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# Incorporating Excess Post-exercise Oxygen Consumption into Accelerometer Energy Expenditure Estimation Algorithms

A Master's Thesis Presented by

Nicholas M. Remillard

# MASTER OF SCIENCE

September 2022

Department of Kinesiology

University of Massachusetts Amherst

# Incorporating Excess Post-exercise Oxygen Consumption into Accelerometer

# **Energy Expenditure Estimation Algorithms**

A Thesis Presented

By

# NICHOLAS M. REMILLARD

Approved as to style and content by:

John Sirard, Chair

Gwenael Layec, Member

Wouter Hoogkamer, Member

Richard Van Emmerik, Department Chair Department of Kinesiology

### Abstract

Incorporating Excess Post-exercise Oxygen Consumption into Accelerometer Energy Expenditure Estimation Algorithms

SEPTEMBER 2022

Nicholas Remillard, B.S., University of Massachusetts Amherst

M.S., University of Massachusetts Amherst

Directed by: John Sirard, PhD

Accelerometers are objective monitors of physical activity (PA) that can be used to estimate energy expenditure (EE). Most accelerometer EE estimation equations are based on steady-state data and do not consider excess post-exercise oxygen consumption (EPOC) after exercise. **PURPOSE**: To quantify the error in accelerometer EE estimates due to EPOC after varying durations of high-intensity treadmill running. METHODS: Nine young, healthy, recreationally active males participated in three study visits. Visit 1 included a treadmill VO<sub>2</sub> peak test to determine the treadmill speed correlating to 80% VO<sub>2</sub> peak for visits 2 and 3. Visit 2 included a seated 20-min baseline and three short (30s, 60s, 120s) vigorous treadmill running bouts each followed by 20 minutes of seated rest. Visit 3 included a supine 60-min baseline and a 30-min treadmill running bout followed by 3 hours of supine rest. Twelve EE estimation equations each using either a non-dominant wrist or right hip ActiGraph GT3X+ accelerometer were compared to the true EE measured by the Parvomedics TrueOne 2400 indirect calorimeter. **RESULTS:** The Freedson 1998 EE estimation equation overestimated EE during the 20min postexercise period after each exercise bout (mean kCals [95% CIs]; 30s: 19.3 [11.4, 27.2], 60s: 16.6 [8.5, 24.7], 120s: 13.4 [5.74, 21.1], 30min: 15.1 [6.69, 23.5]). The Crouter 2009 branching algorithm underestimated EE during the 20min post-exercise period after each exercise bout

(mean kCals [95% CIs]; 30s: -8.59 [-10.6, -6.62], 60s: -11.6 [-13.7, -9.38], 120s: -15.0 [-18.1, -11.8], 30min: -11.0 [-14.3, -7.77]), but was partially corrected by adding in the measured EPOC. **CONCLUSION**: Estimated EE during lying or seated rest from linear accelerometer equations was heavily dependent on the y-intercept of the equation, which represents the estimated resting EE of the wearer, with the Crouter calibration study being the only one to directly measure resting EE. More sophisticated approaches, like the Crouter 2009 and newer machine learning algorithms, have better potential to more accurately estimate EE across various activity types. New accelerometer EE estimations should include resting in their calibration protocols in order to more accurately estimate EE during rest.

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#### Chapter 1:

# Introduction

#### **1.1 Background**

Accelerometers are versatile and widely accepted as objective monitors of physical activity (PA). In practice, accelerometers are commonly used in large epidemiological studies for measurement of PA in free-living settings. Although PA (defined as movement produced by skeletal muscle that results in energy expenditure; EE) is an important measure related to better health, EE is believed to be a more specific physiological measure more strongly linked to mortality and disease risk factors (Hamilton et al., 2007; Mbalilaki et al., 2010). However, direct measures of EE such as direct calorimetry, indirect calorimetry, and doubly-labelled water are currently not feasible in large studies. Direct and indirect calorimeters are often limited to laboratory settings, expensive, and require more extensive staff training than accelerometers. Mobile indirect calorimeter carts can wirelessly transmit data to a computer over short distances, but the mask and unit on participants are cumbersome, limiting range of motion and visibility. Thus, accelerometers may be the best alternative to direct measures of EE due to their objectivity, feasibility, and affordability.

Modern triaxial accelerometers measure the acceleration due to movement of the device along three planes of motion. The raw acceleration from the ActiGraph accelerometer is translated into "activity counts" representing the magnitude of acceleration measured over a unit of time. These activity counts have shown good correlation with measured energy expenditure during ambulatory activities (Freedson et al., 1998). Many researchers have investigated the relationship between activity counts

and energy expenditure using linear correlation, developing linear models to predict energy expenditure from the accelerometer output (Brooks, A. G., Gunn, S. M., Withers, R. T., Gore, C. J., & Plummer, 2005; Freedson et al., 1998; Hendelman, D., Miller, K., Baggett, C., Debold, 2000; Leenders et al., 2003; Nichols et al., 2000; Sasaki et al., 2011; Yngve, A., Nilsson, A., Sjostrom, M., & Ekelund, 2003).

However, common linear-based EE estimation equations, like the Freedson equation used with the research-grade ActiGraph accelerometer, have been found to underestimate EE when applied to settings outside the laboratory (Crouter et al., 2006; Lyden et al., 2011; Imboden et al., 2018). Similarly, commercially available activity trackers from companies such as Nike, Fitbit, and Garmin also underestimate EE, potentially harming wearers' progress toward their exercise and weight management goals (Morris et al., 2019; Evenson et al., 2020; O'Driscoll et al., 2020). Taken together, research and commercial wearables tend to underestimate EE, and are highly variable among individuals (O'Driscoll et al., 2020).

These inaccuracies occur because accelerometer EE estimation algorithms have been primarily developed on steady-state ambulatory data (Freedson et al., 1998; Sasaki et al., 2011; Hildebrand et al., 2014). For example, participants may be walking, jogging, and running on a treadmill at three distinct speeds while they wear an accelerometer and their oxygen consumption is measured. A model using accelerometer output and other variables (i.e. body weight) is then developed to estimate EE as measured by the indirect calorimetry system.

In addition to studying the link between EE and disease risk at a population level, accurate EE measurement at an individual level is important for intervention studies, clinicians, athletes, and recreational exercisers to help meet physical and nutritional needs. Accurate and precise field estimates of EE are needed to better understand the relationship between EE and disease, to help athletes better quantify their programming, to help people lose weight, and satisfy the recreational exerciser that relies on technology to track their energy demands.

#### **1.2 Limitations to Accelerometer-based Energy Expenditure Estimation**

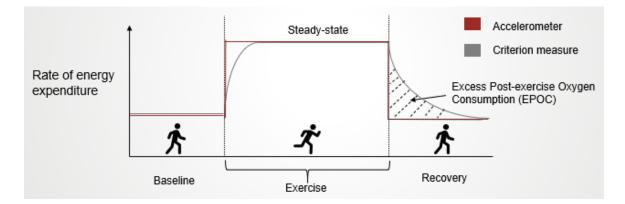
Accelerometers can provide researchers with quantifiable PA information, but because accelerometers do not measure physiological data directly, they rely on predictive algorithms. These algorithms are often based on samples of data no larger than 50 participants that typically consist of healthy adults with normal BMIs. In addition, these algorithms are based solely on motion data – current research-grade accelerometers do not collect other physiological data. Some commercial activity trackers measure heart rate via photoplethysmography , which has become more reliable over time (Falter et al., 2019). Energy expenditure estimation would likely improve with more physiological data, but there is no clear consensus on which data those are (Ainslie, Reilly & Westerterp, 2003). Even if more data beyond motion could be collected, researchers should use the fewest number of sensors possible to minimize participant burden, especially in unsupervised free-living settings. Further advances in wearable technology may be needed before it is feasible to collect different types of physiological data with one device on a large scale.

Current PA levels in large studies are measured in categories of activity intensity: sedentary, light, moderate, and vigorous (CDC, 2020). These binned PA levels are estimated from accelerometer output, often in the form of ActiGraph counts (device

proprietary unit) or ENMOs (Euclydian Norm Minus One) (Van Hees et al., 2013). But EE estimation equations (converting counts or ENMOs to kCals or VO<sub>2</sub>) based on linear regressions tend to underestimate EE in free-living settings at the group and individual levels (Leenders et al., 2006). These regression equations with several predictive factors (i.e. body weight, gender) may work for equations predicting broad activity intensity categories, but not for determining exact EE as a continuous outcome. Despite these drawbacks, current EE estimation equations accurately estimate energy expenditure during steady-state, ambulatory activities. Finding ways to expand these predictive algorithms to free-living settings would be beneficial to researchers, clinicians, coaches, athletes, and consumers alike.

#### 1.3 Excess Post-Exercise Oxygen Consumption (EPOC) as a source of error

One commonly used method in the development of accelerometer EE estimation algorithms is to only capture the steady-state period of an activity bout, excluding the first few and last few minutes of data. The result is an equation that can accurately estimate the EE of someone doing a steady-state ambulatory activity that lasts at least several minutes, but that cannot accurately estimate the EE of someone participating in interval-like activities or someone participating in a short bout of very intense activity (Lyden et al., 2011). The raised metabolism that remains after exercise, or excess postexercise oxygen consumption (EPOC), is not captured by motion data. Because the accelerometer only measures motion, the accelerometer data reflects a low intensity activity although EE remains elevated from the previous bout(s) of exercise. This EPOC period is a potential source of error that could contribute to the underestimation of EE often seen in studies using accelerometer estimation equations. Reducing this error could significantly improve accelerometer EE estimates, providing increased clarity on relationships among PAEE, health, and disease.



**Figure 1**: The rate of energy expenditure (EE) predicted by the accelerometer follows the pattern of the wearer's movement over time, as opposed to the criterion measure (indirect calorimetry) that can capture the individual's elevated metabolism during recovery after the termination of an exercise bout.

#### **1.4 Aims and Hypotheses**

Accurate field estimations of energy expenditure (EE) work well for steady-state ambulatory activities and can be improved by expanding their application through new calibration studies. There is a critical need for new accelerometer EE estimation algorithms that consider EPOC to remove underestimation bias for athletes and exercisers wanting the most accurate information to meet their fitness goals and for researchers seeking to understand EE's relationship with disease risk. Our long-term goal is to develop more accurate and feasible accelerometer EE estimation algorithms. The overall objective of this study is to pave the way for reducing EPOC as a source of error for accelerometer-based EE estimations. Our hypothesis is that an accelerometer estimation algorithm incorporating EPOC will not be significantly different from the criterion measure. The rationale for this study is that many accelerometer EE estimation algorithms have been developed and validated during steady state aerobic activities but accuracy is reduced when extrapolated to free-living activities and total daily EE. Incorporating EPOC estimations into EE estimations would help to eliminate underestimation biases observed in many studies, and which may be especially exaggerated in highly active individuals who perform relatively large amounts of high intensity activity producing prolonged periods of EPOC.

We will test our central hypothesis and address the two <u>specific aims</u> below by having participants undergo a vigorous treadmill run like that of a 10km training run, and several short bouts of vigorous intensity activity. A portable indirect calorimetry device and multiple accelerometers will be worn simultaneously during all exercise.

1. Quantify the EPOC magnitude after vigorous exercise sessions that vary in duration, including short durations simulating free-living activity (30s, 60s, 120s) and a longer bout of structured exercise (30min).

Using portable indirect calorimetry to measure EE, we hypothesize that, holding intensity constant, EPOC magnitude will increase proportionally with exercise duration.

2. Incorporate measured EPOC into accelerometer EE estimates.

Averaging the EPOC magnitude from exercise sessions across participants, we hypothesize that accelerometer EE estimates that include EPOC magnitude will not be significantly different from criterion measured EE, while estimates that do not include EPOC magnitude will significantly underestimate EE.

If our hypothesis is correct, our results would show that further improvements in accelerometer EE estimation algorithms are needed, and the methods developed in this study for including EPOC in estimation models can be expanded upon. For active individuals exercising regularly, incorporating an estimate of EPOC magnitude after a given exercise session would help correct the current underestimation of research-grade accelerometers and many consumer wearables. Based on the current proposed work, future studies can develop comprehensive EPOC prediction models on varying types of exercise intensities, durations, and exercise modalities for further versatility. Increasing the accuracy of EE estimation algorithms will strengthen studies using these estimation methods to connect PAEE and disease, improving our understanding of PAEE's relationship to obesity, cardiovascular disease, and metabolic disorders, and other diseases.

#### Chapter 2:

## Literature Review

The purpose of this thesis is to provide the first steps in eliminating EPOC bias from accelerometer-based energy expenditure estimation algorithms. Therefore, this literature review begins with a description of how indirect calorimetry is used to measure energy expenditure (EE), then reviews the application of accelerometers to measuring PA and EE, summarizes the mechanisms of EPOC, and identifies gaps in our knowledge that will be addressed by this thesis.

Understanding the difference between PA and EE is crucial to this literature review. Physical activity, defined as a behavior produced by skeletal muscle that requires energy expenditure, is important to humans' physical and mental health. Generally, decreasing levels of PA are associated with increased risk of disease, but more specific data, such as EE, is needed to better understand relationships between type and intensity of PA with various diseases. National guidelines focus on time spent in varying levels of PA intensity rather than EE goals per week because of the difficulty in obtaining reliable EE estimates. Valid and reliable measures of EE are needed for use in population-wide studies to determine these specific relationships between PA and disease.

#### 2.1 Measuring Energy Expenditure using Indirect Calorimetry

Indirect calorimetry, measuring oxygen input and carbon dioxide output, is considered the most accurate method to measure EE next to direct calorimetry (Gupta et al., 2017; Mtaweh et al., 2018). Although EE can be expressed in VO<sub>2</sub>, EE is often measured in kCals, or kilocalories, representing energy that can be measured as heat. The abbreviated Weir equation (1949) is still the most common equation to convert oxygen intake (VO<sub>2</sub>) and carbon dioxide output (VCO<sub>2</sub>) to kCals (Gupta et al., 2017):

kcals/min =  $3.9 \text{ L/min O}_2 + 1.1 \text{ L/min CO}_2$ .

To derive this equation, Weir used previously established energy values (in kCals) for the metabolism of each macronutrient (Table 1). Weir concluded that 1 liter of oxygen consumed generates 3.941 kCals and 1 liter of carbon dioxide produced generates 1.106 kCals. Additionally, the original equation included a protein correction factor that necessitated obtaining a urine sample to measure nitrogen levels. Assuming that 12.3% of total kCals produced come from protein metabolism, about 1% of the energy given in the Weir equation would be deducted, and so even for someone with a large protein intake, the maximum error of the Weir equation without the protein correction factor would be 2-3% (Matarese et al., 1997).

	Carbohydrate	Protein	Fat
B.Q. Kg.cal. per litre of O <sub>2</sub> Vol. of O <sub>2</sub> metabolizing	$1 \\ 5.047 \\ x \\ x$	0·802* 4·463* <i>y</i>	0·718† 4·735† z
	ewy modified by L Cuthbertson (1931 7).		

TABLE 1. Symbols and numerical values used in discussing the metabolism of carbohydrate, protein and fat

**Table 1**: Energy required to metabolize each macronutrient (Weir, 1949)

Researchers measuring EE using indirect calorimetry commonly use the Weir equation without the protein correction factor due to difficulty in obtaining urinary nitrogen data. The abbreviated Weir equation, measuring EE in kCals, requires only the subject's rate of  $O_2$  consumed and rate of  $CO_2$  produced. Open-circuit systems, which can be air-tight rooms or mobile metabolic carts, are the most common type of indirect calorimeter using paramagnetic or galvanic  $O_2$  sensors and infrared  $CO_2$  sensors (Mtaweh et al., 2018). Open-circuit systems measure the concentration of  $O_2$  and  $CO_2$ , gas volume, temperature, and time (Matarese et al., 1997; Mtaweh et al., 2018). Subjects using the device breathe room air and expire into a sampling system, which then vents the expired air back into the room. The difference between inspired and expired gas concentrations (namely of  $O_2$  and  $CO_2$ ) and the rate of ventilation is used to determine  $VO_2$ .

Gas collection systems can be breath-by-breath or a mixing chamber. Breath-bybreath devices measure gas exchange at the mask the individual wears and average data over time, avoiding problems of incomplete mixing of gases, unstable inspired O<sub>2</sub> fraction, and the effects of water vapor (Matarese et al., 1997). Breath-by-breath analyses can typically measure in time intervals as small as 5 seconds. Some metabolic carts contain a small mixing chamber that is quickly flushed during high intensity exercise, and so can obtain data in small time intervals similar to a breath-by-breath system.

Mixing chamber systems direct expired gas into a chamber where analyzers sample the gas at select intervals. Many whole-room calorimeters include a separate mixing chamber and take longer than breath-by-breath systems for expired gases to mix properly. For this reason, large mixing chambers are better for longer periods of metabolic testing that include steady-state periods.

#### 2.1.2 Indirect Calorimetry Limits

Indirect calorimeters currently cannot be used in free-living settings or studies wishing to look at EE over a prolonged period. Indirect calorimeters can only be used in controlled settings under the supervision of a researcher trained to set up and calibrate the device. In addition, even wireless mobile carts have a limited telemetry range of up to about 1000m (CareFusion, CA). Thus large population-wide studies and researchers wishing to capture EE over several days in free-living settings cannot feasibly use indirect calorimeters. These researchers must rely on EE estimates from questionnaires, and more recently, accelerometers, which have been validated against indirect calorimetry.

#### 2.2 Accelerometer Energy Expenditure Estimation

Subjective measures like questionnaires, diaries, and interviews are prone to various types of error, difficult to validate, and are not internally consistent or reliable (LaPorte et al., 1985). For example, recall surveys are limited by recall bias and memory limitations, including participants' subjective experiences of exercise intensity. The validity and reliability of questionnaires are not established enough to be considered sensitive to small but important differences in PA between groups or over time (LaPorte et al., 1985). Objective monitors, such as accelerometers, provide an alternative that is more reliable easier to validate against a criterion measure than subjective measures.

The LSI (large-scale integrated motor activity monitor) preceded accelerometers as one of the first objective monitors and had severe limitations: the mercury switches in the device were insensitive to low and high levels of activity, and only measured the quantity of movement (Patterson et al., 1993). Early prototypes of accelerometers looked to be more promising than LSIs as potential objective monitors for use in large studies because of their better sensitivity to low and high levels of activity and the ability to measure intensity of activity in addition to the quantity (LaPorte et al., 1985). In addition, acceleration of the human body and energy expenditure (EE) measured by indirect calorimetry are well correlated during locomotion (Reswick et al., 1978). Early accelerometers, such as the Caltrac, were calibrated on oxygen consumption data and designed to output the wearer's METs (metabolic equivalent) (Wong et al., 1981; Montoye et al., 1983). The Caltrac's estimated EE output had a strong linear relationship with measured VO<sub>2</sub>, but consistently over and underestimated EE during walking and running, respectively (Pambianco et al., 1990; Haymes & Byrnes, 1993). Despite these limitations, the Caltrac proved accelerometers were at least sensitive to small changes in walking speeds and could reliably differentiate between a range of speeds (Washburn et al., 1988; Nichols et al., 1992).

The Caltrac accelerometer's major limitations included the inability to select device sensitivity based on a study's protocol and its output being in MET scores (Sallis et al., 1993; Miller et al., 1994; Richardson et al., 1995). Redmond and Hegge (1985) developed what would later be known as the ActiGraph accelerometer, incorporating adjustable sensitivity measures in the device and producing outputs in movement scores representing changes in PA. The ActiGraph's movement scores were able to differentiate

intensity between a range of daily activities with high reliability and had strong correlations with measured VO<sub>2</sub> in laboratory and simulated free-living settings (Patterson et al., 1993). These initial findings, improved upon the performance of the Caltrac, and indicated that the ActiGraph could be used in the field where indirect calorimetry is impractical.

The ActiGraph became the most popular research-grade accelerometer due to its affordability and versatility (Troiano et al., 2014; Montoye et al., 2018). The ActiGraph's output can be raw acceleration signal or in counts, a proprietary unit representing movement within a selected epoch window (ActiGraph Corp, Pensacola, Fl). Both the Freedson 1998 equation (Freedson et al., 1998) and Hildebrand 2014 equations (Hildebrand et al., 2014) are among the most common accelerometer EE estimation equations (Montoye et al., 2020), Freedson making use of count output and Hildebrand raw acceleration output.

The Freedson equation is one of the earliest equations for converting ActiGraph counts into PA levels and kCals (Freedson et al., 1998). Freedson and colleagues had participants walk and run at three speeds on a treadmill, comparing the ActiGraph counts with VO<sub>2</sub> to create intensity thresholds based on METs. These count thresholds were designed to estimate broad MET-based PA intensity categories: light (<3.00 METs), moderate (3.00-5.99 METs), hard (6.00-8.99 METs), and very hard (>8.99 METs) to account for the national focus on time spent in categories of PA intensity level. These categorical PA intensity thresholds (instead of continuous EE) have been applied to ActiGraph data from large epidemiological studies to relate time spent in each intensity category to disease risk (Loprinzi et al., 2012) while continuous EE output has not.

Freedson et al. (1998) developed a separate equation using the treadmill data to estimate kCals. The ActiGraph counts at each treadmill speed was used as a predictor to estimate kCals, measured by indirect calorimetry, to develop a linear regression model on 35 of the 50 participants, then tested on the remaining fifteen. Only the steady-state data, defined as the last 3 minutes of the exercise period for each speed, was considered for the regression model.

kcal·min<sup>-1</sup> = 
$$(0.00094 * \text{cnts} \cdot \text{min}^{-1}) + (0.1346 * \text{mass in kg}) - 7.37418$$
  
(R<sup>2</sup> = 0.82, SEE = ± 1.40 kcal·min<sup>-1</sup>)

Other popular EE estimation algorithms have also only used steady-state data for their estimation equations, regardless of modelling technique used. Sasaki, John & Freedson (2011) used mean data from minutes 3-6 within their exercise bouts. Hildebrand et al. (2014) used minutes 2.5-4.5 from 5-minute long bouts for input in their model.

Sasaki, John & Freedson (2011) developed a regression equation to estimate METs (another form of EE, easily converted kCals) based on the vector magnitude of all three ActiGraph axes. Sasaki and colleagues performed a similar calibration study as Freedson, walking (4.8 and 6.4 kph) and running (9.7 and 12 kph) on a treadmill while wearing a hip-placed ActiGraph GT3X+ accelerometer and used an Oxycon Mobile indirect calorimeter as the criterion measure of EE. Thirty-six participants were included in the sample for the development of the EE estimation model:

METs = 
$$0.000863(VM3) + 0.668876$$
  
(R<sup>2</sup> =  $0.78$ , SEE =  $\pm 1.3$  METs)

Hildebrand et al.'s (2014) equations (Table 2) differ from other popular EE estimation equations because they utilized raw ActiGraph output at a 60Hz sampling rate in the form of ENMOS. ENMOS, or Euclidian norm minus one, were developed by removing the gravitational component of acceleration from the raw accelerometer data (Van Hees et al., 2013). Van Hees and colleagues created the ENMO as an accelerometer output accessible to all researchers across devices, so researchers would not be limited by the accelerometer manufacturer's proprietary algorithms. Hildebrand et al. used ENMO data from ActiGraph accelerometers placed on the wrist and on the hip to develop separate wrist-based and hip-based EE estimation linear regression models. Hildebrand et al.'s regression VO<sub>2</sub> output can be converted to kCals using the Weir equation or binned in MET-based PA intensity categories.

TABLE 3. Regression equations developed for the prediction of intensity (METs) from AG and GA placed on the hip and wrist, respectively, in adults and children. 95% CI for  $\alpha$ R<sup>2</sup> Equation 95% CI for B  $\dot{V}O_{2} = 0.0554 \text{ m}a + 6.67$ 0.0551-0.0557 6.61-6.72 0.81 Adults (n = 30)AG hip GA hip<sup>4</sup>  $\dot{V}O_2 = 0.0530 \text{ m}q + 6.86$ 0.0527-0.0533 6.81-6.92 0.79 AG wrist  $\dot{VO}_2 = 0.0320 \text{ mg} + 7.28$ 0.0318-0.0322 7.22-7.34 0.75 GA wrist  $\dot{VO}_2 = 0.0323 \text{ m}g + 7.49$ 0.0321-0.0325 7.43-7.54 0.76 Children (n = 30)AG hip  $\dot{V}O_2 = 0.0559 \text{ m}g + 10.03$ 0.0556-0.0563 9.96-10.10 0.78 GA hip<sup>4</sup>  $\dot{V}O_2 = 0.0498 \text{ m}g + 10.39$ 0.0495-0.0501 10.31-10.47 0.75 0.0353-0.0358 10.75-10.91  $\dot{V}O_{2} = 0.0356 \text{ m}a + 10.83$ 0.71 AG wrist  $\dot{V}O_2 = 0.0357 \text{ m}a + 11.16$ 11.08-11.24 GA wrist 0.0355-0.0360 0.72

 $\dot{V}O_2$  is expressed in milliliters per kilogram per minute (mL  $O^2 \cdot kg^{-1} \cdot min^{-1}$ ).

**Table 2**: Hildebrand ENMO-based EE estimation equations (Hildebrand et al., 2014)

Most research-grade accelerometer EE estimation algorithms only include the acceleration data, but some also included heart rate data in their EE estimation to address acceleration-only limitations (Rennie et al., 2000; Strath et al., 2001; Romero-Ugalde et al., 2017). Both Strath (2001) and Romero-Ugalde (2017) compared hip-worn accelerometer-only EE estimation models with their proposed combined accelerometer-HR EE estimation models, finding that the combined models more accurately predicted

EE. Other researchers have proposed that including more physiological data, such as HR, skin temperature, and sweat rate may improve EE estimation models (Ainslie, Reilly & Westerterp, 2003). A major concern regarding heart rate monitoring techniques is the increase in heart rate due to stress, heat, and stimulants; HR does not increase solely due to PA (Meijer et al., 1989). In addition, the HR monitors used in accelerometer-HR combination calibration studies are placed on the chest, which in large epidemiological studies adds to participant burden and introduces more potential user error and non-compliance with the wear protocols. Although wrist-based HR measures have become more accessible in recent years, validation studies report inconclusive evidence that wrist-based HR measures are as accurate as traditional and clinical measures and often cite the need for more research to make valid conclusions (Sartor et al., 2018; Cadmus-Bertram et al., 2017).

#### 2.3 Excess Post-exercise Oxygen Consumption (EPOC)

Originally termed  $O_2$  debt, the phenomenon now known as EPOC has been studied since the early 1900s beginning with Hill, Long & Lupton (1924)'s lactic acid theory. As metabolic testing became more prevalent, researchers noticed the lag in VO<sub>2</sub> at the onset of exercise and the continued elevation of VO<sub>2</sub> after the termination of exercise (Gaesser & Brooks, 1984). The traditional theory asserted that the lactic acid built up during exercise was transformed into glycogen during recovery, powered by oxygen. This glycogen would be used to replenish the glycogen stores used during the lag in VO<sub>2</sub> at the onset of exercise. As time went on, more evidence that lactate removal and EPOC are not temporally or causally linked was identified, based on the finding that

lactic acid reached its highest concentration after the EPOC period and that metabolism of lactate after exercise depends on the biochemical profiles of muscle fibers, which varies between and within species (early experiments were conducted on frogs) (Margaria, Edwards & Dill, 1933; Gaesser & Brooks, 1984). In addition, lactate metabolism is always occurring after exercise, and so cannot be attributed with a particular phase of EPOC.

After lactate was determined not to be the primary contributor to EPOC, researchers have identified several other metabolic processes that cause EPOC in humans, such as the rephosphorylation of creatine and ADP, an increase in catecholamine levels that indirectly elevates mitochondrial respiration, and activation of ATP pumps working to reestablish sodium and potassium gradients (Brooks et al., 1971; Gaesser & Brooks, 1984). At a broader level, the return of tissue temperature to baseline levels is closely associated with the return of VO<sub>2</sub> to baseline; increased tissue temperature decreases mitochondrial efficiency, requiring more oxygen for the same quantity of ATP production (Hagberg, Mullin & Nagle, 1980). The total effect of all the biological variables contributing to EPOC can be simplified: metabolism will return to baseline levels when all the factors affecting mitochondrial respiration have returned to normal levels. Despite the lingering uncertainty surrounding physiological mechanisms of EPOC, many researchers have sought increased understanding of the effect of different exercise protocols on the EPOC magnitude and time coefficients.

#### **2.3.2** Intensity and duration of activity affect EPOC magnitude

EPOC can account for a significant number of calories contributing to total daily energy expenditure (Knab et al., 2015). The EPOC magnitude is also a clear marker of recovery and is more sensitive than other recovery measures, such as heart rate recovery (Mann et al., 2014). The EPOC magnitude is primarily affected by the intensity and duration of an exercise session. Physical activity or exercise at a threshold of at least 50-60% VO<sub>2</sub> max is necessary to induce a detectable EPOC that will last several hours. At a given intensity, there is a linear relationship between EPOC magnitude and exercise duration (Børsheim & Bahr, 2003). But the relationship between EPOC magnitude and exercise duration changes according to varying intensities, as shown below in Figure 2. Notably, exercise of an intensity below 50% VO<sub>2</sub> max did not cause an increase in EPOC magnitude. Above the 50% VO<sub>2</sub> max threshold, duration is often found to have a positive linear relationship with EPOC magnitude. Intensity, holding duration constant, has an exponential relationship with EPOC magnitude (Gore & Withers, 1990; Laforgia, Withers & Gore, 2006).

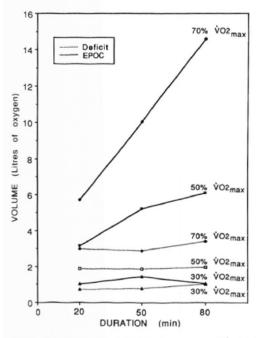


Fig. 1. The mean  $O_2$  deficit and excess post-exercise oxygen consumption (EPOC) of the nine subjects resulting from 20, 50 and 80 min of treadmill exercise at 30, 50 and 70% maximal  $O_2$  consumption ( $\dot{V}_{O_{2max}}$ )

Figure 2: EPOC values by intensity and duration (Gore & Withers, 1990)

EPOC can also be broken down into a fast and a slow component, first observed by Hill, Long & Lupton (1924), and recognized to have separate time constants reflecting their respective slopes by Margaria, Edwards & Dill (1933). These two phases are characterized by different properties and affected by exercise intensity and duration separately (Knuttgen, 1970; Ozyener et al. 2001). The fast component is characterized by a steep slope, during which heart rate and VO<sub>2</sub> decrease rapidly toward baseline (often within minutes). The slow component is characterized by a less steep slope that gradually returns to baseline over a longer period of time (up to and over an hour, depending on exercise intensity and duration). When EPOC is broken down into its fast and slow components, Hagberg, Mullin & Nagle (1980) found intensity significantly affected the fast component. Duration significantly affected the slow component, but only at the highest intensity studied (80% of VO2 max; Figure 3).

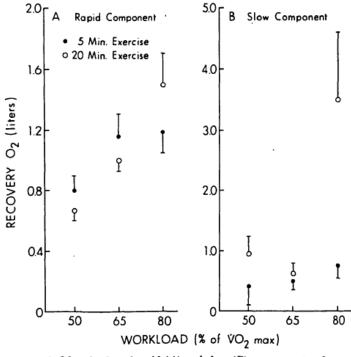


FIG. 1. Magnitudes of rapid (A) and slow (B) components of recovery O<sub>2</sub>. Only significant difference (P < 0.01) between 2 exercise durations at same work intensity was found in slow component at 80% of  $\dot{V}O_{2}$  max.

Figure 3: EPOC values by intensity and duration (Hagberg, Mullin & Nagle, 1980)

This finding is consistent with other studies that find EPOC increasing in magnitude as duration increases, keeping intensity constant (Gore & Withers, 1990; Laforgia et al., 1997, Phelain et al. 1997). Mann et al. (2014) found EPOC magnitude to be significantly greater at 80% VO<sub>2</sub> max than at 70% VO<sub>2</sub> max (effect size: 0.9), but not between 70% VO<sub>2</sub> max and 60% VO<sub>2</sub> max (effect size: 0.5), suggesting that there is a

nonlinear relationship between exercise intensity and EPOC magnitude when duration is kept the same. This literature demonstrates two major points, (1) that EPOC consists of two distinct components, the fast and slow phases, and (2) that exercise intensity and duration have varying relationships to each phase and to the overall EPOC magnitude.

The effect of intensity and duration on EPOC magnitude can be seen in Table 3, which summarizes measured EPOC magnitudes according to different intensities and durations of exercise across seven studies. Intensity and duration demonstrate a dose-response relationship among this data: EPOC magnitude increases with duration and with intensity.

Author	Year	Participant Number	Participant VO2 max (ml/kg/min)	Modality	Intensity (% VO2 max)	Duration (minutes)	EPOC Duration Measured (hours)	Behavior during EPOC	Measurement Method	EPOC (L O2)	Estimated kCals (L O2 * 5 kCals/L O2)
Mann	2014	20 M	60.2 (4.8)	Treadmill	60%	20	0.25	Seated	Cont Ind Cal	6.09*	30.45
Mann	2014	20 M	60.2 (4.8)	Treadmill	70%	20	0.25	Seated	Cont Ind Cal	6.73*	33.65
Mann	2014	20 M	60.2 (4.8)	Treadmill	80%	20	0.25	Seated	Cont Ind Cal	7.86*	39.3
Smith	1993	8 M	50.3 (4.7)	Cycling	40%	30	0.52	Bedrest	Disc Ind Cal	16.3 (0.03)	81.5
Smith	1993	8 M	50.3 (4.7)	Cycling	50%	30	0.70	Bedrest	Disc Ind Cal	22.1 (0.03)	110.5
Smith	1993	8 M	50.3 (4.7)	Cycling	70%	30	0.79	Bedrest	Disc Ind Cal	28.1 (0.06)	140.5
Gore	1990	9 M	63.0 (5.7)	Treadmill	50%	20	8	Bedrest	Disc Ind Cal	3.14 (3.58)	15.7
Gore	1990	9 M	63.0 (5.7)	Treadmill	50%	50	8	Bedrest	Disc Ind Cal	5.19 (3.83)	25.95
Gore	1990	9 M	63.0 (5.7)	Treadmill	50%	80	8	Bedrest	Disc Ind Cal	6.10 (4.22)	30.5
Gore	1990	9 M	63.0 (5.7)	Treadmill	70%	20	8	Bedrest	Disc Ind Cal	5.68 (4.89)	28.4
Gore	1990	9 M	63.0 (5.7)	Treadmill	70%	50	8	Bedrest	Disc Ind Cal	10.04 (3.26)	50.2
Gore	1990	9 M	63.0 (5.7)	Treadmill	70%	80	8	Bedrest	Disc Ind Cal	14.59 (2.94)	72.95
Bahr	1987	6 M	54.1 (1.5)	Cycling	70%	20	12	Bedrest	Disc Ind Cal	5.1 (1.2)	25.5
Bahr	1987	6 M	54.1 (1.5)	Cycling	70%	40	12	Bedrest	Disc Ind Cal	6.8 (1.7)	34
Bahr	1987	6 M	54.1 (1.5)	Cycling	70%	80	12	Bedrest	Disc Ind Cal	14.4 (1.2)	72
Phelain	1997	8 F	47.4 (1.5)	Cycling	50%	77.8	3	Bedrest	Cont Ind Cal	4.8 (1.6)	24
Phelain	1997	8 F	47.4 (1.5)	Cycling	75%	50.9	3	Bedrest	Cont Ind Cal	9.0 (1.7)	45
Laforgia	1997	8 M	69.2 (4.0)	Treadmill	70%	30	9	Bedrest	Disc Ind Cal	6.9 (3.8)	34.5
Knab	2011	10 M	43.5 (12.8)	Cycling	72%	45	14.2	Seated	WRC	38.0 (14.3)	190

**Table 3**: Reported EPOC magnitudes by study.

\*EPOC values are estimated from reported data and include male and female participants \*\*kCals estimated using modified Weir equation (kCals = 5 \* L O<sub>2</sub>) Disc Ind Cal: Discontinuous Indirect Calorimetry; Cont Ind Cal: Continuous Indirect Calorimetry; WRC: Whole-Room Calorimeter

Notably, the number of calories EPOC contributes varies greatly according to exercise intensity and exercise duration. Table 3 displays a range from 15.9 kCals (intensity: 50% VO<sub>2</sub> max; duration: 20 minutes; Gore & Withers, 1990) to 190.0 kCals (intensity: 72% VO<sub>2</sub> max; duration: 45 minutes; Knab et al., 2011). Also, the duration for which EPOC was measured after exercise likely affects the reported EPOC magnitude values (Gore & Withers, 1990: 8 hours; Knab et al., 2011: 14.2 hours). Understanding how long EPOC should be measured after a given exercise session would be possible if more studies reported when or if post-exercise metabolic rate returned to baseline levels. Of the studies in Table 3, only Smith & Naughton (1993) reported the time EPOC took to reach a non-significantly different rate from baseline after their 30-minute cycling session, defined as VO<sub>2</sub> returning to  $\pm 12$ mL O<sub>2</sub>/min for five consecutive 2-minute measurement periods (minutes; mean (SD); 40% VO<sub>2</sub> max: 31.2 (1.9), 50% VO<sub>2</sub> max: 42.1 (2.6), 70% VO<sub>2</sub> max: 47.6 (2.9)).

Though not reported in the studies included in Table 3, there is evidence that fitness, measured by VO<sub>2</sub> max, has an inverse relationship with EPOC magnitude for a given intensity and duration (Matsuo et al., 2012). Researchers studying EPOC should take the fitness level of their participants into consideration when designing protocols and making conclusions.

#### **2.3.4** Predicting EPOC

Varying relationships among multiple factors complicates attempts to predict EPOC based on a given exercise session. One study by Jones et al. (2020) attempted to use heart rate as a proxy for modeling EPOC, but found that post-exercise heart rate and oxygen consumption were poorly correlated in their sample ( $\mathbb{R}^2 < 0.01$ ). Jones et al. used data from a small and homogeneous sample of 14 active and healthy college students, which may have contributed to their poor correlation. In contrast, a conference abstract by Rusko et al. (2003) developed a proprietary model for predicting the EPOC magnitude based on heart rate data before and during an exercise session, using data from 48 peerreviewed articles (158 different subjects, with duration ranging from 2 to 90 minutes and intensity ranging from 18% to 108% of VO<sub>2</sub> max). Rusko et al.'s EPOC prediction model yielded an  $\mathbb{R}^2$ -value of 0.79 utilizing only R-R interval measurement (heart rate) validated on a sample of 32 healthy adults across a range of intensities (40%, 70%, and 100% VO<sub>2</sub> max). Because Rusko et al.'s EPOC prediction model is proprietary, other researchers cannot directly replicate their modeling techniques.

Jung et al. (2021) developed a multiple linear regression model to predict EPOC magnitude across different exercise protocols. Variables considered for the model were sex, age, height, weight, BMI, fat-free mass, fat mass, percent body fat, and heart rate. Only fat-free mass and heart-rate sum were included in the final model, yielding R<sup>2</sup> values ranging from 0.831 to 0.955. These results, in combination with Rusko et al.'s (2003) model, suggest that heart rate during exercise is an important factor in predicting EPOC. Including anthropometric data in EPOC prediction models, such as fat-free mass, may improve a model's ability to predict EPOC (Rusko:  $R^2 = 0.79$  with no anthropometric data vs. Jung:  $R^2 > 0.83$ ).

Jones, Rusko, and Jung's models predicted the EPOC magnitude based on physiological and anthropometric data. None of these models used motion data, such as accelerometer data, to aid in their prediction and did not model the time coefficients of the EPOC curve or separate the fast and slow components. Therefore, additional research in this area could take advantage of wearable accelerometer devices to estimate EPOC using acceleration data alone or in combination with physiological variables such as heart rate, aerobic fitness, and body composition. The inclusion of additional physiological variables creates feasibility concerns about free-living or consumer applications because of limitations in obtaining these data outside of a laboratory setting.

#### 2.4 Accelerometers Inaccurately Estimate Free-living Energy Expenditure

Estimated EE from free-living or unstructured accelerometer data should be interpreted with caution because most algorithms were calibrated on small samples performing ambulatory activities in lab-based settings (Freedson et al., 1998; Crouter et al; 2006; Sasaki et al., 2011; Hildebrand et al., 2014). Several studies have compared the most widely used EE equation, the Freedson equation (1998), to indirect calorimetry in semi-structured settings designed to simulate free-living scenarios, with the purpose of determining the Freedson equation's validity to estimate EE outside of ambulatory activities (Crouter, Churilla & Bassett, 2006; Lyden et al., 2011; Bai et al., 2016; Imboden et al., 2018).

Crouter, Churilla & Bassett (2006) tested several ActiGraph EE estimation algorithms against indirect calorimetry among various activities, including sedentary tasks, walking at various speeds, running at various speeds, playing basketball, racquetball, washing windows and dishes, raking, vacuuming, and sweeping. The ActiGraph equations tended to underestimate EE during non-ambulatory tasks and overestimated sedentary tasks (Figure 4).

Fig. 5 Measured (Cosmed K4b2) and predicted energy expenditure (Actigraph kcal equations) for 18 different activities, in ascending order of energy expenditure. Lower case letters in parenthesis after each activity name denotes which equation is significantly different (P < 0.05) from the Cosmed K4b2 for that activity (a Actigraph Freedson kcal equation; b Actigraph Brooks kcal equation; and c Actigraph Brooks kcal equation with body mass). Error bars are absent for clarity

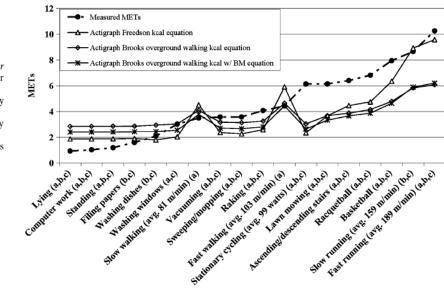
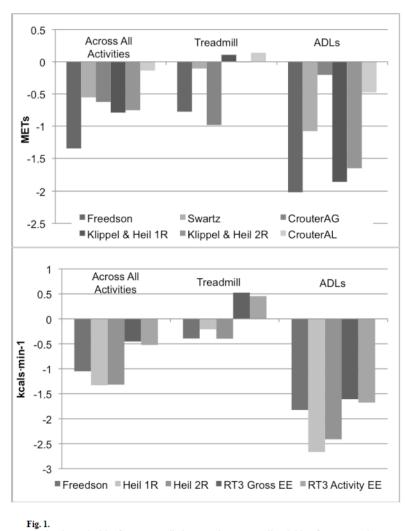


Figure 4: METs per method for each activity (Crouter, Churilla & Bassett, 2006)

Lyden et al. (2011) had participants perform two routines in random order: treadmill activities (at three speeds) and five activities of daily living (ADLs), of which two of the five were chosen from a list of 14 activities representing common household and leisure time activities, all at a self-selected pace. Similar to Crouter, Churilla & Bassett (2006), accelerometer EE estimations underestimated during all ADL activities (Figure 5).



Panel 1 shows the bias for MET prediction equations across all activities, for TRDs and ADLs. Panel 2 shows the bias for kcal prediction equations across all activities, for TRDs and ADLs. All predicted EE values were significantly different from indirect calorimetry except when treadmill activities were analyzed using the Klippel and Heil (2003) 2R MET equation

Figure 5: Bias values of accelerometer-predicted EE (Lyden et al., 2011)

Imboden et al.'s (2018) protocol allowed participants to choose 12 activities from a list of 21, and were able to select the pace, duration, and order of activities. Participants wore an ActiGraph accelerometer plus multiple consumer monitors (AG: ActiGraph, FZ: Fitbit Zip, FO: Fitbit One, FF: Fitbit Flex, JU: Jawbone UP24). All accelerometer EE estimation algorithms (AG using the Freedson equation) underestimated the entire 80minute session (see Table 4).

PA monitor	Steps		Energy expenditure (	kcal)	Active minutes	
	Mean±SD	%Bias	Mean±SD	%Bias	Mean±SD	%Bias
Subgroup not cycling (n=9)						
Criterion	3399±585	n/a	304±47	n/a	29±8	n/a
FZ	2954±709*†	-13	282±47*	-12	n/a	n/a
FO	2935±717*†	-14	245±64†	-19	n/a	n/a
FF	2977±686†	-12	274±61*	-16	12±10*†	-62
UL	2838±897†	-17	241±70†	-25	23±7†	-26
AG	2822±685†	-17	226±37†	-26	25±6	-25

\*Indicates significant difference from the AG.

†Indicates significant difference from criterion measure.

AG, ActiGraph GT3X+; FF, Fitbit Flex; FO, Fitbit One; FZ, Fitbit Zip; JU, Jawbone UP24; n/a, not applicable; PA, physical activity.

Table 4: Mean and %	bias of accelerometer-	predicted EE (1	Imboden et al., 2018)	)
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Bai et al. (2016) developed a semi-structured protocol consisting of three components (20 minutes of sedentary activity, 25 minutes of self-selected treadmill walking or running, and 25 minutes of self-selected resistance exercise) and analyzed each component separately in addition to the whole protocol. The analysis of individual components allowed Bai et al. to conclude that the Freedson equation (denoted GT3X+ in Tables 5 and 6) overestimated EE during sedentary and aerobic activities and underestimated EE during resistance exercise, in agreement with Crouter, Churilla & Bassett (2006) and Lyden et al. (2011). But the Freedson equation did not significantly differ from indirect calorimetry for the whole protocol, potentially due to the cancellation of error between the three components of the protocol (Tables 5 and 6). Because all participants followed the three components in the same order and were allowed to selfselect exercise intensities, EPOC resulting from the aerobic component may have impacted results of the resistance exercise component. If there was an EPOC significantly

raising the metabolic rate, the existing overestimation found during the resistance component would have resulted at least in part to EPOC. If EPOC resulting from the aerobic exercise was controlled, Bai et al. may have found greater overestimation by the monitors during the resistance exercise component.

TABLE 1	. Total EE	and me	n bias	between	OM	and	the	activity	monitors.
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	п	Total EE (kcal)	Limits of Agreement	Mean Bias	95% Confidence Interval
OM	52	316.8 (81.6)			
GT3X+"	49	305.9 (78.7)	-104.3 to 128.1	11.9 (59.3)	-5.1 to 28.9
BMC	49	351.0 (98.9)	-122.7 to 51.7	-35.5 (44.5)	-48.3 to -22.7
FBF	52	337.2 (79.5)	-126.3 to 85.5	-20.4 (54.0)	-35.4 to -5.4
JU24	51	290.7 (99.0)	-101.3 to 147.5	23.1 (63.5)	5.2 to 40.9
MS	50	395.5 (123.0)	-243.3 to 98.5	-72.4 (87.2)	-97.2 to -47.6
NFS"	52	274.5 (60.9)	-65.7 to 150.3	42.3 (55.1)	27.0 to 57.7

Data are presented as mean (SD).

The Work-Energy Theorem and Freedson's 98 regression were used to estimate activity EE in GT3X+. "Added estimated resting EE.

			EE (kcal)				AE		RE	
	п	SB	AE	RE	MB	ES	MB	ES	MB	ES
OM	52	36.5 (8.8)	195.5 (63.5)	84.8 (23.2)						
GT3X+ <sup>a</sup>	49	42.6 (20.4)	216.5 (67.0)	46.8 (15.5)	-5.8 (21.5)	0.42	-20.5 (47.5)	0.32	38.3 (25.4)	1.96
BMC	49	40.6 (7.8)	210.0 (68.8)	100.4 (35.7)	-3.9 (6.1)	0.49	-16.3 (30.3)	0.22	-15.4 (25.9)	0.53
FBF	52	31.0 (24.3)	247.9 (67.5)	59.5 (15.5)	5.5 (22.5)	0.34	-51.9 (44.1)	0.80	25.5 (20.6)	1.31
JU24	51	25.5 (5.0)	231.4 (96.6)	38.0 (9.9)	11.0 (8.1)	1.60	-35.3 (58.4)	0.45	47.2 (22.1)	2.82
MS	50	31.1 (7.2)	309.2 (120.4)	55.9 (12.2)	5.7 (7.6)	0.68	-109.5 (83.8)	1.24	30.8 (21.7)	1.63
NFS"	52	29.9 (5.4)	185.0 (57.2)	58.2 (16.3)	6.5 (7.1)	0.94	9.2 (43.7)	0.17	26.3 (22.9)	1.34

Data are presented as mean (SD).

The Work-Energy Theorem and Freedson's 98 regression were used to estimate activity EE in GT3X+. \*Added estimated resting EE.

AE, aerobic exercise; ES, effect size; MB, mean bias; RE, resistance exercise; SB, sedentary behavior.

Table 6: EE Mean Bias per Protocol Component. Freedson equation denoted as GT3X+. (Bai et al., 2016)

#### **2.5 Literature Review Summary**

Accelerometers have become the preferred tools to measure PA and EE because of their objectivity and reliability. Past researchers have modeled accelerometer output against indirect calorimetry to estimate EE, which works well in the settings in which they were calibrated. Most often, these calibration studies take place during steady-state ambulatory activities such as walking and running. Researchers have begun to apply accelerometer EE estimates (via intensity categories, such as light, moderate, and vigorous) to large-scale epidemiological studies because EE is theorized to be closely associated with disease risk. Accelerometer estimates of EE as a continuous output (such as kCals/day) currently underestimate EE and should be improved before applied to freeliving settings like epidemiological studies. One potential reason contributing to accelerometer-based underestimation of EE is the exclusion of EPOC from accelerometer-based EE estimates.

## Chapter 3:

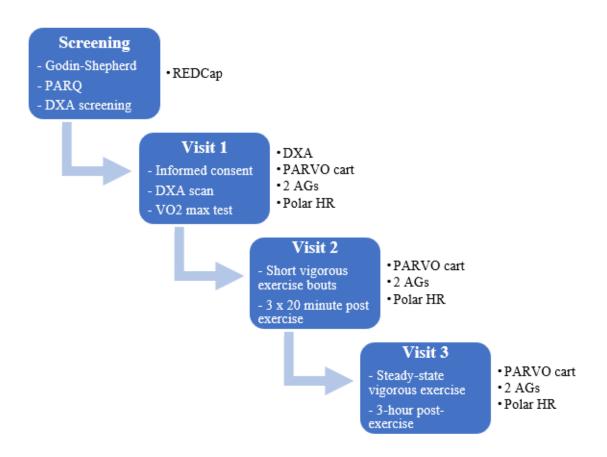
## Methods

### 3.1 Study Overview

The purpose of this thesis was to incorporate measured EPOC into accelerometer EE estimation algorithms to improve their accuracy. We focused on active, healthy college-aged males performing vigorous exercise sessions that vary in duration, including short durations simulating free-living activity (30s, 60s, 120s) and a longer bout of structured exercise (30min). Oxygen consumption was measured using a metabolic cart during the short vigorous bouts the 30-minute bout. Oxygen consumption was measured for 3 hours after the 30-minute exercise session, allowing for measurement of exercise VO<sub>2</sub> and a majority of the EPOC magnitude due to both fast and slow components. The mobile cart allows for quantification of sensitive changes in the EPOC curve following exercise. The post-exercise VO<sub>2</sub> was compared to a pre-exercise VO<sub>2</sub> baseline measured at the beginning of each visit to quantify EPOC. Participants also wore hip and wrist accelerometers and a heart rate monitor for the duration of all protocols.

All participants completed three total visits at the Institute of Applied Life Sciences (IALS) inside the Center for Human Health and Performance (CH2P). Participants were instructed not to perform any exercise for 24 hours prior to the beginning of each visit. Each subsequent visit took place between 2 and 14 days of the prior visit. Visit 1 included consenting the participant, taking height and weight measurements, performing a DXA scan, and an incremental VO<sub>2</sub> peak test on a treadmill. At visit 2, participants performed vigorous exercise bouts of different durations (30 seconds, 1 minute, 2 minutes) at 80% vVO<sub>2</sub> peak (treadmill velocity at 80% VO<sub>2</sub> peak).

At visit 3, participants performed a baseline supine rest for 60 minutes, followed by a 30minute treadmill run at 80% vVO<sub>2</sub> peak and then a 3-hour supine rest.



**Figure 6: Visitation Flowchart -** Sequence of events and equipment needed for each visit. REDCap, Research Electronic Data Capture; DXA, dual-x-ray absorptiometry; AGs, ActiGraph accelerometers; HR, heart rate.

### 3.2 Data Collection

#### Participants

Ten young (18-30 years) males with normal BMI (18.0-24.9 kg/m<sup>2</sup>) were recruited for this study from the University of Massachusetts Amherst campus. Only males were included for this study because controlling for differences in metabolic rate due to the menstrual cycle were outside the scope and timeline of this project. Participants were recreationally active for two months prior to beginning the study. An active activity status was determined by a score of at least 24 points on the Godin-Shepherd Leisure Time questionnaire (Godin, 2011; Appendix B). Participants were healthy by self-report and free from any chronic disease (cardiovascular, pulmonary, neurological) and serious musculoskeletal injury in the lower extremities (including but not limited to; broken bones, ruptured or torn ligaments and tendons that required more than 6 months of rehabilitation or surgery) that may have impeded them from performing vigorous exercise. The Physical Activity Readiness Questionnaire, or PARQ (Warburton et al., 2011; Appendix D), was used to determine if participants were healthy to participate in exercise.

## Equipment

For criterion measures, the ParvoMedics TrueOne 2400 metabolic cart (Parvo cart; Salt Lake City, UT) and the Room Calorimeter at the University of Massachusetts's CH2P (MEI Research, St. Louis Park MN) were used to measure oxygen consumption (VO<sub>2</sub>), which can be converted to energy expenditure (calories). The Parvo cart was worn as a mask covering the nose and mouth connected to a gas collection tube. The Parvo cart is one of the research field's standard criterion measures for collecting laboratory-based oxygen consumption data in humans and has been validated for use in exercise protocols (Bassett et al., 2001). Participants were asked to fast for at least 4 hours prior to all testing involving the Parvo cart.

Before beginning any data collection with indirect calorimetry, devices need to be warmed up and calibrated. Both the flow transducer, measuring the amount of air flow, and the gas analyzer need to be calibrated once the gas analyzers have warmed up for 15-30 minutes. The flow transducer was calibrated before data collection sessions by manually injecting a known volume of air repeatedly through the device. The gas analyzers were calibrated before each measurement against standard reference gases of known concentration. Calibrations were considered complete when the device measured within a 3% error margin of the known gas volume and gas concentrations.

The GE Lunar iDXA (GE Healthcare, Madison, WI) in the CH2P is a valid and precise measurement of body composition (Hild, Oldroyd & Truscott, 2011) and was operated by certified technicians provided by the CH2P. Fat mass and fat-free mass (kg) were obtained from the iDXA and height and weight from the Seca 719/220 Scale and Stadiometer for use in descriptive statistics and for use in EPOC magnitude prediction models.

Participants wore one ActiGraph GT3X+ accelerometer (ActiGraph LLC, Pensacola FL) on the right iliac crest (hip) and another on the non-dominant wrist. The ActiGraph GT3X+ accelerometer is a research-grade triaxial accelerometer that measures raw accelerations and is widely used in the field. Motion data from the accelerometer was collected during all protocols and used in twelve accelerometer-based EE estimation algorithms (Table 7). A Polar heart rate monitor (Polar Electro) was used to measure heart rate, secured onto the participant with a chest strap. Heart rate data is included for potential future analyses (Jones et al., 2020; Jung et al., 2021; Rusko et al., 2003), but will not be included in the analyses of this project's aims. Participants wore the ActiGraph accelerometers and Polar heart rate monitor during all visits. The Parvo cart was utilized during all visits. The room calorimeter was utilized during visits 3 and 4 for one participant whose data was analyzed separate from the sample.

MET Predictions	Equations	Calibration activities
		2 walking speeds, 1 running
Freedson 1998	(0.000795*counts/min) + 1.439008	speed
		2 walking speeds, 2 running
Sasaki 2011	(0.000863*VM3) + 0.668876	speeds
	counts/min $\leq 8$ , EE = 1.0 MET; counts/min	
	>8 and CV counts/10s <=10, EE = 2.294275*(exp(0.00084679 *counts/10s)); or	
	$2.294275^{\circ}(exp(0.00084079^{\circ}counts/108)), of CV counts/10s > 10, EE = 0.749395 +$	
	(0.716431 * (ln(counts/10s))) - (0.179874 *	
	$(\ln(\text{counts}/10\text{s}))^2) + (0.033173 *$	lifestyle and ambulatory
Crouter 2010	(ln(counts/10s))^3)	activities, includes lying down
		lifestyle and recreational
Swartz 2000	(0.0006863*counts/min) + 2.606	activities
Brooks 2005	(0.000370*counts/min)-(0.012*BM) + 3.33	1 walking speed
		standing, 2 walking speeds and 1
Yngve 2003	(0.0008249*counts/min) + 1.136	running speed
Leenders 2003 hip	(0.0006*counts/min) + 2.240	5 walking speeds
		lifestyle and ambulatory
Hendelman 2000	(0.000638*counts/min) + 1.602	activities
	(0.00171*counts/min)+ (1.957*height)-	
Heil 2003	$(0.00171 \text{ counts/min})^{+} (1.937 \text{ neight})^{-}$ $(0.000631 \text{ counts/min} \text{ height})^{-} 1.883$	2 walking speeds
VO2 Predictions		
Hildebrand 2014		lifestyle and ambulatory
Hip	(0.0554*mg) + 6.67	activities
Hildebrand 2014		lifestyle and ambulatory
Wrist	(0.0320*mg) + 7.28	activities
		2 walking speeds, 1 running
Nichols 2000	(0.002545*counts/min) + 6.057359	speed
kCal/min		
Predictions	(0.00094*counts/min) + (0.1346*BM) -	2 wallting grouds 1 minning
Freedson 1998	(0.00094*counts/min) + (0.1346*BM) - 7.37418	2 walking speeds, 1 running speed
Brooks 2005	(0.000452*counts/min) + (0.051*BM) - 0.774	1 walking speed
Leenders 2003 hip	(0.00001*counts/min) + 0.0378	5 walking speeds
Leenders 2003 mp		
wrist	(0.00000646*counts/min) + 0.0495	5 walking speeds
	• • •	·

**Table 7**: ActiGraph EE estimation equations

\*all EE equations give output in units per minute; counts/min: ActiGraph axis 1 counts; VM3: vector magnitude of all three axes; mg: ENMO units; BM: body mass in kg; lifestyle activities: activities of daily living including household chores; recreational activities: leisure-time sports and structured exercises

Visit 1

Participants that were deemed eligible after the phone screening completed visit 1 at the Center of Human Health and Performance (CH2P) in the Institute of Applied Life Sciences on the University of Massachusetts Amherst campus. Study personnel read through the informed consent with the participant and answered any questions. Once consenting was complete, study personnel took height and weight measurements, in duplicate. Participants then underwent a DXA scan operated by trained and certified CH2P staff.

After completing the DXA scan, participants were led into the Exercise Training Room and fitted for a mouth-piece to use with the Parvo metabolic cart. Study personnel assisted the participant in putting on a Polar heart rate monitor using the chest strap and placing the ActiGraph accelerometers on the correct locations on the non-dominant wrist and right hip. Once all equipment was comfortably on the participant, the VO<sub>2</sub> peak test began. All treadmill controls and speed changes were performed by study personnel. This incremental treadmill VO<sub>2</sub> peak test has been previously used by Bartlett et al. (2011) with a similar young, active male population. The participant began with a 5-minute warm up period consisting of a 3-minute walk at 4.8 kph and 2-minute jog at 8.0 kph. After warm up, study personnel increased the treadmill speed to begin the VO<sub>2</sub> peak test.

The first part of the VO<sub>2</sub> peak test consisted of three 2-minute stages at 10 kph, 12 kph, and 14 kph (6.2 mph, 7.5 mph, and 8.7 mph). After completing the 14 kph stage, the treadmill was inclined by 2% in grade every 2 minutes until exhaustion (when the participant decided to stop the test). Reaching VO<sub>2</sub> max is defined as the highest VO<sub>2</sub> value obtained in a 10-second period when meeting the end-point criteria: (1) heart rate is

within 10 beats per minute of age-predicted maximum (220-age), (2) the respiratory exchange ratio (RER) is equal to or greater than 1.15, and (3) VO<sub>2</sub> plateaus despite an increase in workload. This test was considered a VO<sub>2</sub> peak (rather than max) test because the termination criteria consisted of the participant going to voluntary exhaustion. Once the VO<sub>2</sub> peak test was complete, study personnel removed equipment from the participant, sat the participant after a 5-minute walking cooldown, and monitored their condition. Gatorade was provided to the participant when testing was complete to rehydrate and help replenish electrolytes and carbohydrates.

The purpose of conducting the VO<sub>2</sub> peak test was to calculate the treadmill speed corresponding with 80% of each participant's VO<sub>2</sub> peak. After visit 1, the averages of the VO<sub>2</sub> values from the last minute of each of the first five stages (4.8, 8.0, 10.0, 12.0, 14.0 kph) of the VO<sub>2</sub> peak test were linearly correlated with the treadmill speed to calculate the treadmill speeds correlating to 70% and 80% VO<sub>2</sub> peak. The speed correlating with 80% VO<sub>2</sub> peak (80% vVO<sub>2</sub> peak) was used as the treadmill speed for all exercise during visit 2 and was used as the starting speed for visit 3. The speed correlating with 70% VO<sub>2</sub> peak (70% vVO<sub>2</sub> peak) was calculated to use as a minimum speed during visit 3 that participants should attempt to remain above for the duration of the run.

Visit 2

After completing visit 1, participants completed visit 2 between 2 and 14 days after visit 1. Visit 2 (short duration running bouts) occurred at the CH2P Exercise Training Room. Participants were asked to fast for at least 4 hours prior to this visit. At the beginning of the visit, study personnel fit the participant with the Parvo cart mask, Polar heart rate monitor, and ActiGraph accelerometers at the hip and wrist locations (same as visit 1). The participant first sat quietly for 20 minutes to establish a seated VO<sub>2</sub> baseline measurement.

After establishing a baseline measurement, study personnel increased the treadmill speed to the speed that corresponds to 80% of the participant's VO<sub>2</sub> peak (80%) vVO<sub>2</sub> peak) calculated using data from the VO<sub>2</sub> peak test during the first visit. Grade was not considered when calculating the treadmill speed corresponding to 80% VO<sub>2</sub> peak. The participant straddled the treadmill as research personnel increased the belt speed to 8.0 kph, then the participant was instructed to hop onto the moving belt. Speed was increased to 80% vVO<sub>2</sub> peak when the participant was jogging on the belt, and a stopwatch began when the participant reached the desired speed. This protocol was designed to isolate the short bout duration as much as possible while keeping participant safety in mind. This protocol was repeated for three separate short bouts; 30, 60, and 120 seconds. The order of bout duration was randomized and balanced across participants (six possible combinations were distributed among the participants, with four repeats). Study personnel instructed the participant to straddle the treadmill at the end of the bout while the belt came to a stop and to immediately sit for 20 minutes after each short bout. This process (straddle treadmill, run at 80% vVO<sub>2</sub> peak for short duration, sit for 20 minutes) was conducted three times for each short bout duration (30, 60, and 120 seconds).

A 20-minute EPOC measurement period following these short bouts was chosen for three reasons: (1) to capture the fast component and most of, if not all, the slow component of EPOC, (2) not to induce too much participant burden, (3) there was no

literature found on how long EPOC lasts after very short intense bouts of exercise. After measuring VO<sub>2</sub> for the third 20-minute EPOC period, study personnel removed the equipment from the participant. Total duration of this visit was about 100 minutes.

Visit 3

After completing visit 2, participants completed visit 3 between 2 and 14 days after visit 2. Visit 3 occurred at the Center of Human Health and Performance (CH2P) Exercise Training Room.

Researchers asked participants to use a mode of transportation that involves the least amount of physical activity as possible. Participants were instructed to not perform any exercise or strenuous activities the day prior and to maintain an overnight fast before the third visit (instructed not to consume breakfast, including caffeine).

Participants were asked to arrive in the morning around 7:00am to the Exercise Training Room. Upon arrival, study personnel fit the participant with the Polar heart rate monitor, the ActiGraph accelerometers at the hip and wrist locations and the Parvo mask just like in visits 1 and 2. Once fitted, participants began a supine VO<sub>2</sub> baseline measurement at 7:15am lasting 60 minutes. At 8:15am, the Parvo mask was removed from the participant, who was allowed a restroom break and a small drink of water. At this time the Parvo cart gas analyzers were recalibrated to prevent drift. After recalibration, participants began a 10-minute warm up on the treadmill. The first 5 minutes was a walking warm up at 4.8 kph, then 5 minutes of jogging at 8.0 kph. After the warm up, the exercise session began. Participants began the run at 80% vVO<sub>2</sub> peak

(the speed that corresponds with 80% of the participant's VO<sub>2</sub> peak) for 30 minutes. Participants were asked to begin at their 80% vVO<sub>2</sub> peak treadmill speed and instructed that they could decrease the speed if they felt it necessary in order to complete the run. Participants were asked to not decrease the treadmill speed below their 70% vVO<sub>2</sub> peak treadmill speed, as that would classify as terminating the data collection session. All treadmill speed changes were supervised by research personnel. The treadmill had a safety clip that can be attached to the participant; if the participant began to fall too far back on the treadmill, the cord would be pulled so that the treadmill belt immediately stops.

The treadmill belt was stopped at the end of the 30 minutes and the participant was asked to resume a supine resting position for 20 minutes. After 20 minutes, research personnel reset the Parvo gas analyzers to baseline using room air, which allows for the participant to continue wearing the Parvo mask and for the least disruption possible. After another 40 minutes (60 minutes after termination of exercise), the Parvo mask was removed for full recalibration while the participant was asked if they would like a drink of water or like to use the restroom. After this second recalibration, participants resumed supine rest for the remaining 2 hours. During all supine resting, participants were allowed to watch their choice of pre-approved television shows or movies (documentaries or something similar preferred) in order to prevent participants falling asleep and boredom.

## Visit 3 Timeline:

7:00am - participant arrived

7:15am - baseline supine rest began

8:15am - full recalibration and short break

8:20am - began exercise (10min warmup + 30min run)

9:00am - ended exercise, resumed supine resting position

9:30am – full recalibration and short break

10:35am - short recalibration (room air sampling) and optional break

12:05pm - finish session

### 3.3 Data Analysis and Statistics

Data were analyzed as a group across participants. Descriptive analyses were used to assess data distributions and analyses were specific to the data needs. A p-value below 0.05 was considered a significant difference. All data analysis was conducted in custommade R scripts (R Core Team, Vienna, Austria) using existing statistical packages.

**Aim 1:** *Quantify the EPOC magnitude after vigorous exercise sessions that vary in duration, including short durations simulating free-living activity (30s, 60s, 120s) and a longer bout of structured exercise (30min).* 

EPOC magnitude was calculated for each participant as the baseline  $VO_2$  subtracted from the total post-exercise  $VO_2$  value. The post-exercise period was defined between the termination of exercise and the end of the measurement period or return to baseline value (a return to within 95% confidence interval bounds of the baseline rate for more than five consecutive data points) if it occurred. To calculate time to return to baseline (TTB), each participant's raw VO<sub>2</sub> data from the Parvo metabolic cart was fit to a bi-exponential function (f(x) = a\*exp(b\*x) + c\*exp(d\*x)) in the cftool package in MATLAB (*MATLAB* (*MATLAB* (*Version 9.8.0.1380330 (R2020a) Update 2*, 2020) to extrapolate the long duration effect of the slow component if baseline was not reached before the end of the measurement period (Hagberg et al., 1980; Özyener et al., 2001). The TTB was defined as how long (in minutes) the modeled VO<sub>2</sub> took to reach the mean pre-exercise supine resting VO<sub>2</sub> value. We hypothesized that both the EPOC magnitude and TTB would increase with exercise duration.

#### Aim 2: Incorporate measured EPOC into accelerometer EE estimates.

The average EPOC magnitude across participants for each exercise bout was calculated using the methods described under Aim 1 and added to the ActiGraph EE estimation equations (Table 7). Equations with MET and VO<sub>2</sub> outputs were converted to kCals (using conversion factor 1 L  $O_2 = 5$  kCals) for comparability. The averaged ActiGraph EE estimates with and without EPOC were subtracted from the measured EE to calculate bias for both ActiGraph estimations. All data processing and statistics were conducted in R software (R Core Team, 2020). We hypothesized that (1) the bias of the ActiGraph EE estimates including EPOC would not be significantly different from zero, demonstrating that EE estimate without including EPOC would be significantly below zero, demonstrating underestimation of EE by the existing ActiGraph EE estimates.

#### Chapter 4:

# Results

All participants included in this thesis were young, healthy, recreationally active males. Participant characteristics are listed in Table 8.

Table 8: Participant Characteristics; RMR calculated from visit 3 pre-exercise baseline VO<sub>2</sub> (Mean ± SD)

Age (years)	Height (m)	Weight (kg)	BMI (kg/m^2)	VO2 peak (mLO2/kg/min)	80% vVO2 peak (kph)	70% vVO2 peak (kph)	BF%	FFM (kg)	RMR in mLO2/kg/min
20.9 <u>+</u> 2.9	1.8 <u>+</u> 0.1	75.4 <u>+</u> 5.3	24.4 <u>+</u> 1.0	50.9 <u>+</u> 3.8	12.7 <u>+</u> 1.1	11.2 <u>+</u> 1.0	16.3 <u>+</u> 4.2	60.7 <u>+</u> 6.2	3.5 <u>+</u> 0.3

Table 9 provides values for the EPOC magnitudes and TTBs after each exercise bout. The total post-exercise VO<sub>2</sub> magnitudes and EPOC (reported in both VO<sub>2</sub> and kCals) values significantly increased (p < 0.05) with each increase in exercise bout duration. A short increase in exercise duration, from 30 seconds to 60 seconds at 80% vVO<sub>2max</sub>, was enough to elicit a small but significant increase in EPOC EE (p = 0.04). Each of the short bout TTBs were significantly different from the long duration exercise TTB (p < 0.05), but none of the short bout TTBs were significantly different from each other (p > 0.05).

**Table 9**: EPOC magnitude (post-exercise  $VO_2$  – baseline  $VO_2$ ) and TTB averaged across participants during the post-exercise measurement period (Mean <u>+</u> SD)

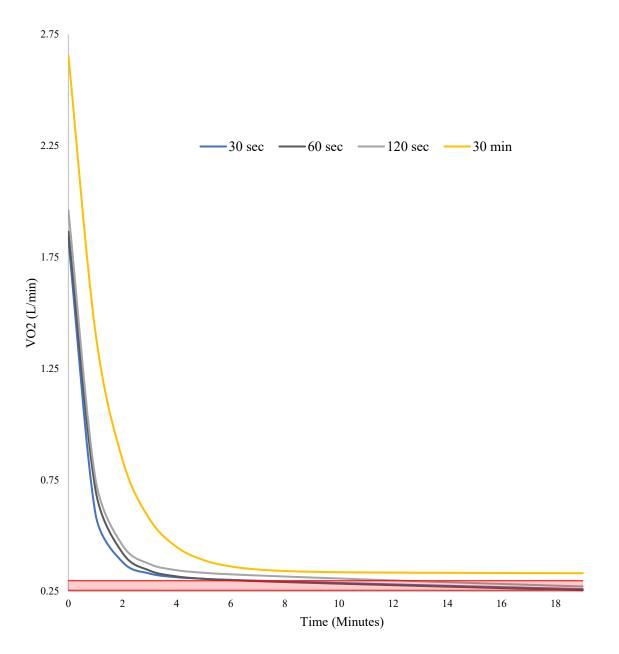
	Exercise Bouts					
	30s	60s	120s	30min		
Total Post-exercise VO <sub>2</sub> (L O <sub>2</sub> )	6.9 <u>+</u> 0.7**	7.1 <u>+</u> 0.8**	7.5 <u>+</u> 0.8**	61.0 <u>+</u> 7.3**		
EPOC (L O <sub>2</sub> )	1.1 <u>+</u> 0.5**	1.3 <u>+</u> 0.7**	1.7 <u>+</u> 0.7**	10.1 <u>+</u> 4.1**		
EPOC (kCals)	5.5 <u>+</u> 2.4**	7.3 <u>+</u> 3.1**	10.2 <u>+</u> 3.5**	50.4 <u>+</u> 20.4**		
Time to Return to Baseline (minutes)	13.3 <u>+</u> 5.1*	13.0 <u>+</u> 5.2*	19.0 <u>+</u> 7.8*	354.4 <u>+</u> 223.8**		

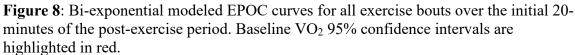
\*significantly different (p<0.05) from the 30min bout only

\*\*significantly different (p<0.05) from all other exercise bouts

Figure 8 graphically displays the bi-exponential EPOC curves for each exercise duration during the first 20 minutes of the post-exercise period. When averaging the modeled VO<sub>2</sub> values across participants, there is little difference between the time it took for VO<sub>2</sub> values to return to baseline levels (TTB) across the short-duration exercise bouts (13.3 minutes, 13.0 minutes, and 19.0 minutes for the 30sec, 60sec, and 120sec bouts respectively), but the EPOC magnitudes did increase significantly with each increasing bout duration (see Table 9). The TTB according to the biexponential model for the 30-minute exercise bout was 354.4 minutes on average, which extends beyond the x-axis of Figure 8 and the post-exercise measurement period (180 minutes after the termination of exercise).

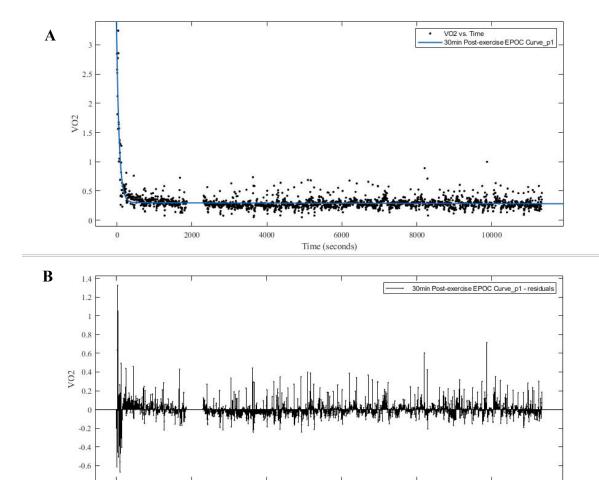
Table 10 contains the average biexponential coefficients and goodness of fit parameters across all participants for each condition (see supplementary Tables S1 and S2 for individual coefficients and parameters). Overall, the bi-exponential model demonstrated a good fit for the EPOC data during the post-exercise periods after the short bouts ( $R^2 = 0.89$ , 0.88, 0.91 for 30sec, 60sec, and 120sec respectively) but was not as good a fit for the post-exercise EPOC data after the 30-minute bout ( $R^2 = 0.66$ ). Figure 9 displays the fitted curve and its residuals over the raw data for participant 1 during the post-exercise period after the 30-minute run.





**Table 10**: Average biexponential model  $(f(x) = a^* \exp(b^*x) + c^* \exp(d^*x))$  coefficients and goodness of fit parameters for EPOC models for each exercise duration.

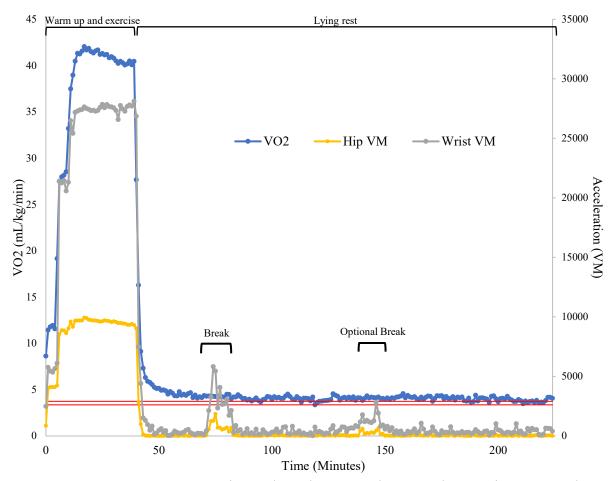
		30 sec	60 sec	120 sec	30 min
ţ	a	1.50	1.54	1.61	2.33
Model efficient	b	-0.03	-0.02	-0.02	-0.01
Model Coefficients	c	0.32	0.32	0.35	0.34
C	d	-1.91E-04	-2.05E-04	-2.29E-04	-1.55E-05
of ers	SSE	1.16	1.41	1.31	35.06
Goodness of Fit Parameters	R Squared	0.89	0.88	0.91	0.66
foodr Para	Adjusted R Squared	0.88	0.88	0.91	0.66
G Fit	RMSE	0.07	0.08	0.07	0.12



**Figure 9**: Example bi-exponential EPOC curve fitted over the 30-minute post-exercise period for participant 1. Panel A contains the fitted curve (see Table 10 for the fit parameters) with the raw  $VO_2$  data points. Panel B contains the residuals.

Time (seconds)

Figure 10 displays raw VO<sub>2</sub> data averaged across participants from the Parvo metabolic cart with time-synchronous acceleration data (vector magnitude, VM, was chosen to represent the sum of the acceleration signal from all 3 axes) from the hip and wrist ActiGraph accelerometers. In red is displayed the 95% confidence interval bounds of the averaged pre-exercise baseline VO<sub>2</sub> to compare to the raw VO<sub>2</sub> data (blue). Figure 10 includes continuous data from the warm up, the 30-minute exercise session, and the entire post-exercise period. There are two periods where the acceleration signals spike, denoting the times when participants were allowed to take a break to use the bathroom. During the breaks, participants were removed from the Parvo metabolic cart (and data during the break was extrapolated using linear regression from 10 minutes before and after the break for each participant) but the ActiGraph accelerometers remained on the participants.

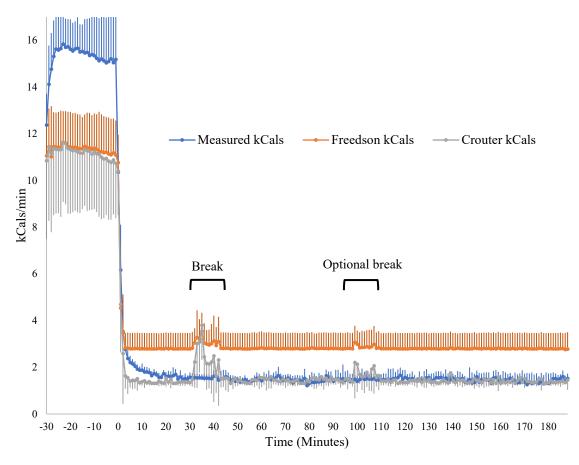


**Figure 10**: Raw oxygen consumption and accelerometer data over the 30-minute run and post-exercise period averaged across participants.

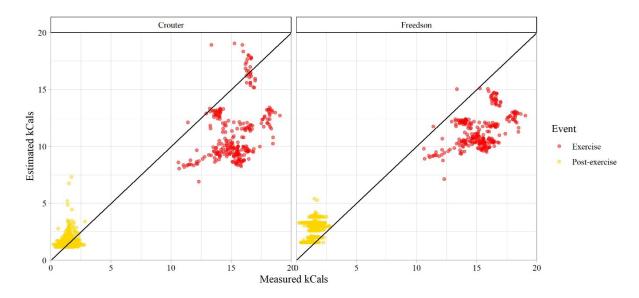
Figure 11 (30-minute bout) and 13 (short exercise bouts) display the measured EE rate in kCals/min (calculated from the Parvo metabolic cart VO<sub>2</sub>) and the estimated kCals/min from the Crouter and Freedson ActiGraph EE equations for each exercise bout. Figures 12 (30-minute bout) and 14 (short exercise bouts) are identity plots comparing the Crouter and Freedson estimated kCals to the Parvo-measured kCals during the exercise and post-exercise periods of each exercise bout.

During the 30-minute exercise session, both the Crouter (-4.07 kCals/min) and Freedson (-3.92 kCals/min) algorithms underestimated the EE rate. During the postexercise period after the 30-minute run, the Crouter algorithm was similar to the measured kCals/min and the Freedson algorithm overestimated the EE rate (+1.34 kCals/min). During the 30-second exercise period, both the Crouter and Freedson algorithms overestimated the EE rate (3.58 kCals/min and 2.88 kCals/min, respectively). During the 20-minute post-exercise period after the 30-second run, the Crouter algorithm underestimated the EE rate by 0.43 kCals/min, and the Freedson algorithm overestimated the EE rate by 0.97 kCals/min. During the 60-second run, both the Crouter and Freedson algorithms overestimated the EE rate (3.28 kCals/min and 3.73 kCals/min, respectively). During the 20-minute post-exercise period after the 60-second run, the Crouter algorithm underestimated the EE rate by 0.58 kCals/min, and the Freedson algorithm overestimated the EE rate by 0.83 kCals/min. During the 120-second exercise period, both the Crouter and Freedson algorithms were not biased in EE rate. During the 20-minute post-exercise period after the 120-second run, the Crouter algorithm underestimated the EE rate by 0.75 kCals/min, and the Freedson algorithm overestimated the EE rate by 0.67 kCals/min.

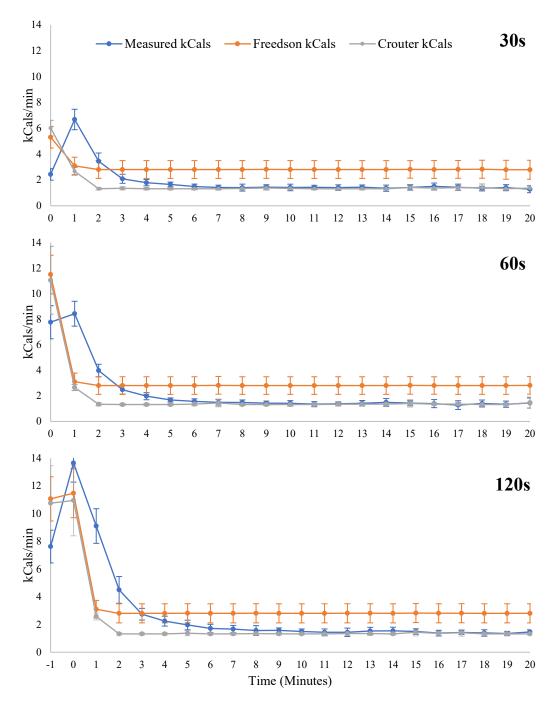
Notably, during the 30-second and 60-second exercise bouts in Figure 13, the peak  $VO_2$  value occurs after the termination of the exercise bout, contributing to error in the rate of accelerometer EE during the first two minutes of the post-exercise period. This may be due to the exercise bout duration, which is short enough to notice the delay in gas exchange between the working muscles and the lungs.



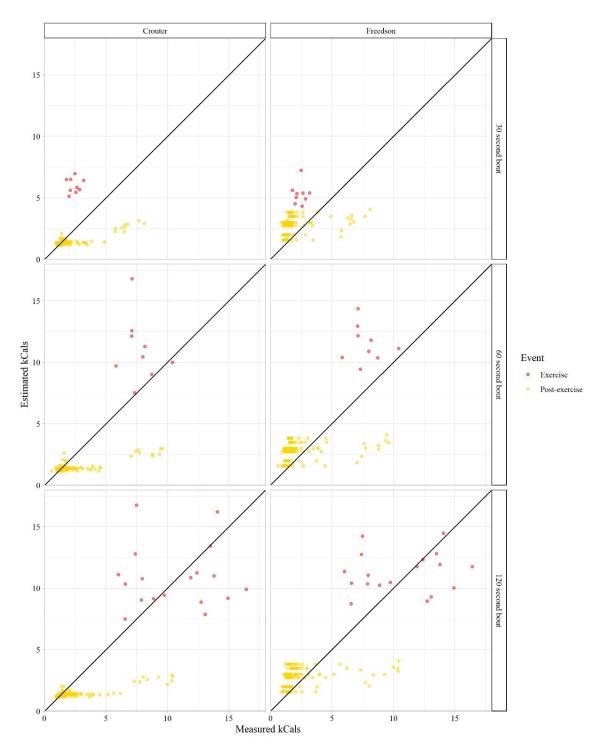
**Figure 11**: Measured vs. Crouter and Freedson estimated kCals/min over the 30-minute run and post-exercise period averaged across participants. Minute zero is the last exercise data point. SD error bars are depicted on only one side of the data for readability of the figure.



**Figure 12**: Identity plot for the Crouter and Freedson accelerometer EE estimation algorithms during the 30-minute exercise and 180-minute post-exercise. Each data point represents the kCals during one minute of data.



**Figure 13**: Measured vs. Freedson and Crouter estimated kCals/min over each short bout (run and post-exercise periods) averaged across participants. SD for each averaged data point is depicted as error bars. Minute zero is the last exercise data point (120s panel includes one extra data point at time -1 minutes).



**Figure 14**: Identity plots for the Crouter and Freedson accelerometer EE estimation algorithms during the short exercise bouts and 20-minute post-exercise periods. Each data point represents the kCals during one minute of data.

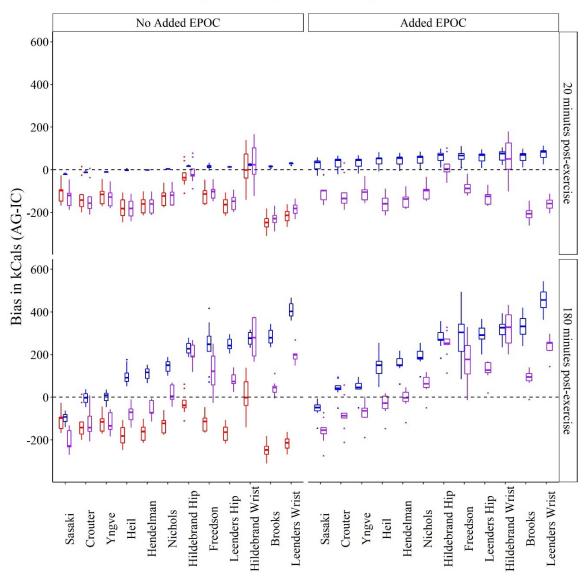
Figure 15 demonstrates the bias of the accelerometer EE estimates in kCals (from lowest to highest post-exercise values) during the long-duration (30 minutes) exercise data collection session, with the following 20 minutes and 180 minutes post-exercise periods. The top panel including only 20-minutes of post-exercise data was included to compare to the short bouts and to display the effect on data collection duration on bias results. The whole session, exercise only, and post-exercise periods are separated to demonstrate which portion of the session drove the whole session bias. The x-axis includes selected accelerometer EE equations and the y-axis is bias, calculated as the measured EE subtracted from the accelerometer EE estimate. The right panels display the bias from the accelerometer EE estimates with the addition of the measured EPOC EE (in kCals) attributable to the exercise. The EPOC kCals added to the whole session and post-exercise bias values were the EPOC kCals measured during 20 minutes of post-exercise and 180 minutes of post-exercise.

Table 11 lists the bias values displayed in figure 14 and notes which equations were unbiased during each part of the data collection period according to 95% confidence intervals. The size of the confidence intervals in this analysis were large (average range between lower and upper 95% bounds: 79.1 kCals) and so reduces the interpretation of the results.

For the entire 180-minute post-exercise data collection without adding EPOC, only the Nichols equation did not demonstrate bias on average (18.9 kCals, 95% CI: [-12.6, 50.5]). When cutting the post-exercise period to only 20 minutes, only the Hildebrand hip (-10.3 kCals, 95% CI: [-50.4, 29.8]) and Hildebrand wrist (29.3 kCals, 95% CI: [-41.8, 100.4]) equations were unbiased.

Isolating the 30-minute exercise period, the Hildebrand hip (-27.2 kCals, 95% CI: [-67.7, 13.3]) and the Hildebrand wrist (5.05 kCals, 95% CI: [-62.6, 72.7]) were not biased. Isolating the 180-minute post-exercise period after the 30-minute run, the Crouter (-2.68 kCals, 95% CI: [-25.1, 19.8]) and Yngve (1.53 kCals, 95% CI: [-19.3, 22.4]) equations were not biased. When cutting the post-exercise period to only 20 minutes, only the Heil (-1.94 kCals, 95% CI: [-4.49, 0.616]) and Hendelman (-0.33 kCals, 95% CI: [-1.49, 0.827]) equations were not biased.

After adding EPOC to the 180-minute post-exercise whole session values, the Heil (-34.3 kCals, 95% CI: [-74.1, 5.45]) and Hendelman (-7.92 kCals, 95% CI: [-45.1, 29.2]) equations became unbiased. When cutting the post-exercise period to only 20 minutes, for the whole session values the Hildebrand hip (11.9 kCals, 95% CI: [-29.2, 53.1]) and Hildebrand wrist (51.5 kCals, 95% CI: [-17.3, 120.3]) equations became unbiased. All equations overestimated during the 20-minute and 180-minute isolated post-exercise period with added EPOC, except for the Sasaki equation (-54.6 kCals, 95% CI: [-84.5, -24.7]) which underestimated EE.



Event  $\rightleftharpoons$  Exercise  $\rightleftharpoons$  Post-exercise  $\doteqdot$  Whole session

**Figure 15**: Bias is kCals (AG-IC) by method over the 30-minute exercise and the following 20-minute and 180-minute post-exercise periods. The right panels display each AG bias with added EPOC EE to each AG EE estimate.

		No EPOC Added	EPOC Added			
Method	Whole Session	Exercise	Post-exercise	Whole Session	Post-exercise	
Sasaki	-124.5 (-163.4, -85.6)	-103.8 (-142.3, -65.3)	-20.7 (-22.3, -19.1)	-102.3 (-141.5, -63.0)	23.6 (2.95, 44.2)	
Crouter	-133.1 (-189.0, -77.1)	-122.0 (-177.8, -66.2)	-11.0 (-14.3, -7.77)	-110.8 (-167.5, -54.2)	33.3 (11.5, 55.1)	
Yngve	-126.1 (-162.4, -89.8)	-115.6 (-151.8, -79.4)	-10.5 (-11.6, -9.4)	-103.9 (-140.3, -67.5)	33.8 (13.1, 54.4)	
Heil	-181.8 (-214.4, -149.1)	-179.8 (-214.3, -145.4)	-1.94 (-4.49, 0.616)*	-159.5 (-191.5, -127.6)	42.3 (20.4, 64.3)	20 1
Hendelman	-164.7 (-195.0, -134.4)	-164.4 (-195.3, -133.5)	-0.33 (-1.49, 0.827)*	-142.5 (-172.5, -112.5)	43.9 (22.8, 65.1)	ninute
Nichols	-122.7 (-155.1, -90.2)	-127.2 (-160.1, -94.3)	4.56 (3.44, 5.67)	-100.4 (-132.9, -68.0)	48.8 (27.8, 69.9)	s pos
Hildebrand Hip	-10.3 (-50.4, 29.8)*	-27.2 (-67.7, 13.3)*	16.9 (15.0, 18.8)	11.9 (-29.2, 53.1)*	61.2 (39.6, 82.8)	20 minutes post-exercise
Freedson	-102.4 (-132.0, -72.9)	-117.5 (-150.8, -84.2)	15.1 (6.69, 23.5)	-80.2 (-110.1, -50.3)	59.4 (34.5, 84.2)	cise
Leenders Hip	-152.1 (-179.8, -124.4)	-166.2 (-195.1, -137.4)	14.2 (12.3, 16.1)	-129.9 (-157.1, -102.6)	58.5 (36.9, 80.0)	
Hildebrand Wrist	29.3 (-41.8, 100.4)*	5.05 (-62.6, 72.7)*	24.2 (20.2, 28.3)	51.5 (-17.3, 120.3)*	68.5 (48.8, 88.2)	
Brooks	-226.5 (-254.4, -198.5)	-242.0 (-272.5, -211.6)	15.6 (12.4, 18.7)	-204.2 (-230.7, -177.8)	59.8 (37.7, 82.0)	
Leenders Wrist	-182.8 (-206.0, -159.6)	-212.6 (-238.2, -187.0)	29.7 (26.4, 33.1)	-160.6 (-182.6, -138.6)	74.0 (51.8, 96.2)	
					,	
Method	Whole Session	Exercise	Post-exercise	Whole Session	Post-exercise	
<b>Method</b> Sasaki	Whole Session -202.7 (-242.5, -162.8)	Exercise	<b>Post-exercise</b> -98.9 (-119.2, -78.5)	Whole Session -158.4 (-203.6, -113.2)	<b>Post-exercise</b> -54.6 (-84.5, -24.7)	
Sasaki	-202.7 (-242.5, -162.8)	-103.8 (-142.3, -65.3)	-98.9 (-119.2, -78.5)	-158.4 (-203.6, -113.2)	-54.6 (-84.5, -24.7)	
Sasaki Crouter	-202.7 (-242.5, -162.8) -124.7 (-179.1, -70.3)	-103.8 (-142.3, -65.3) -122.0 (-177.8, -66.2)	-98.9 (-119.2, -78.5) -2.68 (-25.1, 19.8)*	-158.4 (-203.6, -113.2) -80.4 (-140.8, -20.0)	-54.6 (-84.5, -24.7) 41.6 (9.36, 73.8)	180
Sasaki Crouter Yngve	-202.7 (-242.5, -162.8) -124.7 (-179.1, -70.3) -114.1 (-150.8, -77.4)	-103.8 (-142.3, -65.3) -122.0 (-177.8, -66.2) -115.6 (-151.8, -79.4)	-98.9 (-119.2, -78.5) -2.68 (-25.1, 19.8)* 1.53 (-19.3, 22.4)*	-158.4 (-203.6, -113.2) -80.4 (-140.8, -20.0) -69.8 (-112.7, -26.8)	-54.6 (-84.5, -24.7) 41.6 (9.36, 73.8) 45.8 (14.5, 77.1)	180 minu
Sasaki Crouter Yngve Heil	-202.7 (-242.5, -162.8) -124.7 (-179.1, -70.3) -114.1 (-150.8, -77.4) -78.6 (-109.5, -47.7)	-103.8 (-142.3, -65.3) -122.0 (-177.8, -66.2) -115.6 (-151.8, -79.4) -179.8 (-214.3, -145.4)	-98.9 (-119.2, -78.5) -2.68 (-25.1, 19.8)* 1.53 (-19.3, 22.4)* 101.2 (66.6, 135.9)	-158.4 (-203.6, -113.2) -80.4 (-140.8, -20.0) -69.8 (-112.7, -26.8) -34.3 (-74.1, 5.45)*	-54.6 (-84.5, -24.7) 41.6 (9.36, 73.8) 45.8 (14.5, 77.1) 145.5 (99.1, 192.0)	180 minutes po
Sasaki Crouter Yngve Heil Hendelman	-202.7 (-242.5, -162.8) -124.7 (-179.1, -70.3) -114.1 (-150.8, -77.4) -78.6 (-109.5, -47.7) -52.2 (-82.8, -21.6)	-103.8 (-142.3, -65.3) -122.0 (-177.8, -66.2) -115.6 (-151.8, -79.4) -179.8 (-214.3, -145.4) -164.4 (-195.3, -133.5)	-98.9 (-119.2, -78.5) -2.68 (-25.1, 19.8)* 1.53 (-19.3, 22.4)* 101.2 (66.6, 135.9) 112.2 (90.6, 133.8)	-158.4 (-203.6, -113.2) -80.4 (-140.8, -20.0) -69.8 (-112.7, -26.8) -34.3 (-74.1, 5.45)* -7.92 (-45.1, 29.2)*	-54.6 (-84.5, -24.7) 41.6 (9.36, 73.8) 45.8 (14.5, 77.1) 145.5 (99.1, 192.0) 156.5 (123.2, 189.8)	180 minutes post-exe
Sasaki Crouter Yngve Heil Hendelman Nichols	-202.7 (-242.5, -162.8) -124.7 (-179.1, -70.3) -114.1 (-150.8, -77.4) -78.6 (-109.5, -47.7) -52.2 (-82.8, -21.6) <b>18.9 (-12.6, 50.5)*</b>	-103.8 (-142.3, -65.3) -122.0 (-177.8, -66.2) -115.6 (-151.8, -79.4) -179.8 (-214.3, -145.4) -164.4 (-195.3, -133.5) -127.2 (-160.1, -94.3)	-98.9 (-119.2, -78.5) -2.68 (-25.1, 19.8)* 1.53 (-19.3, 22.4)* 101.2 (66.6, 135.9) 112.2 (90.6, 133.8) 146.2 (124.1, 168.3)	-158.4 (-203.6, -113.2) -80.4 (-140.8, -20.0) -69.8 (-112.7, -26.8) -34.3 (-74.1, 5.45)* -7.92 (-45.1, 29.2)* 63.2 (24.1, 102.4)	-54.6 (-84.5, -24.7) 41.6 (9.36, 73.8) 45.8 (14.5, 77.1) 145.5 (99.1, 192.0) 156.5 (123.2, 189.8) 190.4 (156.6, 224.3)	180 minutes post-exercise
Sasaki Crouter Yngve Heil Hendelman Nichols Hildebrand Hip	-202.7 (-242.5, -162.8) -124.7 (-179.1, -70.3) -114.1 (-150.8, -77.4) -78.6 (-109.5, -47.7) -52.2 (-82.8, -21.6) <b>18.9 (-12.6, 50.5)*</b> 204.5 (167.7, 241.3)	-103.8 (-142.3, -65.3) -122.0 (-177.8, -66.2) -115.6 (-151.8, -79.4) -179.8 (-214.3, -145.4) -164.4 (-195.3, -133.5) -127.2 (-160.1, -94.3) -27.2 (-67.7, 13.3)*	-98.9 (-119.2, -78.5) -2.68 (-25.1, 19.8)* 1.53 (-19.3, 22.4)* 101.2 (66.6, 135.9) 112.2 (90.6, 133.8) 146.2 (124.1, 168.3) 231.7 (207.4, 256.0)	-158.4 (-203.6, -113.2) -80.4 (-140.8, -20.0) -69.8 (-112.7, -26.8) -34.3 (-74.1, 5.45)* -7.92 (-45.1, 29.2)* 63.2 (24.1, 102.4) 248.8 (200.6, 296.9)	-54.6 (-84.5, -24.7) 41.6 (9.36, 73.8) 45.8 (14.5, 77.1) 145.5 (99.1, 192.0) 156.5 (123.2, 189.8) 190.4 (156.6, 224.3) 276.0 (239.1, 312.8)	180 minutes post-exercise
Sasaki Crouter Yngve Heil Hendelman Nichols Hildebrand Hip Freedson	-202.7 (-242.5, -162.8) -124.7 (-179.1, -70.3) -114.1 (-150.8, -77.4) -78.6 (-109.5, -47.7) -52.2 (-82.8, -21.6) <b>18.9 (-12.6, 50.5)*</b> 204.5 (167.7, 241.3) 122.8 (51.6, 193.9)	-103.8 (-142.3, -65.3) -122.0 (-177.8, -66.2) -115.6 (-151.8, -79.4) -179.8 (-214.3, -145.4) -164.4 (-195.3, -133.5) -127.2 (-160.1, -94.3) <b>-27.2 (-67.7, 13.3)*</b> -117.5 (-150.8, -84.2)	-98.9 (-119.2, -78.5) -2.68 (-25.1, 19.8)* 1.53 (-19.3, 22.4)* 101.2 (66.6, 135.9) 112.2 (90.6, 133.8) 146.2 (124.1, 168.3) 231.7 (207.4, 256.0) 240.3 (156.0, 324.6)	-158.4 (-203.6, -113.2) -80.4 (-140.8, -20.0) -69.8 (-112.7, -26.8) -34.3 (-74.1, 5.45)* -7.92 (-45.1, 29.2)* 63.2 (24.1, 102.4) 248.8 (200.6, 296.9) 167.0 (85.8, 248.3)	-54.6 (-84.5, -24.7) 41.6 (9.36, 73.8) 45.8 (14.5, 77.1) 145.5 (99.1, 192.0) 156.5 (123.2, 189.8) 190.4 (156.6, 224.3) 276.0 (239.1, 312.8) 284.6 (190.9, 378.3)	180 minutes post-exercise
Sasaki Crouter Yngve Heil Hendelman Nichols Hildebrand Hip Freedson Leenders Hip	-202.7 (-242.5, -162.8) -124.7 (-179.1, -70.3) -114.1 (-150.8, -77.4) -78.6 (-109.5, -47.7) -52.2 (-82.8, -21.6) <b>18.9 (-12.6, 50.5)*</b> 204.5 (167.7, 241.3) 122.8 (51.6, 193.9) 82.3 (54.8, 109.8)	-103.8 (-142.3, -65.3) -122.0 (-177.8, -66.2) -115.6 (-151.8, -79.4) -179.8 (-214.3, -145.4) -164.4 (-195.3, -133.5) -127.2 (-160.1, -94.3) -27.2 (-67.7, 13.3)* -117.5 (-150.8, -84.2) -166.2 (-195.1, -137.4)	-98.9 (-119.2, -78.5) -2.68 (-25.1, 19.8)* 1.53 (-19.3, 22.4)* 101.2 (66.6, 135.9) 112.2 (90.6, 133.8) 146.2 (124.1, 168.3) 231.7 (207.4, 256.0) 240.3 (156.0, 324.6) 248.5 (223.9, 273.1)	-158.4 (-203.6, -113.2) -80.4 (-140.8, -20.0) -69.8 (-112.7, -26.8) -34.3 (-74.1, 5.45)* -7.92 (-45.1, 29.2)* 63.2 (24.1, 102.4) 248.8 (200.6, 296.9) 167.0 (85.8, 248.3) 126.5 (91.1, 162.0)	-54.6 (-84.5, -24.7) 41.6 (9.36, 73.8) 45.8 (14.5, 77.1) 145.5 (99.1, 192.0) 156.5 (123.2, 189.8) 190.4 (156.6, 224.3) 276.0 (239.1, 312.8) 284.6 (190.9, 378.3) 292.8 (256.1, 329.5)	180 minutes post-exercise

**Table 11**: Bias in kCals (Mean and 95% CIs) for the 30-minute run and the 20-minute and 180-minute post-exercise periods with and without adding the measured EPOC EE.

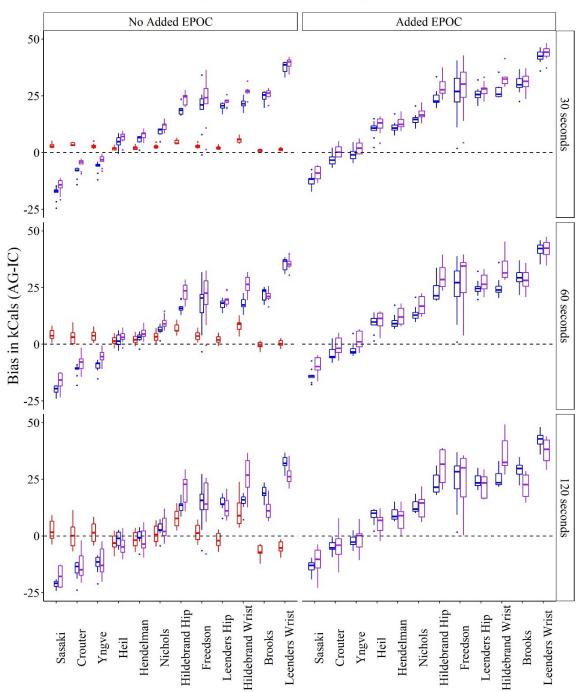
\*not significantly different from zero, unbiased

Figure 16 and table 12 demonstrate the bias of the accelerometer EE estimates in kCals during each short exercise bout (exercise only), 20-minute post-exercise period only, and the whole data collection session (exercise + post-exercise). During all constituent parts (whole session, exercise, and post-exercise) of the 30-second running

bout without adding EPOC, all the accelerometer EE estimation equations demonstrated bias by either over or underestimating EE. When adding EPOC to the 30-second bout whole session analysis, the Crouter (0.53 kCals, 95% CI: [-1.49, 2.55]) and Yngve (1.96 kCals, 95% CI: [-0.2, 4.1]) equations were not biased. Only the Ygnve equation (-0.89 kCals, 95% CI: [-3.01, 1.22]) was not biased when adding EPOC EE to the post-exercise period following the 30-second run.

During the 60-second exercise period, the Brooks (-0.50 kCals, 95% CI: [-1.73, 0.72]) and Leenders wrist (0.49 kCals, 95% CI: [-0.75, 1.73]) equations were unbiased, and during the post-exercise period following the 60-second running bout the Heil equation (1.76 kCals, 95% CI: [-0.48, 3.99]) was unbiased. When adding EPOC to the whole session and post-exercise bias values, the Crouter (-1.0 kCals, 95% CI: [-4.24, 2.23]) and Yngve (1.66 kCals, 95% CI: [-1.19, 4.51]) equations were unbiased for the whole session and only the Yngve equation (-2.15 kCals, 95% CI: [-4.52, 0.22]) was unbiased for the post-exercise period.

During the 120-second whole session bout, the Hendelman (2.17 kCals, 95% CI: [-6.34, 2.00]) and Nicholas (3.74 kCals, 95% CI: [-0.56, 8.04]) equations were unbiased. Isolating the 120-second exercise period, the Crouter (0.44 kCals, 95% CI: [-4.12, 5.0]), Freedson (1.26 kCals, 95% CI: [-1.87, 4.40]), Heil (-2.87 kCals, 95% CI: [-5.84, 0.11]), Hendelman (-1.84 kCals, 95% CI: [-4.82, 1.14]), Leenders hip (-1.94 kCals, 95% CI: [-4.74, 0.85]), Nicholas (0.63 kCals, 95% CI: [-2.60, 3.86]), Sasaki (2.43 kCals, 95% CI: [-1.16, 6.01]), and Yngve (1.38 kCals, 95% CI: [-2.14, 4.90]) equations were unbiased. During the post-exercise period following the 120-second run, the Heil (-1.40 kCals, 95% CI: [-4.04, 1.24]) and Hendelman (-0.33 kCals, 95% CI: [-3.06, 2.39]) equations were unbiased. Adding EPOC to the 120-second whole session bout, the Crouter (-4.37 kCals, 95% CI: [-9.73, 1.00]) and Yngve (-1.04 kCals, 95% CI: [-5.53, 3.45]) equations were unbiased. None of the equations were unbiased when adding EPOC EE to the post-exercise period.



Event  $\rightleftharpoons$  Exercise  $\doteqdot$  Post-exercise  $\doteqdot$  Whole session

**Figure 16:** Bias is kCals (AG-IC) by method over the whole data collection and its constituent parts (exercise and 20-minute post-exercise periods). The right panels display each AG bias with added EPOC EE to each AG EE estimate.

	No EPOC Added		EPOC Added		
Whole Session	Exercise	Post-exercise	Whole Session	Post-exercise	
-14.8 (-17.4, -12.3)	3.07 (2.27, 3.87)	-17.9 (-20.4, -15.5)	-9.3 (-11.7, -6.89)	-12.4 (-14.7, -10.0)	
-5.01 (-6.83, -3.19)	3.58 (3.02, 4.14)	-8.59 (-10.6, -6.62)	0.534 (-1.49, 2.55)*	-3.04 (-5.1, -0.986)	
-3.58 (-5.54, -1.63)	2.85 (2.03, 3.68)	-6.44 (-8.35, -4.52)	1.96 (-0.181, 4.1)*	-0.891 (-3.01, 1.22)*	
6.52 (4.56, 8.49)	1.9 (1.24, 2.55)	4.63 (2.59, 6.66)	12.1 (9.2, 14.9)	10.2 (7.24, 13.1)	
7.81 (6.3, 9.32)	2.14 (1.46, 2.81)	5.67 (4.15, 7.2)	13.4 (11.2, 15.5)	11.2 (9.08, 13.4)	30-9
11.8 (10.3, 13.3)	2.71 (1.96, 3.46)	9.08 (7.61, 10.6)	17.3 (15.1, 19.6)	14.6 (12.4, 16.8)	secon
23.4 (21.4, 25.5)	4.69 (3.92, 5.46)	18.8 (17.1, 20.4)	29.0 (25.6, 32.4)	24.3 (21.1, 27.5)	30-second bout
22.1 (14.1, 30.1)	2.88 (2.15, 3.6)	19.3 (11.4, 27.2)	27.7 (18.4, 37.0)	24.8 (15.6, 34.0)	=
22.4 (21.0, 23.8)	2.14 (1.51, 2.76)	20.2 (18.8, 21.7)	27.9 (25.5, 30.4)	25.8 (23.3, 28.2)	
27.0 (25.2, 28.8)	5.45 (4.5, 6.4)	21.6 (19.7, 23.4)	32.5 (29.7, 35.4)	27.1 (24.3, 29.9)	
25.6 (23.9, 27.3)	0.989 (0.53, 1.45)	24.6 (22.7, 26.4)	31.1 (28.1, 34.2)	30.1 (27.0, 33.3)	
39.1 (37.3, 40.9)	1.47 (0.991, 1.95)	37.7 (35.8, 39.5)	44.7 (41.6, 47.7)	43.2 (40.1, 46.3)	
Whole Session	Exercise	Post-exercise	Whole Session	Post-exercise	İ.
	4.31 (2.47, 6.15)	-21.0 (-23.5, -18.5)	-9.4 (-12.3, -6.47)	-13.7 (-16.1, -11.3)	
	3.28 (0.826, 5.74)	-11.6 (-13.7, -9.38)	-1.0 (-4.24, 2.23)*	-4.29 (-6.77, -1.8)	
-5.6 (-8.16, -3.04)	3.81 (1.98, 5.64)	-9.42 (-11.5, -7.37)	1.66 (-1.19, 4.51)*	-2.15 (-4.52, 0.218)*	
3.39 (1.22, 5.55)	1.63 (0.0638, 3.19)	1.76 (-0.477, 3.99)*	10.7 (7.58, 13.7)	9.02 (5.91, 12.1)	
4.97 (2.78, 7.17)	2.15 (0.574, 3.72)	2.83 (1.06, 4.6)	12.2 (9.39, 15.1)	10.1 (7.62, 12.6)	60-s
9.69 (7.46, 11.9)	3.41 (1.71, 5.1)	6.28 (4.54, 8.02)	17.0 (14.0, 19.9)	13.5 (11.0, 16.1)	60-second bout
22.9 (19.8, 26.1)	6.9 (5.2, 8.59)	16.0 (14.0, 18.1)	30.2 (25.8, 34.6)	23.3 (19.6, 27.0)	1 bou
20.3 (12.2, 28.5)	3.73 (2.09, 5.36)	16.6 (8.5, 24.7)	27.6 (18.1, 37.1)	23.9 (14.4, 33.3)	Ē
19.6 (17.6, 21.7)	2.07 (0.589, 3.56)	17.6 (15.8, 19.3)	26.9 (23.8, 30.0)	24.8 (22.0, 27.6)	
26.1 (22.7, 29.5)	8.08 (5.96, 10.2)	18.0 (15.8, 20.2)	33.4 (29.1, 37.6)	25.3 (21.7, 28.8)	
21.4 (19.3, 23.6)	-0.503 (-1.73, 0.719)*	21.9 (19.8, 24.1)	28.7 (25.2, 32.2)	29.2 (25.7, 32.7)	
35.7 (33.4, 37.9)	0.489 (-0.747, 1.73)*	35.2 (33.1, 37.3)	42.9 (39.3, 46.5)	42.4 (39.0, 45.8)	
Whole Session	Exercise	Post-exercise	Whole Session	Post-exercise	
-21.5 (-27.0, -16.1)	2.43 (-1.16, 6.01)*	-24.0 (-27.6, -20.4)	-11.4 (-16.3, -6.53)	-13.8 (-16.4, -11.2)	
-14.5 (-20.1, -8.95)	0.437 (-4.12, 5.0)*	-15.0 (-18.1, -11.8)	-4.37 (-9.73, 1.0)*	-4.8 (-7.24, -2.37)	
-11.2 (-16.0, -6.44)	1.38 (-2.14, 4.9)*	-12.6 (-15.6, -9.53)	-1.04 (-5.53, 3.45)*	-2.42 (-4.73, -0.12)	
-4.26 (-7.96, -0.569)	-2.87 (-5.84, 0.107)*	-1.4 (-4.04, 1.24)*	5.89 (2.11, 9.67)	8.76 (6.2, 11.3)	
-2.17 (-6.34, 2.0)*	-1.84 (-4.82, 1.14)*	-0.333 (-3.06, 2.39)*	7.99 (3.9, 12.1)	9.82 (7.59, 12.1)	120-s
3.74 (-0.556, 8.04)*	0.628 (-2.6, 3.86)*	3.12 (0.449, 5.78)	13.9 (9.63, 18.2)	13.3 (11.0, 15.5)	econ
20.7 (15.7, 25.8)	7.72 (4.6, 10.8)	13.0 (10.7, 15.3)	30.9 (25.1, 36.7)	23.2 (19.7, 26.6)	120-second bout
14.7 (6.34, 23.1)	1.26 (-1.87, 4.4)*	13.4 (5.74, 21.1)	24.9 (15.4, 34.3)	23.6 (14.8, 32.3)	Ŧ
12.5 (8.59, 16.3)	-1.94 (-4.74, 0.851)*	14.4 (11.9, 16.9)	22.6 (18.6, 26.7)	24.6 (22.2, 27.0)	
25.9 (19.8, 31.9)	10.7 (5.54, 15.9)	15.1 (12.4, 17.9)	36.0 (29.9, 42.1)	25.3 (22.4, 28.2)	
11.0 (0.4.15.0)	( 07 ( 0.25 4 (0))	10 0 (16 2 21 4)	22.0 (18.1, 25.8)	28.0 (26.0. 21.0)	1
11.8 (8.4, 15.2)	-6.97 (-9.25, -4.69)	18.8 (16.2, 21.4)	22.0 (10.1, 25.0)	28.9 (26.0, 31.9)	
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-11.3)</math><math>8.7 (11.4, -51)</math><math>3.28 (0.826, 5.74)</math><math>-11.6 (-1.37, -9.38)</math><math>-1.06 (-4.74, -3.8)</math><math>-2.16 (</math></td>	Whole SessionExercisePost-exercise $14.8 (17.4, -12.3)$ $3.07 (2.27, 3.87)$ $-17.9 (-20.4, -15.5)$ $5.01 (-6.83, -3.19)$ $3.58 (3.02, 4.14)$ $-8.59 (-10.6, -6.62)$ $-3.58 (-5.54, -1.63)$ $2.85 (2.03, 3.68)$ $-6.44 (-8.35, -4.52)$ $6.52 (4.56, 8.49)$ $1.9 (1.24, 2.55)$ $4.63 (2.59, 6.66)$ $7.81 (6.3, 9.32)$ $2.14 (1.46, 2.81)$ $5.67 (4.15, 7.2)$ $11.8 (10.3, 13.3)$ $2.71 (1.96, 3.46)$ $9.08 (7.61, 10.6)$ $23.4 (21.4, 25.5)$ $4.69 (3.92, 5.46)$ $11.8. 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**Table 12**: Bias in kCals (Mean and 95% CIs) for each exercise bout and 20-min postexercise resting period with and without adding the measured EPOC EE.

\*not significantly different from zero, unbiased

Chapter 5:

## Discussion

The purpose of this study was to investigate the bias of accelerometer EE estimation algorithms during the post-exercise period after four different durations of exercise, holding intensity constant. We hypothesized that (1) the accelerometer EE estimation equations would underestimate EE during the post-exercise period due to EPOC not being captured by the accelerometers, and (2) that the underestimation would increase with increasing bout duration, if intensity was held constant, due to the resulting increase in EPOC magnitude.

The results from this study contradict the original hypothesis that accelerometer EE estimation equations would underestimate EE as compared to indirect calorimetry during the post-exercise periods. After the 30-minute run, nine of the twelve algorithms overestimated EE during the post-exercise period, two were not biased, and only one underestimated EE. After the 30-second run, nine of the twelve algorithms overestimated EE and three underestimated EE during the post-exercise period. After the 60-second run, eight of the twelve overestimated EE, one was not biased, and three underestimated EE. After the 120-second run, seven algorithms overestimated EE, two were not biased, and three underestimated EE. With increasing bout duration, EPOC magnitude did increase across participants. Adding the measured EPOC EE from the Parvo metabolic cart reduced the bias of the algorithms that underestimated EE during the post-exercise period, but increased the bias of the algorithms that overestimated EE.

The unexpected overestimation of EE by the accelerometer algorithms occurred because of two factors, (1) the accelerometer EE estimation equations in this analysis (with the exception of Crouter 2006) are standard linear models, and (2) none of the accelerometer EE estimation equations that overestimated EE during the post-exercise period included lying rest in their calibration protocols, producing accelerometer-based estimates of resting EE (y-intercept values) that were above the true resting EE.

Although linear regression is a useful model to understand the basic relationships between two variables, the relationship between motion data (acceleration) from a single body part and EE is much more complex (Freedson et al., 1998; Hildebrand et al., 2014). Linear regression models adequately describe acceleration-EE relationships during specific types of activities, such as ambulatory activities, but do not adequately describe the acceleration-EE relationship during a wide range of activities involving irregular movement patterns like resistance training, common recreational sports, household activities and some sedentary behaviors, plus regular ambulatory movement like locomotion. None of the linear models in this analysis included lying rest during their calibration protocols (Table 7) and only included data from activities demanding higher energy expenditure than that of lying rest. The exclusion of lying rest may be partially due to the public health focus on classifying higher intensity activities. Basing a model on activities with higher EE values resulted in linear models with y-intercepts above the resting metabolic rate of the participants in this study. When one of these linear models is applied to a wearer who is not performing any motion, the output of the linear model is solely the y-intercept. For example, the average resting rate of participant 1 during the post-exercise period was 1.24 kCals/min and the y-intercept of the Freedson kCal

equation for participant 1 is 2.97 kCals/min ([0.1346\*(BM in kg)] - 7.37418). With no input to the accelerometer while the participant is resting, the Freedson kCal equation estimated an EE rate above the resting metabolic rate, resulting in a large overestimation of total EE over the post-exercise period. In contrast, during the post-exercise period after the 30-minute run, the Sasaki algorithm underestimated EE because the y-intercept was below resting EE of participants, the Yngve algorithm did not demonstrate bias because the y-intercept was similar to that of the mean resting EE, and the Crouter algorithm (not based on basic linear regression) did not demonstrate bias because of its branching logic. Crouter (2006) includes separate equations for three different conditions: (1) regular, ambulatory activities, (2) irregular lifestyle activities, and (3) sedentary activities with little to no motion. Depending on the 10-sec CV of the 1-second ActiGraph counts, the minute-by-minute data is then run through the appropriate equation. These results support the use of more complex algorithms that use branching logic or machine learning based on one or several of the many features of accelerometer output to determine activity type before running data through an EE equation. Although, current machine learning models do not always perform better in estimating EE than traditional hip-based linear models (Montoye et al., 2017).

During the exercise portion of the 30-minute run session, ten of the twelve accelerometer EE estimation algorithms underestimated EE and the Hildebrand hip and wrist algorithms were not biased. None of the calibration protocols included as high intensity activity as was performed in this validation protocol, which may be why they were consistently inaccurate, for two primary reasons: the public health focus on categorizing PA intensity zones and for researchers to minimize participant burden. Most EE estimation equations are developed to discern cut-points determining "zones" of PA intensity, and so most researchers would aim to reach the minimum requirement of "vigorous" ( $\geq$  6 METs). In a free-living setting, any activity above the determined cutpoint would simply be classified as "vigorous." Researchers could then quantify how many minutes an individual spends in a given PA intensity zone. In addition, the ActiGraph output counts/min plateaus or decreases (known as the inverted-U phenomenon) with increasing speed or intensity past 10 kph or 6 mph (John et al., 2012). Because participants ran, on average, between 11.2 and 12.7 kph and ten of the twelve ActiGraph EE estimation equations are based on counts/min, the inverted-U phenomenon may have partially driven the underestimation of EE during the run. The Hildebrand algorithms are based on ENMOs rather than counts, and so do not experience the inverted-U phenomenon.

Analyzing the 30-minute run and 180-minute post-exercise period together, six of the twelve algorithms overestimated EE, five underestimated EE, and one was not biased. Overall, these algorithms are not adequate in estimating EE during high intensity (70-80% VO<sub>2</sub> peak) treadmill running, lying rest, or during a period of time that involves high intensity running followed by a long bout of rest. These results are consistent with previous studies that demonstrate that linear models do not accurately estimate EE outside of the range of activities included in the calibration protocols (Bai et al., 2016; Crouter et al., 2006; Lyden et al., 2011).

Extrapolating these results to free-living scenarios, ActiGraph accelerometer EE estimation equations based on linear models are likely to overestimate EE significantly in a largely sedentary population (Crouter et al., 2006), due to the y-intercepts of linear

equations being above true resting EE. The EE estimation equations included in these analyses significantly overestimated EE during activities with little motion, and so are too biased to be used in public health surveillance studies in the United States where adult participants may be spending up to or more than 12 hours per day of sedentary time (Diaz et al., 2018).

Researchers looking to use an accelerometer EE estimation equation must be aware of the effects their protocol activities and population may have on the EE estimates. One of the main findings of this analysis includes the effect that exercise and measurement duration have on EE estimates. There was no single EE equation that performed better than other equations across all conditions. Some equations demonstrated a lack of bias during certain exercise conditions, some demonstrated a lack of bias during certain post-exercise conditions, and others demonstrated a "wash-out" effect (under and overestimation during the exercise and post-exercise periods, respectively, resulted in an unbiased total EE for the whole session), but there was no consistency across all conditions. Thus, researchers should select an equation that best suits their specific needs based on validation studies.

The strengths of this study include (1) oxygen consumption data obtained in small time intervals from the Parvo metabolic cart, allowing for time synchrony between the VO<sub>2</sub> and accelerometer data, (2) participants' behavior during the pre-exercise and postexercise periods were strictly controlled to lying rest, (3) the comparison between preexercise resting VO<sub>2</sub> and post-exercise VO<sub>2</sub> eliminates concerns about day-to-day variability in metabolic rate, (4) the exploration of the effect of short running bouts (30s, 60s, and 120s) of exercise on EPOC, rarely seen in the EPOC literature, and (5) the

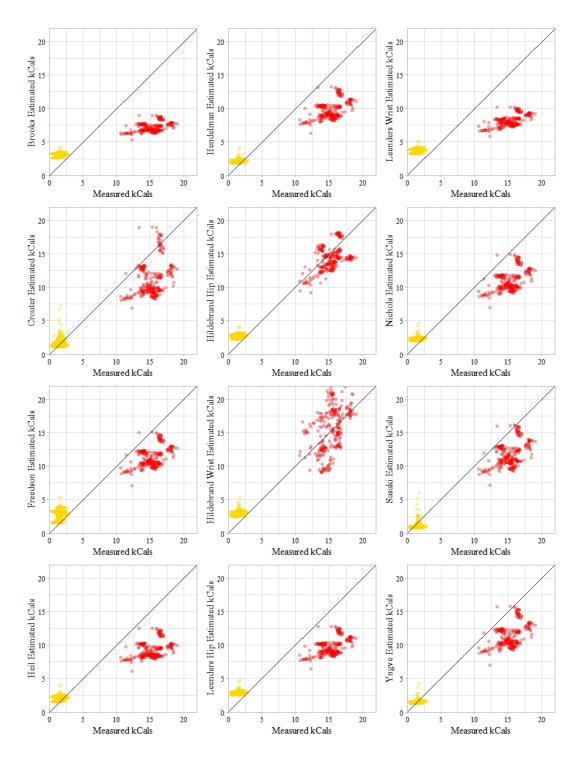
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inclusion of twelve accelerometer EE estimation equations. Limitations of this study include (1) the exclusion of the prolonged slow component of EPOC after the 30-minute run due to the post-exercise data collection time being limited to 180 minutes, (2) the involvement of high participant burden due to the intensity of the exercise (70-80%  $VO_2$ peak) and participants having to wear the Parvo mask for several hours, (3) the removal of participants from the Parvo cart while it was being recalibrated resulted in data gaps in the post-exercise period, (4) only one non-linear regression algorithm was included in this analysis, (5) the sample in this study only included young, healthy and fit males, and (6) accelerometer EE equations that did not estimate kCals directly were translated to kCals using 1 L  $O_2 = 5$  kCals conversion factor, which may not be as accurate at high intensities. The listed limitations were addressed by (1) extrapolating the duration of the EPOC slow component with biexponential models, (2) allowing participants to decrease the treadmill speed as long as intensity remained between 70-80% VO<sub>2</sub> peak and building breaks into the post-exercise protocol, (3) data during the breaks was extrapolated using linear regression from 10 minutes of pre-break data and 10 minutes of post-break data. The fourth and fifth limitations should be addressed in future studies and analyses with the purpose of including more non-linear regression algorithms and different populations.

Future directions should focus on developing EE estimation algorithms with branching logic and tailoring equations to the EE rates of specific types of activity. Developing a branching logic algorithm (also known as decision trees) based on different features of the accelerometer requires expertise in accelerometry and algorithm development and resources to run a comprehensive study with protocols spanning several activity types, which may increase participant burden. Researchers should also validate

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existing EE equations outside of the activities they were calibrated in to determine their generalizability and inform other researchers which equations are appropriate for different settings and populations. Future studies investigating the lasting effects of EPOC should utilize whole-room calorimeters with longer stays to study the prolonged effect of EPOC after intense exercise, which according to the results of this study could last about 354 minutes after a 30-minute run at 80% VO<sub>2</sub> peak. Future accelerometer EE estimation validation studies should also include more machine learning EE models as they are developed in order to investigate their potential for improved performance over linear models.



## **Supplementary Figures and Tables**

**Figure S1**: Identity plots for each ActiGraph EE estimation equation for the 30-minute exercise session. Exercise data points are in red, post-exercise data points are in yellow.

	Participant	a	b	c	d
30sec	1	1.66	-0.02	0.27	-1.45E-05
	2	1.40	-0.02	0.39	-1.54E-04
	3	1.98	-0.03	0.37	-1.65E-04
	4	1.37	-0.04	0.35	-3.63E-04
	5	1.91	-0.04	0.34	-3.23E-04
3	6	1.43	-0.04	0.32	-3.27E-04
	7	1.34	-0.03	0.32	-2.10E-04
	8	1.44	-0.02	0.28	-1.00E-06
	9	1.00	-0.02	0.28	-1.59E-04
	1	2.04	-0.02	0.27	-7.12E-06
	2	1.89	-0.03	0.39	-1.97E-04
	3	1.77	-0.02	0.36	-1.35E-04
ు	4	1.20	-0.03	0.37	-4.62E-04
60sec	5	1.61	-0.02	0.31	-1.92E-04
9	6	1.10	-0.03	0.30	-1.86E-04
	7	1.35	-0.03	0.28	-1.70E-04
	8	1.61	-0.02	0.32	-1.86E-04
	9	1.31	-0.02	0.29	-3.10E-04
	1	2.13	-0.02	0.31	-7.98E-05
	2	1.87	-0.02	0.46	-2.83E-04
	3	1.25	-0.02	0.39	-2.63E-04
ပ္ရ	4	1.38	-0.03	0.34	-2.22E-04
120sec	5	1.99	-0.03	0.37	-2.99E-04
Ŧ	6	1.34	-0.02	0.31	-2.57E-04
	7	1.68	-0.04	0.38	-4.27E-04
	8	1.34	-0.02	0.34	-1.79E-04
	9	1.49	-0.02	0.29	-4.75E-05
	1	2.64	-0.01	0.30	-5.14E-06
	2	2.11	-0.01	0.36	-4.24E-05
	3	2.56	-0.01	0.34	-5.71E-06
.Е	4	2.90	-0.02	0.36	-2.46E-05
30min	5	2.09	-0.01	0.38	-1.22E-05
ĕ	6	2.38	-0.01	0.32	-8.04E-06
	7	2.43	-0.01	0.36	-1.00E-05
	8	2.42	-0.01	0.38	-2.72E-06
	9	1.44	-0.01	0.28	-2.88E-05

**Table S1**: Biexponential model coefficients for all individual participant models.

	Participant	SSE	R squared	dfe	Adjusted R squared	RMSE
	1	2.25	0.87	201	0.87	0.11
	2	1.37	0.87	230	0.87	0.08
	3	1.17	0.92	235	0.92	0.07
ల	4	1.39	0.83	229	0.83	0.08
30sec	5	0.97	0.92	228	0.91	0.07
6	6	0.61	0.92	233	0.92	0.05
	7	1.59	0.79	207	0.79	0.09
	8	0.59	0.94	175	0.94	0.06
	9	0.52	0.91	229	0.91	0.05
	1	1.77	0.93	214	0.93	0.09
	2	1.29	0.92	224	0.92	0.08
	3	0.78	0.95	235	0.95	0.06
ల	4	1.25	0.84	226	0.84	0.07
60sec	5	1.15	0.91	218	0.91	0.07
9	6	1.11	0.84	235	0.83	0.07
	7	2.69	0.70	192	0.70	0.12
	8	0.92	0.94	208	0.94	0.07
	9	1.69	0.87	231	0.87	0.09
	1	1.30	0.95	215	0.95	0.08
	2	3.03	0.86	233	0.85	0.11
	3	0.78	0.93	228	0.93	0.06
ည္ရ	4	0.89	0.89	229	0.89	0.06
120sec	5	0.86	0.95	231	0.95	0.06
1	6	0.95	0.90	228	0.90	0.06
	7	1.81	0.86	220	0.86	0.09
	8	1.13	0.90	221	0.90	0.07
	9	1.02	0.92	225	0.91	0.07
	1	20.11	0.72	2106	0.72	0.10
	2	23.74	0.68	2001	0.68	0.11
	3	37.32	0.63	2124	0.63	0.13
я	4	64.89	0.73	3467	0.73	0.14
30min	5	36.38	0.51	1925	0.51	0.14
ŝ	6	22.22	0.67	2166	0.67	0.10
	7	29.91	0.65	2085	0.65	0.12
	8	50.13	0.68	2745	0.68	0.14
	9	30.81	0.65	3087	0.65	0.10

**Table S2**: Goodness of fit parameters for all individual participant biexponential models.

## Appendix A

#### Informed Consent

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

Researcher(s):	John Sirard, PhD; Nicholas Remillard, B.S.		
Study Title:	Improving Accelerometer Energy Expenditure Predictions		

#### 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. 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. WHAT ARE SOME OF THE IMPORTANT ASPECTS OF THIS RESEARCH STUDY THAT I SHOULD BE AWARE OF?

- 1) Consent is being sought for participation in this research study. Participation is voluntary.
- 2) The purpose of this study is to improve accelerometer energy predictions, specifically by including the metabolism experienced after exercise into prediction models.
- 3) You will be asked to come to the laboratory for three visits. The expected duration of your participation is 10 hours over a period of 2-6 weeks. Visit 1 will include the consent process, height and weight measurements, a DXA scan, and an incremental VO2 peak (aerobic capacity) test on a treadmill. At visit 2, you will perform vigorous exercise bouts of different durations (30 seconds, 1 minute, and 2 minutes) at 80% VO2 peak. At visit 3, you will perform a long-duration (30 minutes) vigorous (80% VO2 peak) exercise session, with 1 hour of laying rest before and 3 hours of rest after.
- 4) There are minimal to moderate risks associated with the procedures in this study. You may experience muscle soreness or discomfort, and there is a slight risk of falling while on the treadmill. These risks are increased during the maximal treadmill test. The DXA scan does involve ionizing radiation, though a small amount (about 5% of a transcontinental flight).
- 5) You will receive no direct benefit from participating in this research study.

#### 3. WHY ARE WE DOING THIS RESEARCH STUDY?

Accelerometers are wearable devices often used to measure physical activity and energy expenditure (calories) in epidemiological studies. The purpose of this study is to improve accelerometer-based energy expenditure estimates by incorporating the elevated metabolism experienced after exercise into accelerometer prediction methods.

#### 4. WHO CAN PARTICIPATE IN THIS RESEARCH STUDY?

Ten participants will participate in this study, each must be a young (18-30 years) male with a BMI between 18.0-24.9 kg/m<sup>2</sup>. You must score at least 24 points on the Godin-Shepherd Leisure-time questionnaire and exercise on a weekly basis. Additionally, you must be healthy by self-report, free from any chronic disease (cardiovascular, pulmonary, neurological), free from musculoskeletal injury (current or prior history) that may impede them from performing vigorous exercise, and be able to ambulate without assistance.

# 5. WHERE WILL THIS RESEARCH STUDY TAKE PLACE AND HOW MANY PEOPLE WILL PARTICIPATE?

This research will be conducted at the University of Massachusetts Amherst at the Center for Human Health and Performance (CHHP). We expect to enroll 10 males in this study, between the ages of 18 and 30 years.

# 6. WHAT WILL I BE ASKED TO DO AND HOW MUCH TIME WILL IT TAKE?

Prior to your first visit, you will be screening by telephone to detail the study protocol in full, give you the opportunity for you to ask questions, obtain your medical history, medication use, and physical activity habits to determine eligibility. The medical history includes questions about chronic diseases, smoking status, medications, and incident history. The medication use section includes questions about your regular use of any prescription or over-the-counter medications to screen for any medications that may affect resting or exercise metabolism, such as hormone therapy or beta-blockers. Physical activity status is assessed using the Godin-Shepherd Leisure Time questionnaire, in addition to questions about running habits within the past 2 months. A score equal to or above 24 points on the Godin-Shepherd Leisure Time questionnaire is determined to be active. Prior to your first visit, you will have answered questions on the Physical Activity Readiness Questionnaire (PARQ) to determine safety for exercise and on a DXA safety screening form to ensure your safety during this scan.

At the beginning of visit 1, study personnel will read through the informed consent with you. You are encouraged to ask questions at any time during this process. To ensure comprehension of the study and what you will be asked to do, we will ask you to describe the study and what you will be asked to do in your own words. Points of confusion or missed elements will be reviewed again to ensure comprehension. Following this process, you and the research staff member will sign the consent form.

**Visit 1 (~2 hours):** If you are deemed eligible after the phone screening, you will complete visit 1 at the Center of Human Health and Performance (CH2P) in the Institute of Applied Life Sciences on the University of Massachusetts Amherst campus. Study personnel will read through the informed consent with you and answer any questions. Once consenting is complete, study personnel will take height and weight measurements. You will then undergo a DXA scan operated by trained and certified CH2P staff.

A DXA (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, DXA scans are performed by a certified technician. 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 or 5% of that you would receive from a cross-country airplane flight.

After completing the DXA scan, you will be led into the Exercise Training Room and fitted for a mouth-piece to use with the Parvo metabolic cart. Study personnel will assist you in putting on a Polar heart rate monitor using the chest strap and placing the ActiGraph accelerometers on the correct locations on the non-dominant wrist and right hip. Once all equipment is comfortably on you, the VO<sub>2</sub> peak test will begin. All treadmill controls and speed changes will be performed by study personnel. You will begin by warming up with a 3-minute walk at 3 mph and a 2-minute jog at 5mph.. After warm up, study personnel will increase the treadmill speed to begin the VO<sub>2</sub> peak test. The first part of the test consists of three 2-minute stages at 10 kmh, 12 kmh, and 14 kmh (6.2 mph, 7.5 mph, and 8.7 mph). After completing the 14 kmh stage, the treadmill will be inclined by 2% every 2 minutes until exhaustion. After exhaustion is reached, you will be asked to complete a 5-minute cooldown at 3mph. Once the VO<sub>2</sub> peak test is complete, study personnel will remove equipment and instruct you to sit to monitor your condition. A Gatorade will be provided to rehydrate, replenish electrolytes and carbohydrates.

Visit 2 (~2 hours): After completing visit 1, you will complete visit 2 between 2 and 14 days after visit 1. Visit 2 will occur at the Center of Human Health and Performance (CH2P) Exercise Training Room again. At the beginning of the visit, study personnel will fit you with the Parvo cart mouth-piece, Polar heart rate monitor, and ActiGraph accelerometers at the hip and wrist locations. You will first sit quietly for 20 minutes to establish a seated  $VO_2$  baseline measurement (you will have the choice to watch TV while sitting quietly). During this visit, you will perform three bouts of exercise in random order including a 30-second bout, a 1-minute bout, and a 2-minute bout. After establishing your seated VO<sub>2</sub> baseline, you will be asked to step onto the treadmill and straddle the treadmill belt. Study personnel will turn on the treadmill to the speed that corresponds with 80% of your VO<sub>2</sub> peak calculated using data from the VO<sub>2</sub> peak test during the first visit. You will step onto the treadmill belt and run at this speed for either 30 seconds, 60 seconds, or 120 seconds. At the end of your bout, you will be asked to straddle the treadmill belt and the treadmill will be stopped. Immediately after the exercise bout, study personnel will instruct you to sit for 30 minutes. After your rest you will repeat the protocol two more times for the other two durations at 80% VO<sub>2</sub> peak. After each of the bouts, study personnel will instruct you to sit and rest for 30 minutes.

Once your final bout and rest is complete, study personnel will remove the equipment and provide you with water and a snack.

Visit 3 (~5 hours): Your third visit will be completed between 2 and 14 days after visit 2. Visit 3 will occur at the Center of Human Health and Performance (CH2P) Exercise Training Room. You will be asked to arrive in the morning at 7:00am to the Exercise Training Room. At the beginning of the visit, study personnel will fit you with the Parvo cart mouth-piece, Polar heart rate monitor, and ActiGraph accelerometers at the hip and wrist locations. You will first sit quietly for 60 minutes to establish a lying VO<sub>2</sub> baseline measurement (you will have the choice to watch TV while laying quietly). After your rest, you will have the opportunity to use the bathroom and drink water before beginning a 10-minute warm up on the treadmill. The first 5 minutes will be a walking warm up at 3mph, the last 5 minutes of the warm up will be an easy jog at 5mph. After the warm up, the exercise session will begin. You will run at the speed that corresponds with 80% of your VO<sub>2</sub> peak for 30 minutes. After 30 minutes the treadmill will be stopped and you will be asked to sit down and rest for 60 minutes. All treadmill speed changes will be made by study personnel, who will give you a verbal warning 15 seconds prior to any speed changes and a 5-second countdown. You can stop the treadmill at any time by pulling the safety cord clipped to your waistband. After 60 minutes, you will have another opportunity to use the bathroom and drink water. Then you will be asked to lay back down for another 2 hours until the termination of this visit. At the end of your visit, you will be provided a snack (protein bar) and an electrolyte drink. During all the lying rest of this visit, you will be allowed to watch your choice of TV to prevent you from falling asleep and to keep you entertained.

#### 7. WILL BEING IN THIS RESEARCH STUDY HELP ME IN ANY WAY?

You may not directly benefit from this research. However, if you are interested in knowing your body composition (DXA) and maximal aerobic capacity (VO<sub>2</sub> peak) test results, study personnel will share and explain these data if you request.

#### 8. WHAT ARE MY RISKS OF BEING IN THIS RESEARCH STUDY?

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 Peak Testing and treadmill running: Risks 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. The emergency cord attached to the treadmill will be utilized so that, in case of exhaustion or a fall, the pulling of the

emergency cord will immediately stop the treadmill belt. Two trained researchers will also be standing next to the treadmill (one behind and one to the side) ready to assist if needed.

DXA Scan: Scans expose you to low-levels of X-Ray ionizing radiation. The risk associated with this type of scan is very low, similar to 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 your lifetime limit for being exposed to ionizing radiation, then no scan will be performed.

Parvo Metabolic cart: Use of the Parvo metabolic cart requires a face mask to be placed you through which you breathe. This mask can cause discomfort when worn for long periods of time. The built-in break periods during visit 3 are designed to minimize this discomfort by allowing you a break from wearing the mask. In addition, the straps securing the mask onto your face will be loosened as much as they can during the rest periods to minimize discomfort.

We believe there are minimal risks associated with this research study; however, a risk of breach of confidentiality always exists and we have taken the steps to minimize this risk as outlined in section 9 below.

#### 9. HOW WILL MY PERSONAL INFORMATION BE PROTECTED?

Your privacy and confidentiality are important to us. The following procedures will be used to protect the confidentiality of your study records. The electronic forms with your name or signature on them (the Telephone Screening Form, the Informed Consent, Physical Activity Readiness Questionnaire (PARQ), DXA safety screening form) will be stored securely on an electronic database that authorized study staff will only access from password protected computers using user-specific log-in credentials. Any physical paper versions that will have your name on them will be stored securely in a locked filing cabinet, which is located in a locked office. All other data collection sheets (electronic or paper) will be labeled with a code instead of your name, so that no potential identifiers can be linked back to you. Data collection sheets and the master key of participant codes and names are kept separate from all other study materials in separate locked filing cabinets, which are also located in a locked office. The master key will be destroyed 6 years after the close of the study. All electronic data will be kept on a password-protected computer in a locked office to prevent access by unauthorized users. Only the members of the research staff will have access to the passwords. At the conclusion of this study, the researchers may publish their findings. Information will be presented in summary format and you will not be identified in any publications or presentations. Your privacy will be protected by conducting the study procedures in a private location with only authorized research team members present.

#### 10. WHAT IF THERE IS AN UNEXPECTED FINDING ON MY DXA 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 physician (for DXA). 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.

# 11. WILL MY INFORMATION (BIOSPECIMENS OR PRIVATE INFORMATION) BE USED FOR RESEARCH IN THE FUTURE?

Identifiers might be removed and the de-identified information or biospecimens may be used for future research without additional informed consent from you.

#### 12. WILL I BE GIVEN ANY MONEY OR OTHER COMPENSATION FOR BEING IN THIS RESEARCH STUDY?

You will be compensated for your participation with \$100 cash. We will prorate the compensation to \$10 cash for visit 1, \$10 for visit 2, \$80 for visit 3.

Since you are being compensated for your participation in this study, your personal information may be released to the accounting officials at University of Massachusetts, Amherst. If payment to a research participant is \$600 or more in any one calendar year, the University of Massachusetts, Amherst is required to report this information to the IRS as taxable income. This information will be kept confidential and will only be used to process payment.

#### **13. WHO CAN I TALK TO IF I HAVE QUESTIONS?**

Take as long as you like before you make a decision. 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 researchers via Nicholas Remillard at nremillard@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.

#### 14. WHAT HAPPENS IF I SAY YES, BUT I CHANGE MY MIND LATER?

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.

#### **15. 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.

#### **16. 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 which I use. I have had the opportunity to ask questions and have received satisfactory answers. I have been informed that I can withdraw at any time. A copy of this signed Informed Consent Form has been given to me.

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:

## **Appendix B**

Godin-Shepherd Leisure Time Questionnaire

#### Figure 1: THE GODIN AND SHEPHARD LEISURE-TIME PHYSICAL ACTIVITY QUESTIONNAIRE

During a typical **7-day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).

9	Times per week
STRENOUS EXERCISE (HEART BEATS RAPIDLY) (e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)	
MODERATE EXERCISE (NOT EXHAUSTING) (e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	
MILD EXERCISE (MINIMAL EFFORT) (e.g., yoga, archery, fishing from river bank, bowling, horseshoeing, golf without using a cart, snow-mobiling, easy walking)	

Adapted from Godin, G. (1983). Psychosocial factors influencing intentions to exercise in young students. Graduate Department of Community Health, University of Toronto, Toronto.

### Weekly leisure--time activity score = (9 × Strenuous) + (5 × Moderate) + (3 × Mild) EXAMPLES FOR COMPUTING THEOVERALL SCORE

- Strenuous = 2 times/wk
  - 1. Moderate = 2 times/wk
    - Mild = 7 times/wk

Total leisure---time activity score

 $= (9 \times 2) + (5 \times 2) + (3 \times 7)$ 

= 18 + 10 + 21 = 49 units

## Appendix C iDXA Screening Form

Study PI

Participant Identifier\_\_\_\_\_

Date of Visit

1) Height (in.)

2) Weight (lbs.)

3) Month and Year of birth

4) Sex Male Female Intersex

a. If female or intersex: 'Are you or might you be pregnant?'

Yes No I don't know \*If 'Yes, ' no iDXA will be performed

b) If 'I don't know': 'Would you be willing to take a urine pregnancy test?'

Yes No \*If 'No, ' no iDXA will be performed

c) If participant consents to a pregnancy test, administer test. Test results:

Positive Negative \*If 'Positive,' refer to UHS Women's Health Clinic

5) Have you ever been told that you have reached your lifetime limit for being exposed to ionizingradiation?

Yes No

\*If 'Yes' no iDXA will be performed

For iDXA technician only:

1. Was a scan performed?

Yes No

2. Were the results placed in .pdf format in the shared folder for MD review? Yes No

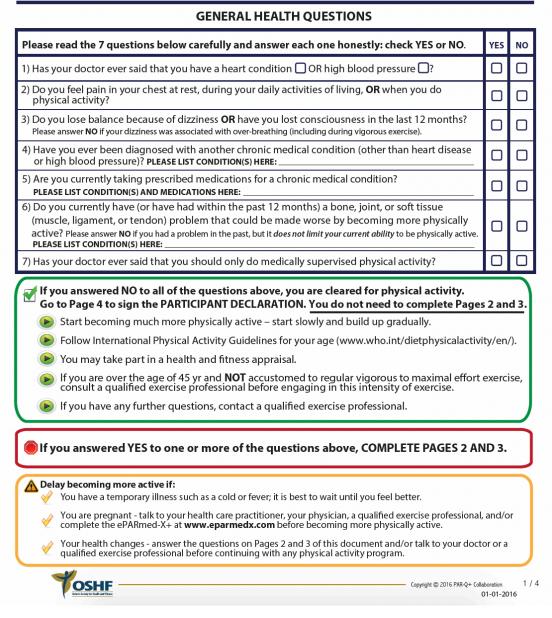
Technician Signature:

## Appendix D PARO

# 2016 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.



# 2016 PAR-Q+ FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

Do you have Arthritis, Osteoporosis, or Back Problems? 1. If the above condition(s) is/are present, answer questions 1a-1c If NO go to question 2 1a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)? 1b. Have you had steroid injections or taken steroid tablets regularly for more than 3 months? 1c. YES NO 2. Do you currently have Cancer of any kind? If NO go to question 3 If the above condition(s) is/are present, answer questions 2a-2b Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck? 2a. 2b. Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)? Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, 3. Diagnosed Abnormality of Heart Rhythm If **NO** go to question 4 If the above condition(s) is/are present, answer questions 3a-3d Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) 3a. YES NO Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction) 3b 3c. Do you have chronic heart failure? YES NO 3d. Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months? YES NO 4. Do you have High Blood Pressure? If **NO** go to question 5 If the above condition(s) is/are present, answer questions 4a-4b Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) 4a. YES NO 4b. Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure) 5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes If the above condition(s) is/are present, answer questions 5a-5e If NO go to question 6 5a. Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies? YES NO Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness. 5b. YES NO 50 Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet? YES NO 5d. Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)? YES NO 5e. Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future? YES NO Copyright © 2016 PAR-Q+ Collaboration OSHF 2/4

01-01-2016

	<b>2016 PAR-Q+</b>	
6.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dement Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome	ia,
	If the above condition(s) is/are present, answer questions 6a-6b If <b>NO</b> go to question 7	
6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer <b>NO</b> if you are not currently taking medications or other treatments)	YES NO
6b.	Do you have Down Syndrome AND back problems affecting nerves or muscles?	YES NO
7.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary Hig Blood Pressure	
	If the above condition(s) is/are present, answer questions 7a-7d If NO 🗌 go to question 8	
7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer <b>NO</b> if you are not currently taking medications or other treatments)	
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES NO
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	
8.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia If the above condition(s) is/are present, answer questions 8a-8c If NO 🗌 go to question 9	
Ba.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer <b>NO</b> if you are not currently taking medications or other treatments)	YES NO
Bb.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	
Bc.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES NO
9.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event If the above condition(s) is/are present, answer questions 9a-9c If <b>NO</b> go to question 10	
9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer <b>NO</b> if you are not currently taking medications or other treatments)	
9b.	Do you have any impairment in walking or mobility?	
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	
10.	Do you have any other medical condition not listed above or do you have two or more medical co	nditions?
	If you have other medical conditions, answer questions 10a-10c If NO 🗌 read the Page 4 re	commendation
10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months <b>OR</b> have you had a diagnosed concussion within the last 12 months?	YES NO
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES NO
10c.	Do you currently live with two or more medical conditions?	YES NO
	PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE:	

# GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

OSHF Date beidy for Real Prices

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2016	PAR-Q+		
vou are ready to become more physicall	IP questions about your medical condition, ly active - sign the PARTICIPANT DECLARATION below: e professional to help you develop a safe and effective physical		
You are encouraged to start slowly and build up 3-5 days per week including aerobic and muscle	o gradually - 20 to 60 minutes of low to moderate intensity exercise, e strengthening exercises.		
	150 minutes or more of moderate intensity physical activity per week.		
If you are over the age of 45 yr and <b>NOT</b> accusted qualified exercise professional before engaging	omed to regular vigorous to maximal effort exercise, consult a in this intensity of exercise.		
You should seek further information before becoming	the follow-up questions about your medical condition: g more physically active or engaging in a fitness appraisal. You should complete recommendations program - the ePARmed-X+ at www.eparmedx.com and/or a the ePARmed-X+ and for further information.		
Delay becoming more active if:			
You have a temporary illness such as a cold or fe	ever; it is best to wait until you feel better.		
You are pregnant - talk to your health care pract and/or complete the ePARmed-X+ <b>at www.epa</b>	You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.		
Your health changes - talk to your doctor or qua activity program.	alified exercise professional before continuing with any physical		
The authors, the PAR-Q+ Collaboration, partner orga	must use the entire questionnaire and NO changes are permitted. anizations, and their agents assume no liability for persons who PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire,		
	IPANT DECLARATION		
• All persons who have completed the PAR-Q+ please	<u>,</u>		
<ul> <li>If you are less than the legal age required for consen provider must also sign this form.</li> </ul>	it or require the assent of a care provider, your parent, guardian or care		
physical activity clearance is valid for a maximum of condition changes. I also acknowledge that a Trustee or other designate) may retain a copy of this form for to local, national, and international guidelines regar	satisfaction and completed this questionnaire. I acknowledge that this f 12 months from the date it is completed and becomes invalid if my e (such as my employer, community/fitness centre, health care provider, r their records. In these instances, the Trustee will be required to adhere rding the storage of personal health information ensuring that the d does not misuse or wrongfully disclose such information.		
NAME	DATE		
SIGNATURE	WITNESS		
SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER			
For more information, please contact	The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+		
www.eparmedx.com			
Email: eparmedx@gmail.com Citation for PAR-Q+ Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration.	Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry		
Email: eparmedx@gmail.com Citation for PAR-Q+	Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the		
Catalon for PAR-Q+ Werburton DER, Jamrik VK, Bredin SSD, and Gleichill N on behaf of the PAR-Q+ Collaboration. The Physical Activity Readines SQuarting for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+), Health & Ritness Journal of Canada 4(2):3-23, 2011. Key References 1. Jamrik VK, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gleichill N. Enhancing th	Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry		
Email: eparmedx@gmail.com Watburton DER, Jammik VK, Bredin SSD, and Gledhill Non behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaite for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (#PARMedX+); Health Stimesa Journal of Canada 4(2):3-23, 2011. Key References 1. Jammik VK, Watburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing th 2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard	Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jammik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services. ne effectiveness of clearance for physical activity participation; background and overall process. APMM 36(51):53-513, 2011. RI: Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM bla Medical Journal. 1975;17:375-378.		

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