95% confidence bounds for the time constant

VO_2 Peak

The VO2 peak tests ranged from 11 to 16 min in duration, and all tests were terminated upon volitional fatigue. For two participants (pt2 and pt6) heart rate data was not recorded due to equipment failure. To further evaluate how muscle energetics may play a role in this relationship VO2 peak is reported here normalized to both total mass ($mL \cdot kg^{-1} \cdot min^{-1}$) and lean mass ($mL \cdot kg\ lean^{-1} \cdot min^{-1}$). Representative changes in oxygen consumption and RER can be found in Fig. 31. Individual VO2 peak data can be found in Fig. 32 and Table 7.

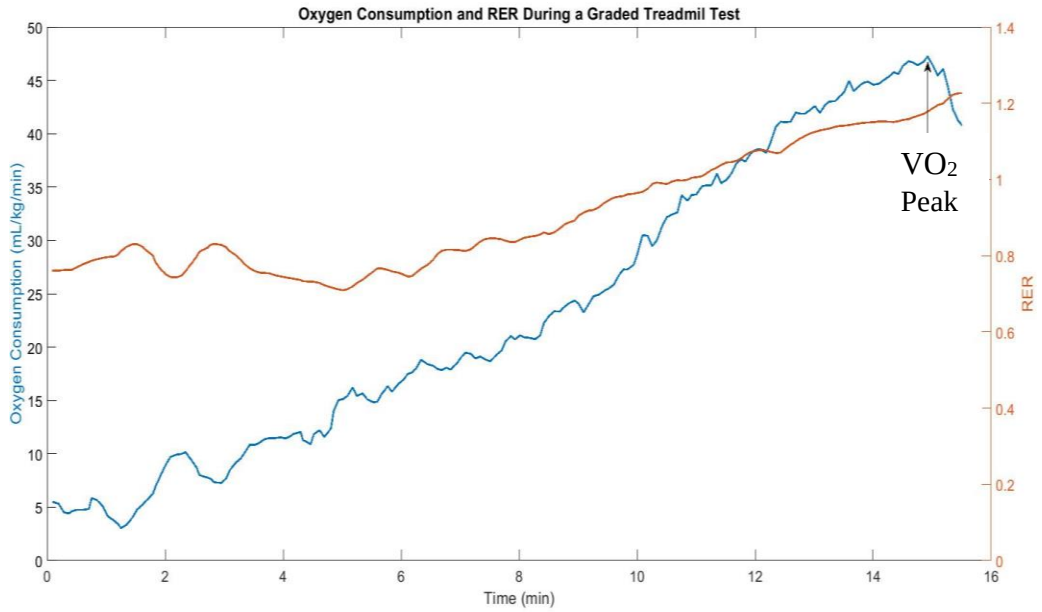


Figure 31. Representative data from the ramp treadmill protocol. Data are from a single participant (pt9). VO_2, volume of oxygen consumption; RER, respiratory exchange ratio.

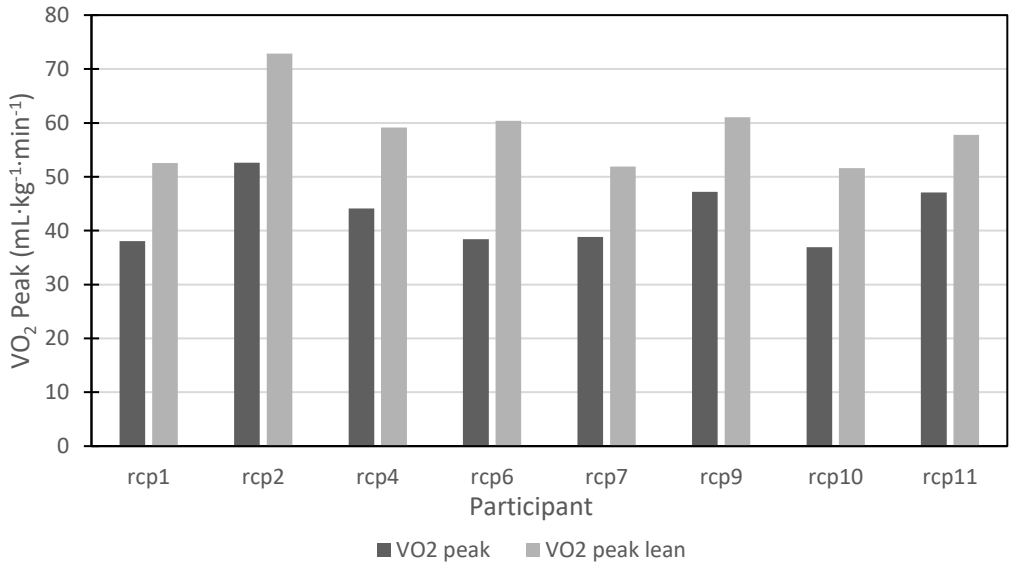


Figure 32. Individual VO_2 peak values from the ramp treadmill protocol. VO_2, volume of oxygen consumption

Table 7. VO_2 peak values from the ramp treadmill protocol

Participant	VO_2 Peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	VO_2 peak lean ($mL \cdot kg^{-1}$ lean$\cdot min^{-1}$)	Max RER	Max HR	Max RPE
pt1	38.0	52.5	1.34	201	10
pt2	52.6	72.8	1.10	-	10
pt4	44.1	59.2	1.23	190	10
pt6	38.4	60.4	1.14	-	10
pt7	38.8	51.9	1.27	202	10
pt9	47.2	61.1	1.22	197	9
pt10	36.9	51.6	1.25	200	10
pt11	47.1	57.8	1.20	179	10
Mean ± SD	42.9 ± 5.7	58.4 ± 7.0	1.20 ± 0.1	195 ± 9	9.9 ± 0.4

VO_2, volume of oxygen consumption relative to total mass; Max RER, maximal respiratory exchange ratio; Max HR, maximal heart rate; Max RPE, maximal rating of perceived exertion; SD, standard deviation.

Muscle Bioenergetic Variables

Oxidative Capacity

The 24-s isokinetic protocol led to an average PCr recovery of 14.1 ± 3.8 mM (Table 8). No participant's muscle pH dropped below 6.8 during any part of the protocol, suggesting that PCr replenishment was not hindered in any case by H+ accumulation. Individual fit variables are shown in Table 8 and a sample PCr fit for a single participant can be seen in Fig. 30.

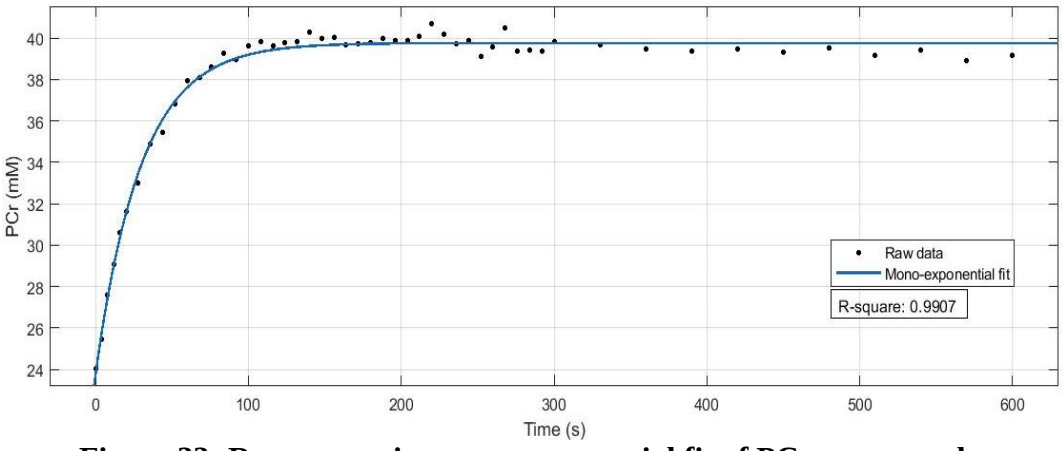


Figure 33: Representative monoexponential fit of PCr recovery data from the oxidative capacity protocol. Data are from a single participant (pt9) with a kPCr of 0.034 s^{-1}. PCr, phosphocreatine.

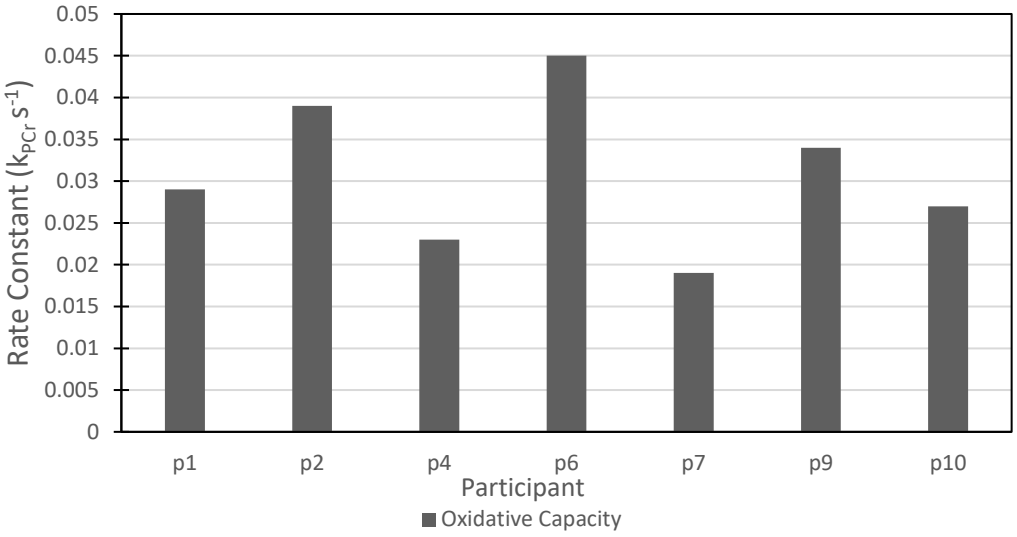


Figure 34. Individual oxidative capacity rate constants.
kPCr (**s^{-1}**), rate constant of PCr recovery;

Table 8. Oxidative capacity individual fit variables

Participant	Rate constant ($k_{PCr,}$ s^{-1})	(95% CB)	r^2	y-intercept (*mM*)	Amplitude (*mM*)
pt1	0.029	(0.026, 0.032)	0.98	25.0	13.4
pt2	0.039	(0.035, 0.044)	0.97	20.1	18.7
pt4	0.023	(0.025, 0.028)	0.99	25.1	14.6
pt6	0.045	(0.036, 0.054)	0.93	30.2	6.2
pt7	0.019	(0.017, 0.020)	0.99	25.0	12.9
pt9	0.034	(0.031, 0.036)	0.99	23.8	16.0
pt10	0.027	(0.025, 0.030)	0.98	21.4	17.9
pt11	0.026	(0.024, 0.028)	0.99	24.4	13.3
Mean ± SD	0.030 ± 0.009		0.98 ± 0.02	24.3 ± 3.0	14.1 ± 3.8

SD, standard deviation; 95% CB, 95% confidence bounds for the rate constant; kPCr, s-1, rate constant of PCr recovery.

Non-Oxidative ATP Production

Data from the first 2-min stage were used to calculate the amplitude of ΔPCr for each participant, thereby reflecting the contribution of ATP production from the (non-oxidative) creatine kinase reaction. The target relative intensity of this stage was 6% of MVIC. The mean MVIC was 282 ± 67 Nm and the mean target load was 17 ± 4 Nm. The measured mean load was 21.9 ± 5.2 Nm, representing 7.8 ± 0.6 % MVIC. Individual fit

variables are presented in Table 9 and an example PCr fit for a single participant is shown in Fig. 31.

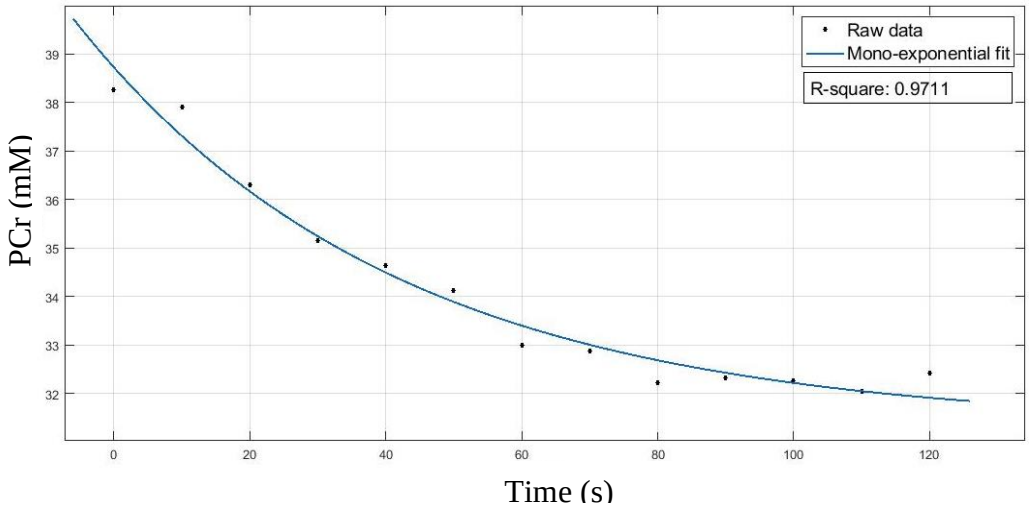


Figure 35. Representative fit of changes in PCr during stage 1 of ramp protocol. Data are from a single participant (pt9) with a ΔPCr of 7.4 mM. PCr, phosphocreatine.

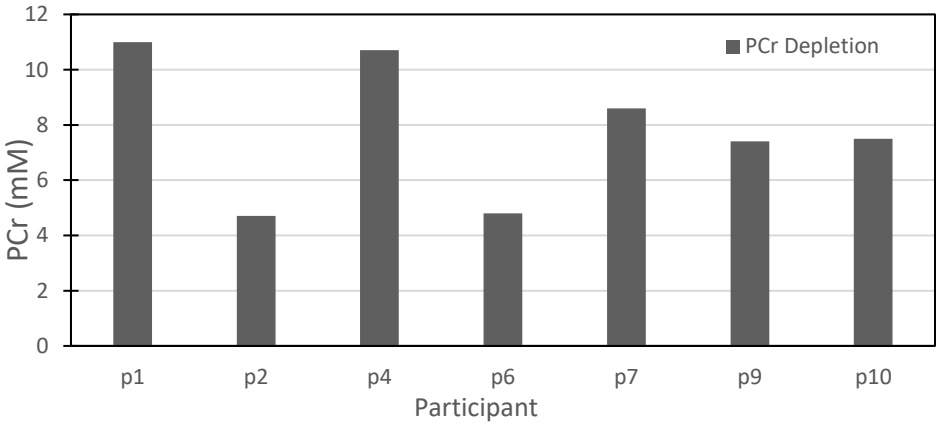


Figure 36. Individual ΔPCr during stage 1 of ramp protocol. PCr, phosphocreatine.

Table 9. Variables from fit of ΔPCr

Participant	Rate constant (k_{PCr}, s^{-1})	(95% CB)	r^2	y-intercept (*Y0*)	Amplitude (*a*)
pt1	0.034	(0.027, 0.041)	0.99	38.4	11
pt2	0.072	(0.037, 0.11)	0.92	37.7	4.7
pt4	0.017	(0.012, 0.023)	0.99	38.4	10.7
pt6	0.093	(0.042, 0.14)	0.91	36.4	4.8
pt7	0.020	(0.016, 0.025)	0.99	38.4	8.6
pt9	0.021	(0.014, 0.029)	0.97	38.7	7.4
pt10	0.023	(0.013, 0.032)	0.97	36.9	7.5
pt11	0.024	(0.017, 0.031)	0.98	36.9	6.9
Mean ± SD	0.038 ± 0.028		0.97 ± 0.03	37.7 ± 0.9	7.7 ± 2.4

SD, standard deviation; 95% CB, 95% confidence bounds for the rate constant; kPCr s-1, rate constant of PCr depletion.

Associations between Single Muscle and Whole-body Energetics

To evaluate these relationships Spearman rank correlation coefficients were calculated. The coefficient output r_s is similar to the *r* variable from a Pearson correlation and represents the strength of association. A strong Spearman correlation suggest a monotonic (directional; up or down) relationship, but not necessarily a linear one. The linearity of relationships was inferred through visual interpretation of the association plots. Probability values (p-values) were calculated for all correlations as well; however, due to the low sample size and this being an exploratory analysis, we did not set an alpha level for significance. The magnitude of ΔPCr showed a positive linear relationship with oxygen deficit (Fig. 37). Muscle oxidative capacity showed a negative linear relationship with EPOC, as shown in (Fig. 38). Muscle oxidative capacity showed a negative linear association with the mean RER from the entire steady-state (RER L23), the last 5 min (RER L5), and the last minute (RER L1) (Fig. 39). Muscle oxidative capacity had a positive linear association with VO2 peak relative to lean mass, but no relationship to VO_2 peak relative to total mass as shown in (Fig. 40). The correlation coefficients of the association between both independent variables and all of the dependent variables (corresponding to both aims) are reported in Table 10.

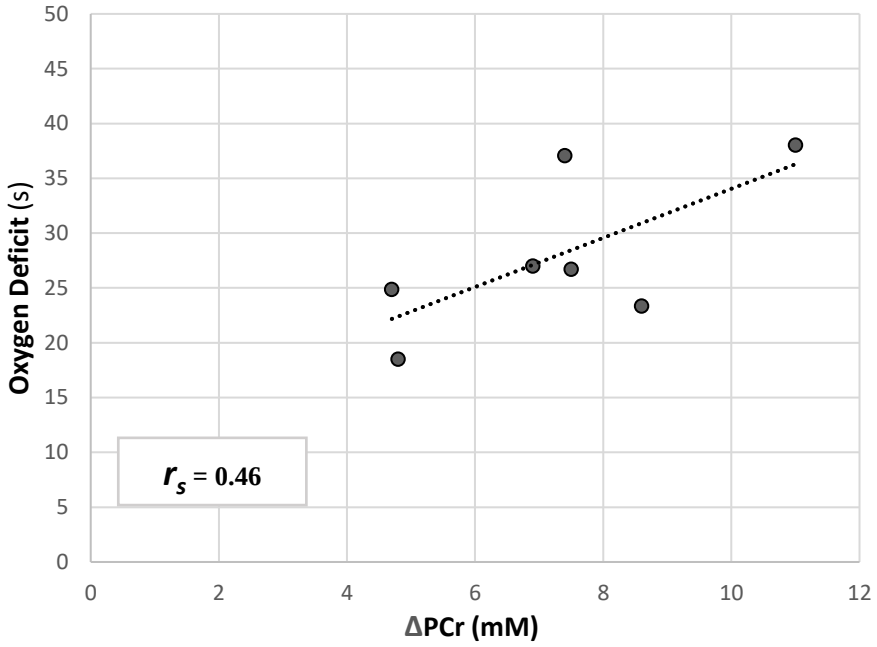


Figure 37. Association between oxygen deficit and ΔPCr. PCr, phosphocreatine; r_s, Spearman's rank correlation coefficient

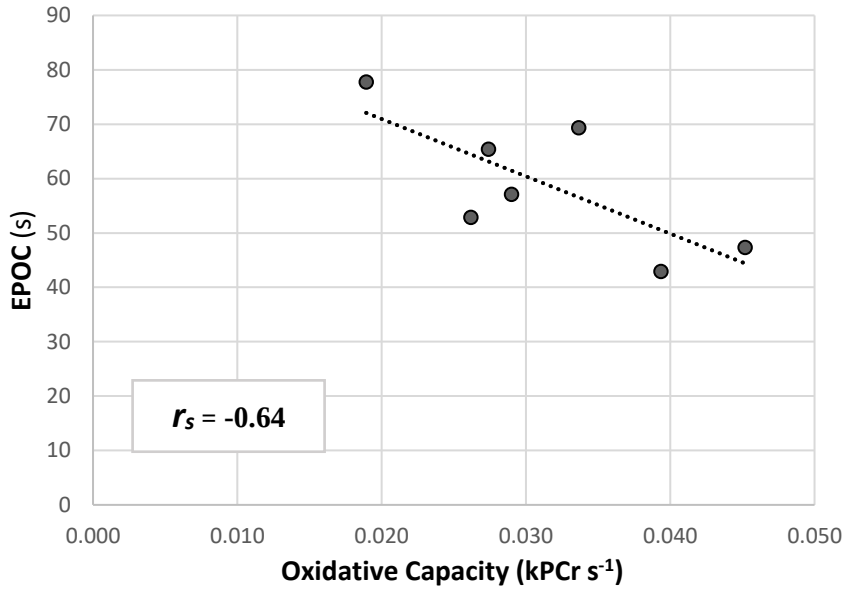


Figure 38. Association between EPOC and muscle oxidative capacity. EPOC, excess post-exercise oxygen consumption; PCr, phosphocreatine; kPCr $\mathbf{s^{-1}}$, rate constant of PCr recovery; r_s, Spearman's rank correlation coefficient

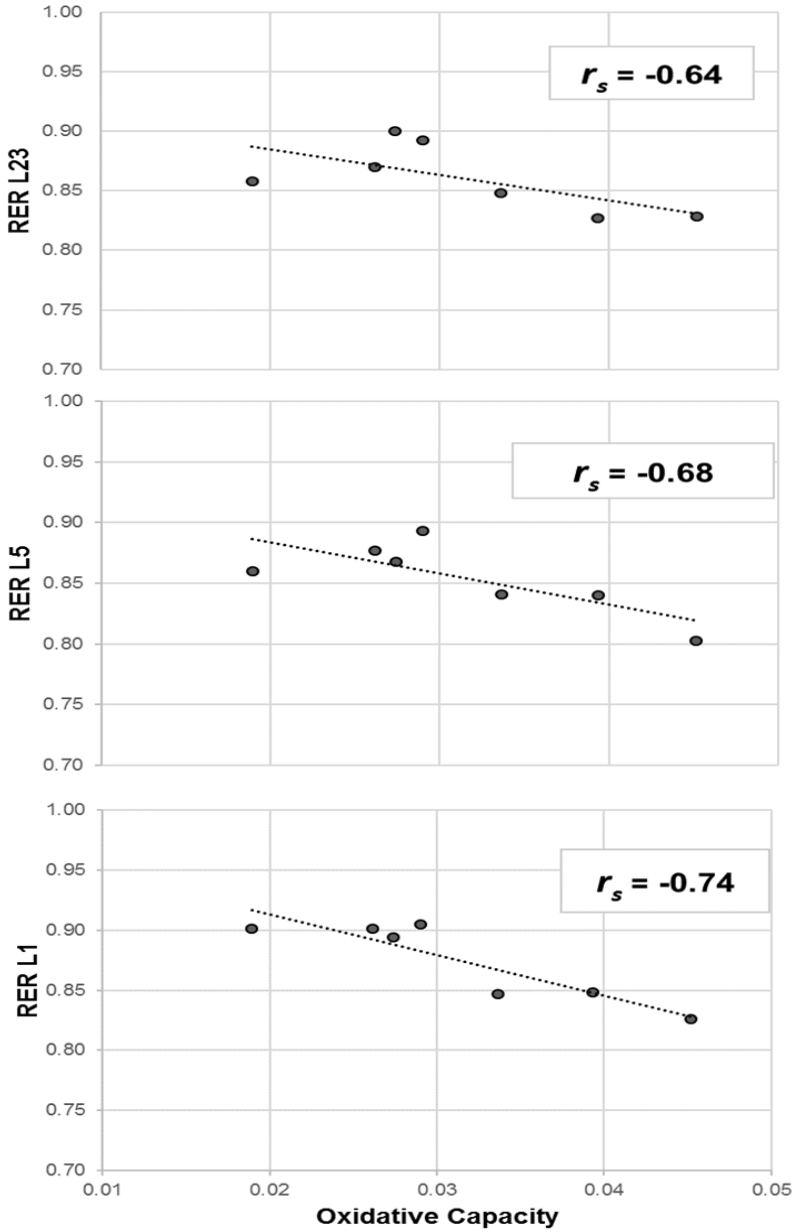


Figure 39. Associations between RER during the 30MTW and muscle oxidative capacity. 30MTW, 30-min treadmill walk; RER, respiratory exchange ratio; RER L23, average RER over the last 23 min of walk; RER L5, average RER over the last 5 min of walk; RER L1, average RER of the last 1 min of walk; PCr, phosphocreatine; kPCr s^{-1}, rate constant of PCr recovery; r_s, Spearman's rank correlation coefficient

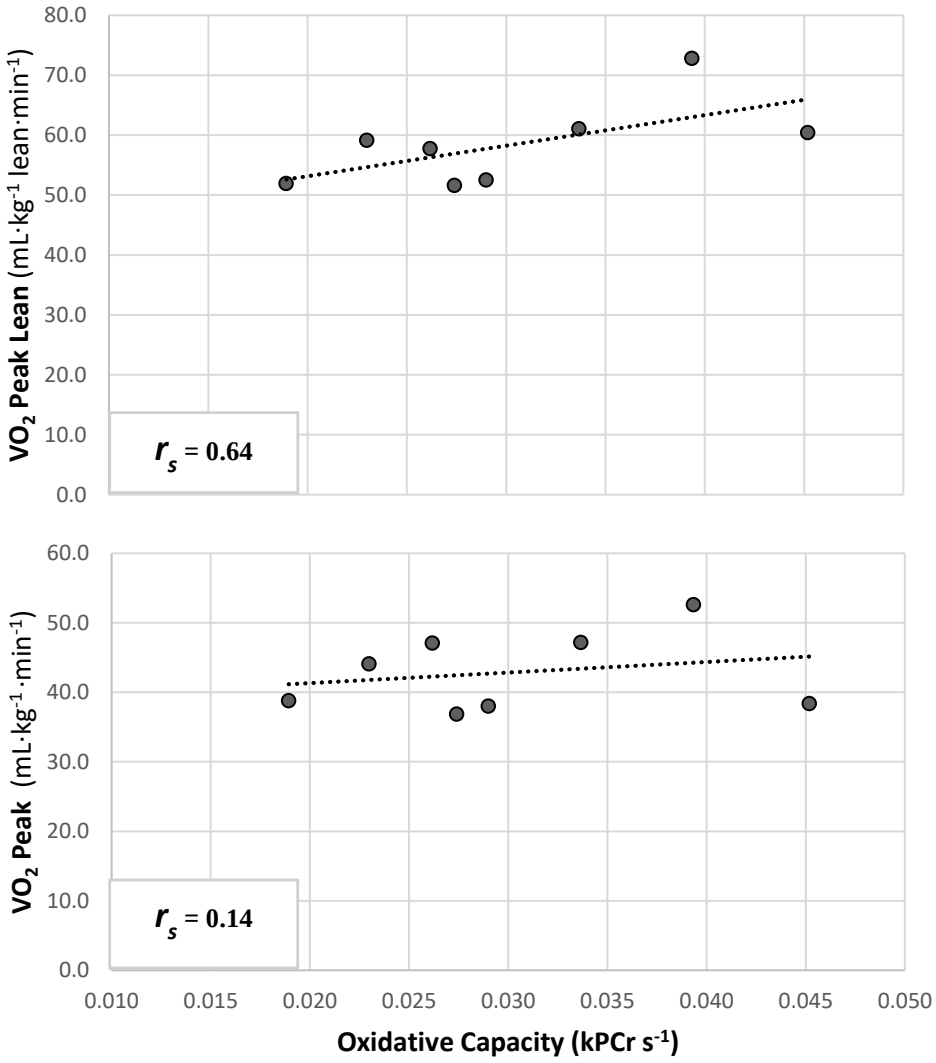


Figure 40. Association between VO_2 Peak normalized to total mass and lean mass and oxidative capacity. VO_2 peak, oxygen consumption; PCr, phosphocreatine; kPCr, s^{-1}, rate constant of PCr recovery; r_s, Spearman's rank correlation coefficient

Table 10. Spearman's Rho correlation coefficients

	ΔPCr	Muscle Oxidative Capacity
Oxygen Deficit (s^{-1})	**0.46 (0.30)**	-.018 (0.71)
EPOC (s^{-1})	0.75 (0.07)	**-0.64 (0.14)**
RER L23	0.75 (0.07)	**-0.64 (0.14)**
RER L5	0.71 (0.09)	**-0.68 (0.11)**
RER L1	0.70 (0.09)	**-0.74 (0.07)**
VO_2 Peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	-0.52 (0.20)	**0.14 (0.75)**
VO_2 Peak ($mL \cdot kg^{-1}$ lean$\cdot min^{-1}$)	-0.62 (0.11)	**0.64 (0.10)**
Oxidative Capacity	-0.62 (0.11)	-

Data are r_s (p-value), bolded values are from aims; PCr, phosphocreatine; EPOC, excess post-exercise oxygen consumption; RER, respiratory exchange ratio; RER L23, average RER over the last 23 min of 30MTW; RER L5, average RER over the last 5 min of 30MTW; RER L1, average RER of the last 1 min of 30MTW; VO_2, oxygen consumption.

CHAPTER 5

DISCUSSION

The purpose of this study was to begin to quantify the links between muscle energetics the whole-body energetic response to daily-living type activity (30MTW). The aims of this study were to determine whether the oxygen deficit at the beginning of whole-body exercise was associated with the rate of muscle non-oxidative ATP at the onset of contractions (ΔPCr), and whether whole-body EPOC, RER, and VO_2 peak were associated with muscle oxidative capacity. Our hypotheses were that there would be a positive association between muscle ΔPCr and O2 deficit, a negative association between muscle oxidative capacity and EPOC as well as RER, and a positive association between muscle oxidative capacity and VO2 peak.

The vastus lateralis muscle was selected for this study because it is a large, commonly-studied muscle that plays an important role in ambulation (during which the whole-body measurements were taken) as well as mobility-related function (22, 76, 86). As no muscle works in isolation, the evaluation of a single muscle is generally completed to gain information about a muscle group, the muscular system, or an organism as a whole. The question here was: Are the energetic characteristics of a single muscle (vastus lateralis) indicative of corresponding characteristics of whole-body energetics? The results presented here suggest that they are.

While attempts were made to maximize the similarity between the muscle and whole-body exercises, single-leg knee extension contractions and treadmill walking are still fairly disparate activities. We believe this gives more weight to the associations presented here, and suggests that the energetic variables described are not just indicators

of task performance, but underlying physiological function. The relative similarity of the participants- all young (25-35 years) healthy males who were not highly trained- should also add weight to the strength of associations, as a study population with a larger spread of characteristics would increase the chance that correlations are driven simply by group based differences.

Muscle and Whole-body Non-oxidative Energy Use are Related at the Onset of Exercise

In Aim 1, we explored the relationship between non-oxidative energy use at the muscle (ΔPCr) and whole-body (oxygen deficit) levels. For whole-body exercise, the 30MTW was used as it is a light-moderate intensity exercise, similar to what a person may perform during free-living walking to the store or going to work. Low-intensity, isotonic contractions were selected for the muscle contraction protocol because we believed this mode of contraction to be most similar to the type of muscular work performed during walking. To approximate the intensity of muscle contractions to those occurring during the 30MTW, only the first 2-min stage of the ramp protocol was used in the calculation of ΔPCr. It should be noted, however, that while the speed of the 30MTW was the same for all participants (1.3 $m \cdot s^{-1}$), the intensity of the first stage of the ramp was set relative to each individual's maximal force output (6% MVIC).

The magnitude of ΔPCr was associated with oxygen deficit (r_s = 0.46). This result suggests that individuals who were more reliant on PCr (a non-oxidative energy source) at the onset of knee extensor contractions were also more reliant on non-oxidative energy sources (PCr and glycolysis) at the beginning of whole-body exercise, such as walking. It is not clear whether greater non-oxidative reliance is due to an enhanced ability to

produce energy non-oxidatively, or a limited ability to rapidly increase oxidative energy production. In support of the former, studies have shown that blocking PCr breakdown and glycolysis in different preparations of isolated muscle leads to faster oxygen uptake kinetics (41, 42, 44, 59). Additionally, the speeding of uptake kinetics (i.e., the shortening of oxygen deficit) that occurs with training has been shown to precede changes in muscle oxidative capacity measured by citrate synthase activity (89). However, the latter is supported in this study by the negative association observed between ΔPCr and muscle oxidative capacity (r_s = -0.62), as well as VO_2 peak normalized to lean mass (r_s = -0.62), suggesting that individuals with a greater capacity to increase oxidative energy production relied less on PCr. Surprisingly, muscle oxidative capacity was not associated with oxygen deficit (r_s = -0.18), which in addition to the other research cited here, suggest that oxygen deficit is a product of a great number of factors and controls that warrants further investigation.

Although oxygen concentration (PO_2) in muscle at rest is much higher than what is required for resting oxidative energy production, potentially 50-100 fold, how quickly PO_2 becomes limiting is unclear and likely dependent on exercise intensity (68). It may be the case that during light intensity, whole-body exercise the increase in oxygen uptake is affected more by the ability of the body to deliver oxygen and nutrients than by the inherent characteristics of the muscle, an idea that is a subject of debate in the field (90). The fact that oxidative capacity in the VL is associated with greater reliance on PCr at the onset of muscle contractions but not at the onset of walking (oxygen deficit), could also be due to lower oxidative capacity in other ambulatory muscles. These complexities may

explain why the correlation coefficient for the association between ΔPCr and oxygen deficit was lower than other relationship shown here.

Muscle and Whole-body Oxidative Energy Use are Related

The relationship between oxidative energy use at the muscle (oxidative capacity) and whole-body levels (EPOC, RER, and VO_2 peak) was examined in Aims 2.1-3. The isokinetic muscle oxidative capacity protocol was selected because it causes a significant depletion of PCr without greatly disturbing pH, or blood flow (as maximal isometric protocols do), which should help ensure that the post-contraction rate of PCr recovery represents the maximal ability of the muscle to generate ATP oxidatively (87). Given that muscle cells are responsible for up to 90% of oxygen consumption during exercise and 30% during rest, the two levels are inextricably linked (110). Additionally, the same physiological factors - mitochondrial content, capillary density, and concentration of oxidative enzymes - are major determinants for both the independent and dependent variables (13, 31, 90, 100). Still, the relevance of a single muscle's oxidative capacity to whole-body energetics is not evident enough that it may be assumed. The correlations presented here shed light on the strength and direction of these relationships.

Muscle Oxidative Capacity is Associated with EPOC

Muscle oxidative capacity was associated with the time constant of EPOC (r_s = -0.64), suggesting that individuals who have a higher maximal rate of muscle oxidative energy production may return to resting VO_2 levels more rapidly following exercise. The time constant for EPOC represents the time it takes for oxygen consumption to recover by 63% following exercise, and reflects of the body's ability to return to homeostasis.

Metabolic recovery after exercise is dependent on restoration of metabolite concentrations to resting levels, and re-synthesis of PCr makes up a substantial portion of EPOC (38). It is then reasonable to expect that the ability to replenish PCr quickly (via a high oxidative capacity) would lead to a faster recovery (a lower EPOC time constant). However, this would imply that either muscles work at or near their oxidative capacity during recovery from low-intensity work, or that a lower oxidative capacity is related to lower rates of oxidative energy production even at submaximal intensities (during recovery). It should be noted that the beneficial effects of training on muscle, such as mitochondrial biogenesis, increased cytochrome oxidase activity, and greater activation of mitochondrial complexes has been demonstrated to lead to greater improvements in oxygen deficit than EPOC (109). In light of which, the authors pointed out that EPOC is less dependent on muscle oxygen consumption than deficit, as it also reflects the slowly decreasing oxygen consumption of the heart and lungs among other variables. It is then surprising that we found in this study a strong relationship between muscle oxidative capacity and EPOC, but not oxygen deficit.

An enhanced ability to produce energy oxidatively may also lead to a lesser reliance on PCr at the onset of activity, as observed in Aim 1, thus decreasing the amount of PCr that needs to be replenished in recovery. Our analysis shows that the strongest association among our variables was the positive linear relationship between ΔPCr and EPOC (r_s = 0.75). This relationship indicates that those who deplete PCr to a greater degree at the onset of exercise take longer to recover following exercise, and that reliance on non-oxidative energy production at the beginning of exercise may be a more important determinant for exercise recovery than oxidative capacity. This is not surprising given

that EPOC was originally termed oxygen debt and was believed to only consist of 'repayment' of oxygen deficit; an idea that has since been disproven, but was not entirely baseless (38).

Muscle Oxidative Capacity is Associated with RER

Muscle oxidative capacity was associated with the mean RER from the entire steady-state (RER L23, r_s = -0.64), the last 5 min (RER L5, r_s = -0.68), and the last minute (RER L1, rs = -0.74) of the 30MTW. These correlations suggest that individuals with a higher oxidative capacity relied more on fat oxidation than those with a lower oxidative capacity throughout the 30MTW. In the example plot in Fig. 27, RER takes longer to plateau than does VO_2, suggesting that RER changes over the course of VO_2 steady-state; a similar pattern was observed in all participants. It is interesting to note that the association between muscle oxidative capacity and RER appears to strengthen when the RER mean was calculated from the latter portions of the test, especially considering that the group means for RER L23, L5 and L1 were not significantly different (Table. 4).

Oxidative capacity is more than just a measure of the muscle's ability to replenish PCr, it is an indicator of the tissue's ability to produce ATP through oxidative metabolism (87). Fat is the preferred fuel source for oxidative metabolism at rest and during low-intensity activities. Carbohydrate is the predominant fuel source at higher intensities, at least partially because it can be metabolized at a faster rate (55, 90). In this way, cells rely more on fat until the energy demand is too great to be met by its slower rate of oxidation. A higher oxidative capacity may keep energy demand from reaching this point, which would explain the association between oxidative capacity and RER. Likewise, an adaptation seen with training is an increase in fat oxidation across relative intensities (13,

31). The results here suggest that this effect may be evident even at relatively low intensities (i.e., the 30MTW). It is also important to consider that the 30MTW walk represented a different relative intensity (as a percentage of VO_2 peak) across participants. The relationship between muscle oxidative capacity and RER may be a function of the higher VO_2 peak, and therefore lower relative intensity, in individuals with a higher oxidative capacity (14).

A surprising finding was that ΔPCr had a higher correlation with RER (L23 r_s = 0.75, L5 = 0.71, and L1 = 0.70) than muscle oxidative capacity did. Given that ΔPCr is a purely anaerobic measure, and RER (at steady-state) is a purely aerobic measure, the reason for this relationship is not clear. However, individuals who had a greater ΔPCr also had a lower VO_2 peak per lean mass (r_s = -0.62); so the relationship between ΔPCr and RER may also be an artifact of the relative intensity of the 30MTW. Other factors, such as energy balance and dietary consumption, can also affect RER, however this was controlled for by the standardized lunch consumed 2.5 hr. before the 30MTW by all participants (80).

Muscle Oxidative Capacity is Associated with VO_2 Peak

Muscle oxidative capacity was associated with VO2 peak relative to lean mass (r_s = 0.64), but not VO_2 peak relative to total mass (r_s = 0.14). This suggests that individuals with a higher oxidative capacity tend to have a greater ability to increase whole-body oxygen consumption per unit of lean tissue, but not per unit of total mass (fat and lean mass); meaning that variations in body fat between participants confound this relationship, which is not surprising given the relatively low metabolic rate of adipose tissue (32). This is in contrast to several previous studies that have found an association

between VO_2 peak normalized to total mass and muscle oxidative capacity measured via *in vitro* respiration (r^2 = .81-.89) (111), near-infrared spectroscopy (r^2 = .57) (63), and ^{31}P-MRS (r^2 = .54) (2). Discrepancies in findings may be due a smaller range in body composition (63), or greater number of participants (2, 111), which may have negated or balance the impact of inter-individual differences in body composition. In another study (58), an inverse relationship was shown- a higher VO_2 peak was associated with a slower PCr recovery- but this was possibly due to sex based differences in VO_2 peak (women tend to have a higher percentage of body fat). It has been suggested that VO_2 normalized to total body mass may have greater implications for exercise performance, while VO_2 normalized to lean mass (more metabolically active tissue) is more relevant when discussing physiological capacity (39).This is logical as an increase in body fat would increase the workload of a graded treadmill test, but not mechanical function or oxidative capacity of the muscle.

Maximal oxygen consumption is dependent on the ability of the pulmonary and cardiovascular systems to deliver oxygen to the body's cells, the capacity of those cells to use oxygen to generate ATP, and the rate at which ATP production can be stimulated. However, studies that have measured oxidative capacity in isolated muscle cells suggest that the summed maximal oxygen consumption of all the body's muscle cells is greater than what has been measured at the whole-body level (5, 10). This suggests that oxygen consumption is likely limited by the ability of the body to deliver oxygen to the muscle, not the muscle's oxidative capacity. Despite this, ours and other studies have shown evidence of a positive relationship between muscle oxidative capacity and peak oxygen

consumption (3). It is reasonable then, that although muscle oxidative capacity may not be the factor limiting VO_2 peak, it can be used as an indicator for aerobic fitness.

It should be noted that performance in a graded exercise test and the $VO_{2\ peak}$ value obtained is dependent on participant motivation, nutritional status, the testing environment, exercise experience, and testing modality (e.g., treadmill, cycle ergometer .etc.), among other factors (55, 90). If not well controlled, these factors can lead to greater variability and lower accuracy in this measure. In addition, performance of a graded exercise test may not be possible for certain populations, including the elderly. Due to this, Alexander et al., 2003 (4) suggest that submaximal measures of oxygen consumption such as the oxygen deficit and EPOC may be more suitable indicators of aerobic fitness in these populations. This is one of the reasons that Aims 2.1 and 2.2 focused on submaximal whole-body measurements that minimize or eliminate some of these issues in identifying the link between muscle and whole-body energetics.

Limitations

This study was completed on a small number of participants, who were all male, younger, relatively healthy, and not representative of the population as a whole. This could be easily remedied through a study with a greater number of participants including both sexes and multiple age groups. With the 7 or 8 participants involved in these analyses, a Spearman's r_s greater than ~0.76 would have been necessary to reach the generally accepted alpha level of significance of $p < 0.05$. No associations detected here met that criterion. Again, a greater number of participants could help with this. Since this was an exploratory study, associations were also assessed using Pearson's and Kendall's methods and these data can be found in *Appendix E.* Pearson correlations resulted in

considerably higher coefficients and lower p-values and the opposite was true for Kendall's. Oxygen consumption data is inherently subject to a fair amount of variability and noise. As a result completing several 30MTWs and averaging the oxygen consumption measurements would have been useful for increasing the signal to noise ratio. A study by Benson et al. in 2017 (11), specifically suggests averaging four exercise bouts when analyzing the oxygen deficit, and even proposed a minimally important time constant difference of ~5 s (which was not found in our study). As mentioned in the results, the timing of the end of the 30MTW was not recorded for two participants, and thus had to be estimated. The first four participants were allowed phone access during recovery, which may have unintentionally increased VO_2 during this period. Several participants who were not allowed phone access during recovery were close to falling asleep post-walk, which may have unintentionally decreased their resting VO_2. If this study were repeated, better control of the environment, wakefulness of participants, and timing of the whole-body energetic measurements would be desirable. To account for the thermic effect of food and other dietary responses a standardized lunch was fed to participants for all visits. This lunch, however, may not have the same effect on participants with different body sizes, free-living diets, and energy expenditures. Individualization of future standardized meals may be more effective if robust methods to elicit similar responses can be implemented.

Summary

The goal of this study was to investigate both non-oxidative and oxidative energetic characteristics at the muscle and whole-body levels. Associations were

observed between ΔPCr and oxygen deficit, EPOC, and RER; as well as between muscle oxidative capacity and EPOC, RER, and VO_2 peak in a group of healthy, young males.

These promising results can be extended by studies evaluating the same relationships with participants of different ages, sex, and habitual physical activity levels. If strong and significant associations are consistently demonstrated, then researchers can begin to make inferences about whole-body energetics from muscle measurements, and vice versa. There is already a well-established relationship between whole-body energetics - including oxygen deficit, EPOC, and VO_2 peak, and energy cost of walking – and performance and fatigability during mobility test (4, 9, 92, 98, 99). Therefore, whole-body energetics may serve as an intermediary point between muscle energetics and mobility-related function. This connection could potentially help researchers understand the role muscle energetics may play in the decline in mobility-related function (Fig. 41). This would be particularly useful in the study of aging and physical inactivity, which is associated with a decline in knee extensor muscle oxidative capacity (36), as well as a decline in walking speed, postural control, and the ability to transition from sitting to standing or walking (9, 11, 75).

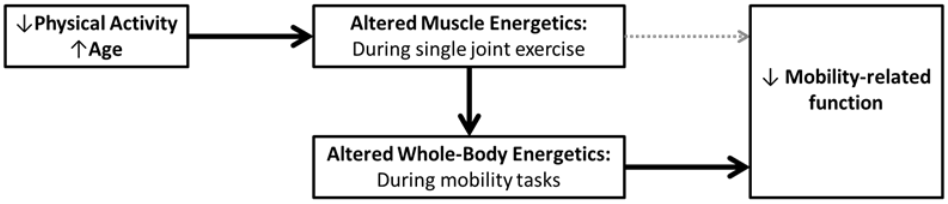


Figure 41. Pathway connecting muscle energetics to mobility-related function. Adapted from Fig. 2.

In conclusion, this study provides preliminary evidence that muscle energetics are indicative of the oxygen deficit, RER, and EPOC in response to daily-living type

exercise. This is a novel finding that links muscle and whole-body energetics despite their being measured using different techniques, in different settings, and in response to different forms of exercise. Given the practicality of indirect calorimetry measurement during mobility tasks, this link can be exploited to improve our understanding of the energetics of movement.

APPENDIX A

INFORMED CONSENT FORM

Consent Form for Participation in a Research Study University of Massachusetts Amherst

Researcher(s): Michael Busa, Ph.D., Jane Kent, Ph.D., John Sirard, Ph.D., John Staudenmayer, Ph.D., Greg Petrucci, Christopher Hayden

Study Title: Room Calorimetry: Energetics of Fatigue Pilot

Funding Agency:

1. WHAT IS THIS FORM?

This form is called a Consent Form. It will give you information about the study so you can make an informed decision about participation in this research. It will also describe what you will need to do to participate and any known risks, inconveniences or discomforts that you may have while participating. We encourage you to take some time to think this over and ask questions now and at any other time. If you decide to participate, you will be asked to sign this form and you will be given a copy for your records.

2. WHO IS ELIGIBLE TO PARTICIPATE?

We are recruiting 8 people to participate in this study. All participants will be free of acute and chronic neurological, musculoskeletal, metabolic, cardiovascular or other major diseases or injuries, and will not have a pacemaker. Additionally, a physician must not have told the participant that they should refrain from physical activity. Additionally, they will not have had surgery in the previous 6 months that would impact their ability to perform light-to-moderate physical activity. Participants will consist of 8 males between the ages of 25-40 years.

3. WHAT IS THE PURPOSE OF THIS STUDY?

The goals of this study are to: 1) Demonstrate that reliable data can be obtained from the Room Calorimeter during 24-hour visits. 2) Determine the sensitivity of the Room Calorimeter system to better understand its capacity and constraints. 3) Compare energy expenditure values from the Room Calorimeter to energy expenditure and physical activity estimates from several research-grade accelerometers. 4) Identify the effects of a treadmill walk on the energetics of healthy young sedentary individuals. 5) Collect data that will assist us in developing hypotheses in the areas of exercise, exercise recovery, mechanical and metabolic interactions, and physical activity assessment.

4. WHERE WILL THE STUDY TAKE PLACE AND HOW LONG WILL IT LAST?

The study will be conducted in the Human Testing Center and the Human Magnetic Resonance Center (S360 and S230 Life Science Laboratory Building), in the Institute for Applied Life Science (IALS) at the University of Massachusetts Amherst. This study is expected to last 18 months. If you participate, you will take part in 4 testing visits; 2 visits will last approximately 24 hours each, 1 visit will last 4.5 hours, and 1 visit will last 2.5 hours.

5. WHAT WILL I BE ASKED TO DO?

Prior to each visit

We ask that the day prior to each visit you avoid alcohol use and strenuous exercise. The day of each visit we also ask that you avoid consuming caffeine.

Visit 1 (4.5-hour Baseline Habituation visit)

This visit will be conducted in the Room Calorimeter suite of the Human Testing Center in IALS, with the exception of height and weight measures and the body composition scan, which will be completed across the hall in the Exam Room. The Room Calorimeter is an instrument built as a standard room and used to measure oxygen consumption and carbon dioxide production by humans in a typical living setting. It is about the size of a large bedroom and contains a TV, toilet, sink, chair, desk, and treadmill. There are two ports (sealed openings in the wall) by the entrance to the room that are used for passing food and other material into the chamber without opening the doors. The room has a window to the outside of the building as well as to the inside of the lab. Both windows are equipped with blinds for privacy. For the first visit, the Room Calorimeter door will not be shut and the instrument will not be running. This habituation visit will allow you to become familiar with the facility, and promote easier acclimation during following visits.

You will be fed a small lunch (~400 calorie meal replacement bar) which will allow us to compare energetic response between participants, there will be several options to choose from. You will be allowed 30 minutes to complete your lunch. You will be oriented to the Room Calorimeter, and shown the button that blurs the cameras in case you are in need of private time (i.e., changing clothes or using the toilet). Video will be recorded during this study, this button will disable recording and live viewing through the camera system. Video access is restricted to Human Testing Center personnel and can only be accessed using an onsite computer. You will also be shown how to work the exit door (a simple push latch). Your height, weight, age, and gender will be recorded, and an iDXA body scan will be completed by a certified technician.

iDXA Scan

An iDXA (dual-energy X-ray absorptiometry) scan, also called a bone density scan, is a common technique used to measure bone density. It can also be used to estimate body

composition (% fat and % lean mass), as in this study. The DXA scan will involve lying on a padded table while a scanning arm passes over you. This test will last no longer than 30 minutes. This completely painless procedure is easily performed and involves minimal radiation exposure.

In accordance with Massachusetts Department of Public Health guidelines, iDXA scans are performed by a certified technician and prescribed and reviewed by Dr. Stuart Chipkin (University of Massachusetts Amherst, 240 Thatcher Rd., Amherst, MA 01003). During the scan you will be exposed to low amounts of ionizing radiation; these levels are approximately 2% of that you would be exposed to during a chest X-Ray and less than you would receive from a cross-country airplane flight.

From this study, it may be found that you have 'normal bone density', 'low bone density', 'osteoporosis', or 'severe osteoporosis'. If your results indicate anything OTHER THAN 'normal bone density,' you will receive a follow-up letter from the research team. In this case, it will be recommended that you follow up with your personal physician. With your written permission, the data that we collect will be made available to your physician for their review and interpretation. These results are not diagnostic and do not indicate a specific risk of bone fracture. This can only be determined by a clinician when also accounting for other factors.

Prior to this scan, you will be asked a series of screening questions to determine if undergoing an iDXA would pose any undue risk. As this is for your protection, it is of the utmost importance that you answer completely and to the best of your knowledge. While the risk with this procedure is extremely low, your safety is paramount.

Two hours after lunch you will be outfitted with the Parvo Medics TrueOne 2400 Metabolic System, which consist of a mask connected to hoses that collect expired air, filters it, and runs it through a gas analyzer on a stationary cart. You will be asked initiate the fitting of the mask and a researcher (while wearing gloves and a face shield) will step in to complete it. You will also be asked to outfit yourself with several research-grade accelerometers (similar to Fitbits); an ActivPal (dominant thigh), a StepWatch (dominant ankle), a SenseWear Armband (upper arm), and an ActiGraph (non-dominant wrist).

Next, you will be asked to complete 30 minutes of walking on a treadmill. The treadmill will be flat and the speed will be increased over the first 30 seconds of this walk until 1.3 $m \cdot s^{-1}$ is reached. At that point, you will be asked if you think you can maintain that speed for 30 minutes. If you cannot, the treadmill speed will be reduced in small (0.045 meters per sec) increments every 10 seconds, until you are confident you are at a speed that you can maintain for 30 minutes. At minutes 7, 17, and 27, the incline of the treadmill will be increased to 3% for 1 minute, in order to provide a slight challenge and to simulate a hill that you might encounter in everyday life. After 1 minute, the incline will be returned to level. At minute 28, you will be asked to rate your exertion on a scale of 0 to 10. The scale is a simple visual rating system that you can point to during the walk, with a 0

meaning “nothing at all” and a 10 meaning “maximal” exertion. During the final 30 seconds of the walk, the treadmill will be gradually slowed to 0.4 meters per second before stopping.

During this treadmill walk, you will be connected to the emergency stop safety cord and instructed on how to use the treadmill safely. At all times, the treadmill will be controlled by study staff. This treadmill walk will be completed on the treadmill in the Room Calorimeter.

Next, you will be asked to sit quietly for a 2-hour, passive recovery period during which time you will be allowed to watch TV, read, or use any mobile devices you may have. You will wear all of the same study devices from the walk during this recovery period. After this passive recovery, you will remove the facemask and all other monitors. You will then be given an ActiGraph (a step counter), which you will be asked to wear on your non-dominant wrist for a seven-day lead-up to your first 24-hour visit to the Room Calorimeter. You will also be asked to take pictures of your meals and any snacks for 3 days prior to your second visit. You will be asked to complete this task and send us the pictures via email at least two days before your 24-hour visit.

Visits 2 and 3 (24-hour Room Calorimeter Stays):

You will be asked to complete two visits of 24 hours each in the Room Calorimeter. They will be completed on separate days within one month of each other. These visits will be conducted in the Room Calorimeter suite of the Human Testing Center of IALS.

You will be asked to arrive in the evening (~7 pm). Upon arrival, you will again outfit yourself with Fitbit-like devices consisting of an ActivPal (placed on your dominant thigh), a StepWatch (dominant ankle), a SenseWear Armband (upper arm), and an ActiGraph (non-dominant wrist). You will be asked to keep these on for the duration of the study unless you are changing your clothes.

The Room Calorimeter is an instrument that allows us to measure your oxygen consumption. This measurement requires the door to the room calorimeter to remain sealed throughout the study. You will be free to leave at any time during the study if you no longer wish to be a participant. You should simply notify the study staff and press on the exit latch to leave the room. Your entire stay will be video recorded through the cameras installed in the calorimeter, sound will not be recorded. We will use these recording to annotate your stay and the timing of certain activities you will perform. You can stop the video from recording at any time by pressing a button on the wall. We will request that you do this anytime you wish to change, use the restroom, or are in need of private time.

A walkie-talkie (and extra batteries) will be provided and there will be a CPR- and First Aid-certified research staff member in communication with you at all times. A schedule will be given to you, and either the walkie-talkie or the intercom system will be used to

provide further instructions during your stay. You will then enter the Room Calorimeter. The schedule is as follows:

7:00 PM:	Enter Room Calorimeter and quick review of facilities and procedures
7:15 PM:	Dinner (served through the food port)
8:00 PM:	Free time until you wish to sleep
12:00 AM:	Time by which you must be in bed
8:00 AM:	Wake up, get out of bed
8:15 AM:	Scripted Activities (see below)
9:15 AM:	Breakfast (served through the food port)
9:45 AM:	Downtime
11:30 AM:	Lunch
12:00 PM:	Two hours of passive (resting) free time
2:00 PM:	30 treadmill walk as described above (without Parvo unit)
2:30 PM:	Passive recovery
7:00 PM:	Exit Room Calorimeter

The “Scripted Activities” will be as follows

Strip bed; make bed (10 min)

Sit (5 min)

Fold basket of laundry (towels; 10 min)

Sit (5 min)

Vacuum floor (10 min)

Sit (5 min)

Wash and dry dinnerware and silverware (10 min)

Sit (5 min)

You will be asked to complete 1,200 steps in the Room during your downtime (before 2:00 pm). The activity tracker on your wrist will display the step count for you. Breakfast and dinner will be based on the meal pictures you provided us. Lunch will be the same

for all four visits. After exiting the Room Calorimeter, you will remove all devices prior to leaving.

Research staff will be present in the Human Testing Center for the entirety of the study (including during your sleep time).

Visits 4 (Exercise Testing):

For Visit 4 you will be given a meal replacement bar ahead of time to take home, identical to the other visits, which we ask you consume in the morning two hours before your visit. That morning you will also be asked to:

1. Wear loose-fitting clothes (jogging attire, shorts, training shoes, etc.)

2. Avoid eating or drinking anything but water and the meal provided.

3. Avoid alcohol, tobacco, and coffee.

4. Avoid exercising.

5. Try to get a good night's sleep the night before.

Upon arriving at the Human Magnetic Resonance Center in the Life Sciences Lab Building, you will meet with the MR Operator to review the MR Safety Form. Before entering the magnet room, you will remove any jewelry, empty your pockets, and walk through a metal detector. You will then be escorted to the MR scanner and positioned on your back with your dominant leg on top of our custom-built leg exercise apparatus, which is similar to a knee extension machine one might use at a gym. The lower leg (shin) will be strapped to the apparatus just above the ankle and at the knee using Velcro straps. Inelastic straps will also be secured over your hips to prevent unwanted movement during the muscle contractions, and a specially-designed copper coil encased in a plastic box will be secured over your thigh muscles using Velcro straps. This coil will be used to measure changes in muscle biochemistry during the contractions.

Lastly, headphones will be provided to limit the amount of noise from the MR scanner, and to enable clear and constant communication between you and the investigators. We will talk with you and explain each set of measures as we go along. These measures are described next.

After you have been secured to the leg-exercise apparatus, we will position you in the center of the scanner and “tune” the magnet to your thigh muscles. We will then take measures of your muscle biochemistry while you lay quietly for about 10-15 minutes. Next, you will complete 2-3 maximal static contractions, each lasting 3-4 seconds, to determine your strength and to establish the workloads that will be performed during the incremental protocol. About 1 minute of rest will be given between each of these contractions. You will then be familiarized with the dynamic contractions (1 contraction every 2 seconds) by performing a few sets of 2-3 contractions. Once you are comfortable with the dynamic contraction procedures, you will perform a 24s maximal isokinetic

contraction protocol. This will consist of one maximal dynamic contraction every two sections, after which you will rest quietly without moving for up to 10 minutes.

Next, you will perform the study protocol, which involves 4 stages of dynamic knee extensions. These contractions are most similar to the types of contractions that would be performed on an exercise machine at a fitness gym. Each stage will last 2 minutes, with 1 contraction being performed every 2 seconds. Workload will increase with each stage, starting at a light intensity and increasing to moderately-high intensity. Following the 4th stage of the protocol, recovery measures will be collected while you rest quietly without moving for up to 10 minutes. Following the MR measures, you will be brought from the Human Magnetic Resonance Center to the Human Testing Center. In the human testing center, you will have your blood pressure taken and will be prepped to complete a graded exercise test to determine your maximal volume of oxygen consumption (VO2 max).

VO2 max tests are one of the most widely used evaluations in the world of exercise physiology and are utilized to evaluate aerobic fitness. During the test, you will be instrumented with the same Parvo Metabolic Cart utilized during the baseline visit as well as a chest strap Polar heart rate monitor. We will be using the BSU/Bruce Ramp treadmill protocol as described by Kaminsky and Whaley (1998). This protocol is designed to be better suited for a wide range of populations through its use of a gradual ramp in intensity, allowing for longer walking periods and smaller transitions between stages. The test begins with a paced walk and gradually increases in speed and/or incline. Heart rate, oxygen consumption, and Rating of Perceived Exertion (RPE) will be monitored throughout the test. The test will be terminated when you feel you cannot continue or if the researcher determines the test should end due to maximal intensity being reached safety concerns or equipment malfunction. A typical test will last between 8-12 minutes depending on your fitness level. After Completion of the VO2 max test, you will have equipment removed and will complete a short cool down.

6. WHAT ARE MY BENEFITS FOR BEING IN THIS STUDY?

It is important that you understand that we anticipate no direct benefit to you from your participation in this study.

7. WHAT ARE MY RISKS OF BEING IN THIS STUDY?

Physical well-being

General Exercise Precautions: During any type of exercise, there are slight health risks, along with the possibility of fatigue, cardiovascular events, muscle soreness, and falls. Study personnel will monitor your performance. The testing will be terminated if you show any signs of poor exercise tolerance (i.e. extreme fatigue, shortness of breath, chest pain, dizziness, etc.).

VO2 Max Testing: Risk include, but are not limited to, abnormal blood pressure, chest pain, shortness of breath, fainting, disorders of the heartbeat (too rapid, too slow or unusual beats) and in rare instances, heart attack. Every effort will be made to avoid or

minimize such occurrences through screening and continuous observations during testing. Emergency equipment and trained personnel are available to deal with unusual situations which may arise.

DXA Scan: Scans expose you to low-levels of X-Ray ionizing radiation. The risk associated with this type of scan is very low, less than that of a New York to Los Angeles flight. You will be screened for the possibility of lifetime limit for radiation exposure. If you indicate that you have reached their lifetime limit for being exposed to ionizing radiation, then no scan will be performed.

Magnetic Resonance Spectroscopy (MRS) and Imaging (MRI): The United States Food and Drug Administration (FDA) has established guidelines for magnetic field strength and exposure to radio waves, and we carefully observe those guidelines. Some people may feel uncomfortable or anxious during the MRS testing procedures. If this happens you may ask to stop the study at any time and we will take you out of the MR scanner (magnet). On rare occasions, some individuals might feel dizzy, get an upset stomach, have a metallic taste, feel tingling sensations, or experience muscle twitches. MRS poses some risks for certain people. Individuals with a pacemaker or some metal object inside the body will be excluded from this study because of the strong magnets used for these studies. Another risk is a metallic object flying through the air toward the magnet and hitting the participant. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. Nothing metal can be brought into the magnet room at any time. Also, once you are in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet.

Psychological well-being

During the 24-hour stay, you will be asked to stay within the Room Calorimeter, which is very similar to a hotel room. Because of the duration and lack of general outside contact, you may become bored. To minimize this you will have access to all modern means of personal communication and entertainment (i.e. phone, computer, TV, internet access). Visitors will also be allowed during your downtime, however, they must remain outside of the chamber. Being in the Room Calorimeter may make you feel uncomfortable, anxious, or claustrophobic, although multiple windows in this space minimize this possibility.

You may be bothered by feelings of claustrophobia during MRS testing. Unlike the case for brain imaging, you will be positioned feet-first in the magnet, and your head will remain near the opening of the magnet. These features minimize the problem of claustrophobia, but anyone reporting significant claustrophobia who wishes to be removed from the scanner will be removed immediately.

8. HOW WILL MY PERSONAL INFORMATION BE PROTECTED?

All digital files will be maintained on an encrypted, secure, password-protected server. None of your personal information will be included in these files. Rather, we will give them a code. The file linking your identity and code will be kept in a locked drawer in a locked room. We expect the data collection for this study to last approximately 1.5 years. All electronic files containing identifiable information will be password-protected. Video recordings will be housed on a secure data server and agents of the sponsor may be able to view these on-site at UMass. At the conclusion of this study, the researchers may publish their findings. Information will be presented in summary format and you will not be personally identified in any publications or presentations.

9. WHAT IF THERE IS AN UNEXPECTED FINDING ON MY IDXA OR MRI SCAN?

The investigators for this research project are not licensed or trained diagnosticians or clinicians. The testing performed in this project is not intended to find abnormalities, and the images or data collected do not comprise a diagnostic or clinical study. However, occasionally in the process of research, investigators may perceive an abnormality, the health implications of which may not be clear. When an unexpected finding is noted, UMASS Amherst researchers will consult with a radiologist (for MRI) or physician (for iDXA). If the radiologist/physician determines that an additional inquiry is warranted, the researcher will then contact you regarding the radiologist's/physician's opinion of the unexpected finding(s).

In such a case, you are advised to consult with a licensed physician to determine whether further examination or treatment would be prudent. Although the images collected for this research project do not comprise a diagnostic or clinical study, the images can be made available to you for clinical follow-up. The costs for any care that will be needed to diagnose or treat an unexpected finding(s) would not be paid for by University of Massachusetts, Amherst. These costs would be your responsibility. If you have further tests done by your licensed physician, those results will then become part of your medical record, which may affect your current and future health or life insurance. Regardless of the health implications, the discovery of an unexpected finding(s) may cause you to feel anxious or worried. You may wish to talk to your physician or a qualified mental health clinician. You can contact the Center for Counseling and Psychological Health (CCPH) at (413) 545-2337 (Mon-Fri from 8-5pm) - on weekends or after 5pm, call (413) 577-5000 and ask for the CCPH clinician on call. You can also contact the Psychological Services Center at 413-545-0041 (Monday-Friday 8am-5pm) or psc@psych.umass.edu. In a serious emergency, remember that you can also call 911 for immediate assistance.

10. WILL I RECEIVE ANY PAYMENT FOR TAKING PART IN THE STUDY?

Yes, you will be compensated in person for your participation in this study, in gift cards. You will receive $25 for the baseline visit, $100 for each 24-hour visit, and $25 for the

exercise testing visit. You will be paid at the end of each portion of the study. Payment for the 24-hour stay will be prorated in six-hour increments. You will receive $25 for each 6-hour block you stay in the study (e.g., those who drop out after 12 hours will receive $50). You will receive the full $100 if and when you complete the full 24-hour visit. Total possible compensation will equal $250.

11. WHAT IF I HAVE QUESTIONS?

Take as long as you like before you make a decision about whether to participate. We will be happy to answer any question you have about this study. If you have further questions about this project or if you have a research-related problem, you may contact the principal investigator, Professor Jane Kent (413-545-9477; jkent@umass.edu). If you have any questions concerning your rights as a research subject, you may contact the University of Massachusetts Amherst Human Research Protection Office (HRPO) at (413) 545-3428 or humansubjects@ora.umass.edu.

12. CAN I STOP BEING IN THE STUDY?

You do not have to be in this study if you do not want to. If you agree to be in the study, but later change your mind, you may drop out at any time. There are no penalties or consequences of any kind if you decide that you do not want to participate.

13. WHAT IF I AM INJURED?

The University of Massachusetts does not have a program for compensating subjects for injury or complications related to human subjects research, but the study personnel will assist you in getting treatment in the unlikely event that you are injured during the testing.

14. CAN WE RECONTACT YOU?

May we contact you in the future to invite you to participate in additional studies like this one?

Yes NO

15. SUBJECT STATEMENT OF VOLUNTARY CONSENT

When signing this form, I am agreeing to voluntarily enter this study. I have had a chance to read this consent form, and it was explained to me in a language that I use and understand. I have had the opportunity to ask questions and have received satisfactory answers. I understand that I can withdraw at any time. A copy of this signed Informed Consent Form has been given to me.

In no way does signing this consent form waive your legal rights nor does it relieve the investigators, sponsor or involved institutions from their legal and professional responsibilities.

Participant Signature

Print Name

Date

By signing below, I indicate that the participant has read and, to the best of my knowledge, understands the details contained in this document and has been given a copy.

Signature of Person Obtaining Consent

Print Name

Date

EMERGENCY CONTACT

As a part of this study, you will be staying in the Human Testing Center for 24 hours. In the event that you fall ill or become injured, please provide an emergency contact.

Emergency Contact Name

Phone Number

APPENDIX B

PARTICIPANT SCREENING FORM

Screened by: _____ Date: _____ Study: _____ Status: _____

RC Pilot Screening Form

This study consist of four visits: One 4.5 hour visit, two 24 hour room calorimeter stays and one 2.5 hour exercise testing visit. During these visits, you will be monitored by accelerometers and indirect calorimetry (room calorimeter and metabolic cart), during your first visit a DXA scan will be completed, and your fourth visit will require a form of MRI. You will be asked to complete a 30-minute treadmill walk during three of the visits. The treadmill walk is designed to produce low levels of fatigue but does not require any running. During the fourth visit you will be asked to perform knee extension exercises inside our MR scanner, and then complete a VO2 max test consisting of a short, but intense, bout of treadmill running. All visits will occur on campus in the Life Science Laboratory Building on Thatcher Way.

The Room Calorimeter is an instrument used to measure oxygen consumption and carbon dioxide production by humans in a free-living setting. It is about the size of a large bedroom and contains a TV, toilet, sink, chair and desk, and a treadmill. There are two ports by the entrance to the room that are used for passing food and other material into the chamber without opening the doors. The room has a window to outside the building as well as to inside the lab along with blinds for privacy. Your entire stay will be video recorded through the cameras installed in the calorimeter, sound will not be recorded. We will use these recording to annotate your stay and the timing of certain activities you will perform. You can stop the video from recording at any time by pressing a button on the wall. We will request that you do this anytime you wish to change, use the restroom, or are in need of private time.

A DXA scan (A dual-energy X-ray absorptiometry (DEXA) scan, also called a bone density scan, is a common technique used to measure bone density. However, as it is in this study it is often also used to estimate body composition. The DXA scan will consist of lying down on a padded table as a scanning arm passes over you. This test will last no longer than 30 minutes. This completely painless procedure is easily performed and requires minimal radiation exposure.

Magnetic Resonance (MR) scans collect information about the body using a magnet and radio waves. While the procedure is much like a CT scan, there is no radiation involved in an MRI exam. The MRI scan in this study will take about one hour including prep time. To be sure that it is safe for you to have an MRI exam, you will be asked to complete standard MRI screening questionnaires. Since the MR machine uses a strong magnet that will attract other metals, you may not take part in this study if you have a pacemaker, an implanted defibrillator, or certain other implanted electronic or metallic devices, shrapnel, or other metal. While in the MR scanner we will ask you to complete several bouts of knee extension exercises on our custom-built ergometer.

VO2 max test are used to evaluate aerobic fitness. During the test, you will be instrumented with a mask that measures oxygen consumption, the same one that will be used during the treadmill walks. The test will begin with a paced walk and gradually increase in speed and/or incline up to your highest tolerable intensity. Heart rate, oxygen consumption, and Rating of Perceived Exertion (RPE) will be monitored throughout the test. The test will end when you feel you cannot continue or if the researcher determines the test should end due to maximal intensity being reached safety concerns or equipment malfunction. A typical test will last between 8-12 minutes.

The purpose of this study is to: 1) Demonstrate that reliable data can be obtained from the room calorimeter during 24-hour visits. 2) Determine the sensitivity of the room calorimeter system to better understand its abilities and constraints. 3) Compare energy expenditure values from the whole room calorimeter with energy expenditure and physical activity estimates from several research-grade accelerometers. 4) Identify the effects of a treadmill walk on the energetics (calorie requirements) of healthy young sedentary individuals. 5) Collect data that will assist us in hypothesis generation in the areas of exercise, exercise recovery, mechanical and metabolic interactions, and physical activity assessment.

We are looking for healthy sedentary males between 25 and 40 years of age with no physical or cognitive limitations that would affect their ability to be moderately active or to follow study instructions. You will receive $250.00 compensation for participating in the full study in the form of gift cards. This will be paid out as $25 for the 4.5hr session, $100.00 for each of the two 24hr stays, and $25 for the 2.5hr session. Are you still interested in partaking in the study?

Y/N

We are recruiting sedentary individuals for this study before we move on it is important to see if you meet those criteria. Have you been completing more than 30 minutes of moderate physical activity three times a week?

Y/N

Also, this study will require you to stay in our room calorimeter, which is about 13'x11' in size, and spend time inside our MR scanner (with your head outside). If you are claustrophobic or uncomfortable being confined to one room for 24 hours then you should not participate in this study. Additionally, you will be asked not to consume caffeine the days of your study visit. Are you still interested in participating?

Y/N

Screened by: _____ Date: _____ Study: _____ Status: _____

Script to read before issuing the PARQ

Before you officially enroll in this research study, I will be asking you to complete a Physical Activity Readiness Questionnaire (PAR-Q). It should take no more than 5 minutes to complete. This questionnaire is a screening tool that will ask you questions about your health history to determine your eligibility for participation in the study.

If you are determined ineligible to participate, your completed questionnaire will be destroyed. If you are determined eligible to participate, your completed questionnaire will be retained. We will protect your information contained in the PAR-Q as confidential information safeguarding it from unauthorized disclosure. Only research personnel will have access to the information contained in your PAR-Q.

Screened by: _____ Date: _____ Study: _____ Status: _____

PAR-Q

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)

Name_____ **Date**_____

	Question	Yes	No
1	Has your doctor ever said that you have a heart condition and that you should only perform physical activity recommended by a doctor?		
2	Do you feel pain in your chest when you perform physical activity?		
3	In the past month, have you had chest pain when you were not performing any physical activity?		
4	Do you lose your balance because of dizziness or do you ever lose consciousness?		
5	Do you have a bone or joint problem that could be made worse by a change in your physical activity?		
6	Is your doctor currently prescribing any medication for your blood pressure or for a heart condition?		
7	Do you know of any other reason why you should not engage in physical activity?		
8	Do you have any chronic pain or injuries (ankle, knee, hip, back, shoulder, etc.)? (If yes, please explain.) _____ _____		
9	Have you had any surgeries, in the past six months? (If yes, please explain.) _____ _____		
10	Has a medical doctor ever diagnosed you with a chronic disease, such as coronary heart disease, coronary artery disease, hypertension (high blood pressure), high cholesterol or diabetes? (If yes, please explain.) _____ _____		

Screened by: _____ Date: _____ Study: _____ Status: _____

TELEPHONE SCREENING FORM: RCP

1) Name_____

2) Phone # (Circle preferred contact): Best time/day to contact:_____

a. Home _____ Message? Yes No _____

b. Cell _____ Message? Yes No _____

3) Email: _____

4) Age: _____ Sex: _____ Height: _____ Weight: _____ BMI: _____*(calculate)*

5) Has your weight changed by more than ±5 pounds in the past two months? _____

6) How did you find out about this study? _____

7) Have you ever participated in a research study before? _____

a. Are you currently participating in any other research studies right now?_____

i. If yes, describe:_____

ii. End date: _____

8) Current health status (general)_____

9) Do you have any physical limitations?_____

10) Are you pregnant?_____

11) Do you, or have you ever had, any of the following:

a. Stroke?_____

b. Peripheral vascular disease?_____

c. Cardiac disease?_____

d. Pulmonary disease?_____

e. Neurological disease?_____

f. Arthritis in the lower leg?_____

Have you used ambulatory devices during the last month (i.e., ankle-foot orthotic, cane, wheelchair, etc.)?

_____

12) Do you smoke or have you ever smoked before? _____ If yes, for how long? _____ Quit? ____

13) Do you have any allergic reactions? _____

Screened by: _____ Date: _____ Study: _____ Status: _____

14) Do you have any other significant medical history? (e.g. hypertension, CAD, etc.)

_____

15) Current medications:

Drug Name	Classification	Dosage	Frequency	Duration	Prescribed for?

a. Do you take any Statins (if yes, please describe)?_____

b. Do you take any beta-blockers, sedatives, tranquilizers, or other medication that may impair physical function? _____

i. If yes, please describe:_____

16) Current physical activity level (regular exercise, none, athlete, etc.)

_____

17) Has your Doctor ever told you not to exercise? _____

a. If yes, please describe:_____

18) What was the date of your last doctor's visit? _____

19) Is fatigue a problem for you? _____ Leg fatigue? _____

20) What type of transportation will you be using? _____ Will you need a parking pass? _____

21) Would you like to be contacted again for future studies (circle one)? Yes No

22) Do you have any food allergies or dietary preferences (e.g. vegan etc.)?

_____

_____

23) Do you have a University LogIn for the Wi-Fi

24) Comments:_____

_____

_____

Go through Magnetic Resonance Safety Questionnaire

Screened by: _____ Date: _____ Study: _____ Status: _____

MR Safety Screening Questionnaire

Participant's Name (print): _____ **Today's date:** _____

Study Name: _____ Investigator: _____ Participant ID: _____

Year of birth: _____ Gender: _____ Height: _____ Weight: _____

Please read the following questions carefully. It is very important for us to know if you have any **metal devices** or **metal parts** anywhere in your body. If you do not understand a question, please ask us to explain!

1. Yes ☐ No ☐ Do you get upset or anxious in small spaces (claustrophobia)?
2. Yes ☐ No ☐ Did you ever have an aneurysm clip implanted during brain surgery?
3. Yes ☐ No ☐ Do you have embolization coils (Gianturco) in your brain?
4. Yes ☐ No ☐ Do you have a Carotid Artery Vascular clamp?
5. Yes ☐ No ☐ Do you have a "shunt" (a tube to drain fluid) in your brain, spine or heart?
6. Yes ☐ No ☐ Do you have a Vagus nerve stimulator to help you with convulsions or with epilepsy?
7. Yes ☐ No ☐ Have you ever had metal removed from your eyes by a doctor?
8. Yes ☐ No ☐ Have you ever worked with metal? (For example in a machine shop)?
9. Yes ☐ No ☐ Do you have implants in your eyes? Have you ever had cataract surgery?
10. Yes ☐ No ☐ Do you wear colored contact lenses or permanent eye liner?
11. Yes ☐ No ☐ Do you have shrapnel or metal in your head, eyes or skin?
12. Yes ☐ No ☐ Do you have implants in your ear (like cochlear implants) or a hearing aid?
13. Yes ☐ No ☐ Do you wear braces on your teeth or have a permanent retainer?
14. Yes ☐ No ☐ Do you have a heart pacemaker or a heart defibrillator?
15. Yes ☐ No ☐ Do you have a filter for blood clots (Umbrella, Greenfield, bird's nest)?
16. Yes ☐ No ☐ Do you have any stents (small metal tubes used to keep blood vessels open)?
17. Yes ☐ No ☐ Did you ever have a device implanted in your body such as a nerve stimulators?
18. Yes ☐ No ☐ Do you have an implanted pump to deliver medication?
19. Yes ☐ No ☐ Do you have metal joints, rods, plates, pins, screws, nails, or clips in any part of your body?
20. Yes ☐ No ☐ Have you ever had a gunshot wound? Or a B-B gun injury?
21. Yes ☐ No ☐ Do you wear a patch to deliver medicines through the skin?
22. Yes ☐ No ☐ Do you have any devices to make bones grow (like bone growth or bone fusion stimulators)?
23. Yes ☐ No ☐ Do you have unremoved body-piercing or a tattoo?
24. Yes ☐ No ☐ Have you ever had any surgery? Please list all:

FOR WOMEN

25. Yes ☐ No ☐ Do you use a diaphragm, IUD, or cervical pessary?
26. Yes ☐ No ☐ Do you think there is any possibility that you might be pregnant?

IMPORTANT INSTRUCTIONS: Before entering the Magnet Room, you must remove all metallic objects including hearing aids, dentures, partial plates, keys, beeper, cell phone, eyeglasses, hair pins, barrettes, jewelry including body piercing jewelry, watch, safety pins, paperclips, money clip, credit cards, bank cards, magnetic strip cards, coins, pens, pocket knife, nail clipper, tools, clothing with metal fasteners, and clothing with metallic threads in the material.

I attest that the above information is correct to the best of my knowledge. I have read and understand the entire contents of this form and have had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

Participant Signature: _____ **Date:** _____

MR Operator Signature: _____ **Date:** _____

APPENDIX C

VO_2 PEAK TEST PROTOCOL

BSU/Bruce Ramp Time Protocol

Increment	*Time*	*Speed*	*Grade*	*Increment*	*Time*	*Speed*	*Grade*
1	0:00	1.7	0.0	33	10:40	4.0	15.2
2	0:20	1.7	1.3	34	11:00	4.1	15.4
3	0:40	1.7	2.5	35	11:20	4.2	15.6
4	1:00	1.7	3.7	36*	11:40	4.2	16.0
5	1:20	1.7	5.0	37	12:00	4.3	16.2
6	1:40	1.7	6.2	38	12:20	4.4	16.4
7	2:00	1.7	7.5	39	12:40	4.5	16:6
8	2:20	1.7	8.7	40	13:00	4.6	16.8
9*	2:40	1.7	10.0	41	13:20	4.7	17.0
10	3:00	1.8	10.2	42	13:40	4.8	17.2
11	3:20	1.9	10.2	43	14:00	4.9	17.4
12	3:40	2.0	10.5	44	14:20	5.0	17.6
13	4:00	2.1	10.7	45*	14:40	5.0	18.0
14	4:20	2.2	10.9	46	15:00	5.1	18.0
15	4:40	2.3	11.2	47	15:20	5.1	18.5
16	5:00	2.4	11.2	48	15:40	5.2	18.5
17	5:20	2.5	11.6	49	16:00	5.2	19.0
18*	5:40	2.5	12.0	50	16:20	5.3	19.0
19	6:00	2.6	12.2	51	16:40	5.3	19.5
20	6:20	2.7	12.4	52	17:00	5.4	19.5
21	6:40	2.8	12.7	53	17:20	5.4	20.0
22	7:00	2.9	12.9	54*	17:40	5.5	20.0
23	7:20	3.0	13.1	55	18:00	5.6	20.0
24	7:40	3.1	13.4	56	18:20	5.6	20.5
25	8:00	3.2	13.6	57	18:40	5.7	20.5
26	8:20	3.3	13.8	58	19:00	5.7	21.0
27*	8:40	3.4	14.0	59	19:20	5.8	21.0
28	9:00	3.5	14.2	60	19:40	5.8	21.5
29	9:20	3.6	14.4	61	20:00	5.9	21.5
30	9:40	3.7	14.6	62	20:20	5.9	22.0
31	10:00	3.8	14.8	63*	20:40	6.0	22.0
32	10:20	3.9	15.0				

*Increments are at same time, speed, and grade as the standard Bruce protocol.

APPENDIX D

PARTICIPANT 7 EPOC FIT DATA ADJUSTMENT FOR PHONE USE

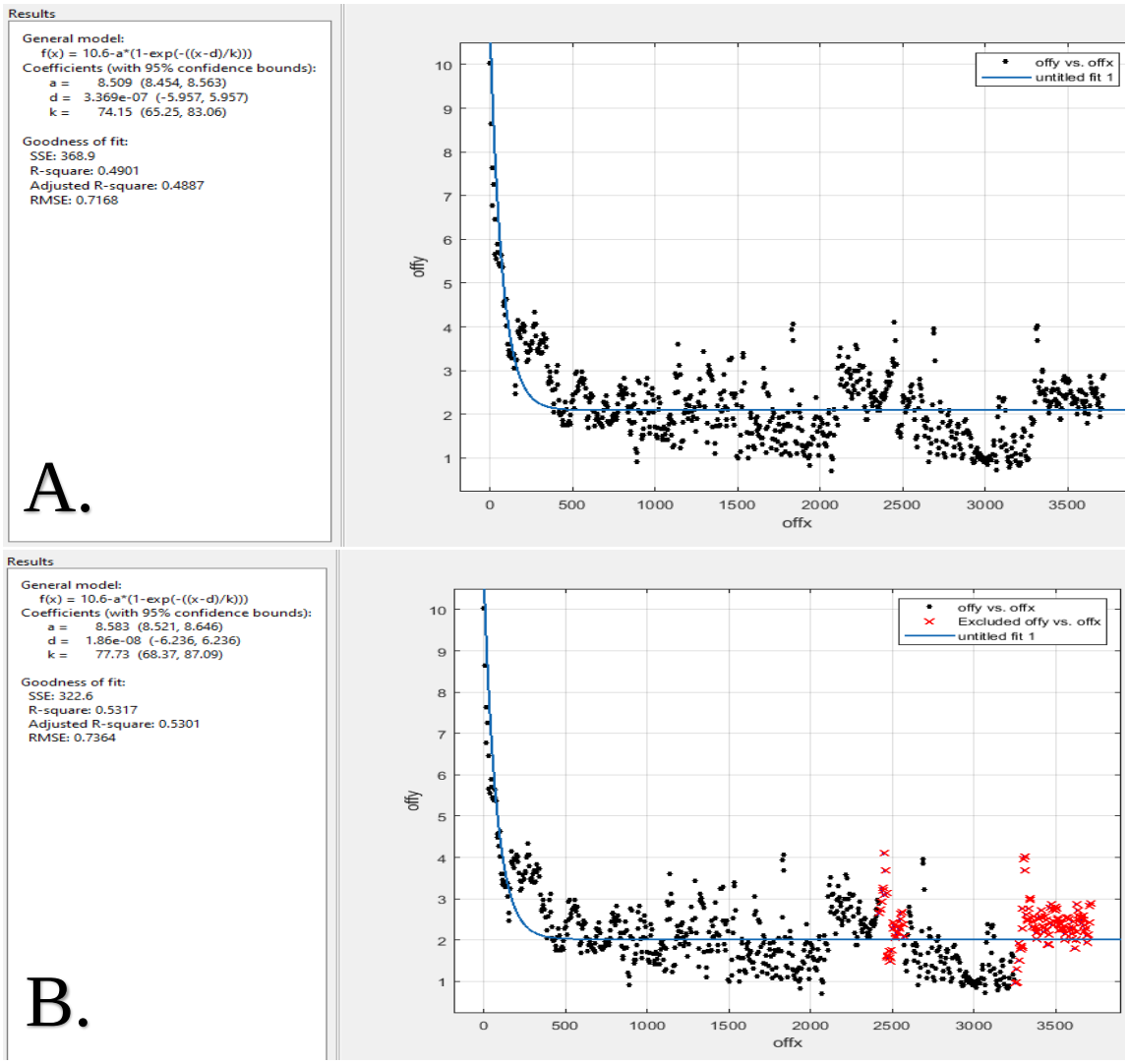


Figure 42. Screenshot of the curve fitting analysis for 30MTW EPOC of participant 7. Data are VO_2 fit over time from a single participant (pt7). Offy, volume of oxygen consumption in $mL \cdot kg^{-1} \cdot min^{-1}$; offx, time in seconds; 30MTW, 30-min treadmill walk.

A.) Unadjusted fit of EPOC.

B.) Adjusted fit EPOC. Data points during documented times when the participant was observed to be holding a cellular phone high in front of their face (red "x's") were excluded from the fit. The results from this fit were used for analysis in this case. It is likely that phone use in this manner occurred with earlier participants, as well. However, the exact times of this use were not documented, and therefore, those data were not removed from the analysis.

APPENDIX E

CORRELATION COEFFICIENTS AND P-VALUES FROM PEARSON'S, SPEARMAN'S, AND KENDALL'S TESTS

Dependent Variable	ΔPCr vs.					
	Pearson R	P-value	Spearman Rho	P-value	Kendall tau	P-value
ΔPCr (mM)	1.00	1.00	1.00	1.00	1.00	1.00
Oxygen Deficit (s)	0.68	0.09	0.46	0.30	0.33	0.38
Oxidative Capacity (s^{-1})	-0.73	0.04	-0.62	0.11	-0.43	0.18
RER L23	0.75	0.05	0.75	0.07	0.62	0.07
RER L5	0.80	0.03	0.71	0.09	0.52	0.14
RER L1	0.76	0.05	0.70	0.09	0.59	0.09
EPOC (s)	0.57	0.18	0.75	0.07	0.62	0.07
VO2 Peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	-0.38	0.35	-0.52	0.20	-0.36	0.28
VO2 Peak Lean ($mL \cdot kg^{-1}$ lean$\cdot min^{-1}$)	-0.61	0.10	-0.62	0.11	-0.43	0.18

Dependent Variable	Oxidative Capacity vs.					
	Pearson R	P-value	Spearman Rho	P-value	Kendall tau	P-value
ΔPCr (mM)	-0.73	0.04	-0.62	0.11	-0.43	0.18
Oxygen Deficit (s)	-0.24	0.60	-0.18	0.71	-0.14	0.77
Oxidative Capacity (s^{-1})	1.00	1.00	1.00	1.00	1.00	1.00
RER L23	-0.65	0.11	-0.64	0.14	-0.43	0.24
RER L5	-0.76	0.05	-0.68	0.11	-0.52	0.14
RER L1	-0.90	0.01	-0.74	0.07	-0.59	0.09
EPOC (s)	-0.74	0.06	-0.64	0.14	-0.43	0.24
VO2 Peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	0.23	0.58	0.14	0.75	0.21	0.55
VO2 Peak Lean ($mL \cdot kg^{-1}$ lean$\cdot min^{-1}$)	0.63	0.09	0.64	0.10	0.43	0.18

EPOC, excess post-exercise oxygen consumption; RER, respiratory exchange ratio; RER L23, average RER over the last 23 min of walk; RER L5, average RER over the last 5 min of walk; RER L1, average RER of the last 1 min of walk; VO_2, volume of oxygen consumption.

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