

University of Montana

## ScholarWorks at University of Montana

---

Graduate Student Theses, Dissertations, &  
Professional Papers

Graduate School

---

2022

### 24-HOUR TOTAL ENERGY EXPENDITURE DURING ULTRA- ENDURANCE CROSS-COUNTRY CYCLING

Patrick S. Dodds

Follow this and additional works at: <https://scholarworks.umt.edu/etd>

**Let us know how access to this document benefits you.**

---

#### Recommended Citation

Dodds, Patrick S., "24-HOUR TOTAL ENERGY EXPENDITURE DURING ULTRA-ENDURANCE CROSS-COUNTRY CYCLING" (2022). *Graduate Student Theses, Dissertations, & Professional Papers*. 11937. <https://scholarworks.umt.edu/etd/11937>

This Thesis is brought to you for free and open access by the Graduate School at ScholarWorks at University of Montana. It has been accepted for inclusion in Graduate Student Theses, Dissertations, & Professional Papers by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact [scholarworks@mso.umt.edu](mailto:scholarworks@mso.umt.edu).

24-HOUR TOTAL ENERGY EXPENDITURE DURING ULTRA-ENDURANCE  
CROSS-COUNTRY CYCLING

By  
Patrick Sebastian Dodds  
B.S. University of Montana  
Missoula MT

Thesis Paper  
presented in partial fulfillment of the requirements for the degree of:

Master of Science  
Integrative Physiology and Athletic Training, Exercise Science

The University of Montana  
Missoula, MT

May 2022

Approved by:  
Scott Whittenburg, Dean of The Graduate School Graduate School

Brent Ruby, Chair WPEM IPAT

John Quindry, Committee Member IPAT

Rich Willy, Committee Member IPAT

## Table of Contents

### Chapter I: Introduction

Introduction	2
Purpose & problem	8
Hypothesis	8
Limitations	8
Delimitation	8

### Chapter II: Review of literature

Carbohydrate intake	13
Doubly labeled water	16
Water turnover and comparison of total energy expenditure and intake	18
Total daily energy intake	20
Table 1. Doubly labeled water studies	22

### Chapter III: Methodology

Methodology	23
Mudslinger 24 ultra-endurance MTB event	26
Experimental design	26

### Chapter IV: Manuscript for submission

International Journal of Sport Nutrition and Exercise Metabolism	27
Manuscript table and figures	37
Manuscript references	41
Comprehensive reference List	45

Dodds, Patrick, M.A., May 2022  
Chair: Brent Ruby  
Co-Chair: Rich Willy, John Quindry

**Chapter Four: Manuscript for International Journal for Sports Nutrition and  
Exercise Metabolism**

**ABSTRACT**

Previous studies have used the doubly labelled water (DLW) method to evaluate the total energy expenditure (TEE) during Ironman, ultra-marathon trail runs and competitive road cycling. However, the technique has not been applied to a 24-hour cross-country mountain bike event. This case study examined the TEE, cycling metrics and *ad libitum* nutrient/fluid intake in a trained male cyclist during a 24-hour cross-country mountain bike race. A trained male cyclist (41 y, 74.1 kg, 172.4 cm) received an oral dose of DLW prior to the 24-hour event for the calculations of TEE and water turnover ( $rH_2O$ ). Nude body weight and urine samples were collected pre, during, and post-race. Total nutrient (TNI) and fluid intake (TFI) and cycling metrics (speed, power output, cadence, HR) were continuously quantified during the event. The rider completed 383 km coupled with a vertical gain of ~7,737 m (GPS) during the 24-hour event. Average speed, power and HR were  $16.3 \pm 2$  km·hr<sup>-1</sup>,  $122 \pm 29$  W, and  $134 \pm 18$  bpm, respectively. TEE and TNI were 41 and 23.5 MJ, respectively. Total carbohydrate intake was 1192 g with an average hourly intake of  $58 \pm 22$  g·hr<sup>-1</sup>. Total body weight was 75.3 and 72.3 kg pre- and post-race, respectively with an *ad libitum* total fluid intake of 13.3 L. These data provide foundational measures of TEE, TEI and TFI during an ultra-endurance cross-country mountain biking event which can provide for future race/training strategies.

## **Chapter I: Introduction**

### **Introduction**

Humans are capable of a wide range of energy expenditure which has manifested itself differently throughout history. Early humans relied on the persistence hunting of wild animals, often tracking their prey to the point of collapse from heat exhaustion [1], [2]. Out of necessity, hominids evolved to maintain high levels of total daily energy expenditure (TDEE) to support migration, escape, scavenging and hunting (MESH) [3], [4]. The development of agriculture, farming equipment and readily available food in modern societies has decreased the need to maintain high levels of TDEE out of necessity. Regardless, the relationship between adequate daily physical activity, overall health, and the propensity of metabolic disease is well established [5], [6]. Humans have developed other ways to utilize their ability to have elevated energy expenditures. Large scale, popular endurance events such as the Tour de France and Western States 100, where the athletes are observed to maintain a TDEE of  $32.7 \pm 1.6 \text{ MJ}\cdot 24^{-1}$  ( $7,815 \pm 386 \text{ kcals}\cdot 24^{-1}$ ) and  $68.2 \pm 12.4 \text{ MJ}\cdot 24^{-1}$  ( $16,310 \pm 2,960 \text{ kcals}\cdot 24^{-1}$ ), respectively, [4], [7] demonstrate that humans can maintain very high levels of TDEE inversely proportionate to the duration of the task or event.

TDEE is quantified as the sum of resting metabolic rate (RMR), dietary induced thermogenesis (DIT), and the energy expended during physical activity (EEA). RMR is defined as the basal rate of energy expenditure immediately following sleep. DIT is the increase in metabolic rate following ingestion of a meal [8], associated with digestion and estimated at approximately 10% of the TDEE. Physical activity is defined as voluntary

movement of skeletal muscle which increases energy expenditure above the RMR [9]. Speakman and Selman demonstrate that for most individuals who undertake a sedentary lifestyle, the majority of TEE is accounted for by the RMR (60%) [10]. However, for those that engage in physical activity, training, and competition, EEA can account for 80-90% of TEE [11], [12], increasing TDEE by 2-6.9 x RMR [4], [13]–[15]. Research also indicates that TDEE can be elevated significantly (8-12 x RMR) during endurance and ultra-endurance events over 12 – 24 hours [4], [16].

Levine et al. [9] highlights different methods for measuring energy expenditure. Two of the more popular lab-based methods are indirect and direct calorimetry. Direct calorimetry measures the heat lost from the body while indirect calorimetry measures expired gas concentrations and volumes to calculate the metabolic cost of the time frame over which the gas was collected. Forms of indirect calorimetry systems include ventilated open and closed-circuit systems. Ventilated open-circuit systems measure the subject's expired breath with a metabolic cart device (either stationary or portable), whereas closed-circuit systems require the subject to be placed in a room or respiratory chamber of known volume before the air in the chamber is sampled and measured over extended measurement periods (24 hours).

Levine et al. [9] discusses other methods of measuring energy expenditure including isothermal systems, heat sink or adiabatic systems and convention systems. An isothermal system calorimeter consists of a chamber lined with a layer of insulating material with both inner and outer aspects of the chamber being in thermal equilibrium

achieved by circulating fluid through the walls. The change in temperature gradient within the walls of the chamber is proportional to the non-evaporative heat loss from the subject in the calorimeter. Heat sink or adiabatic systems measure heat extracted from the subject by a liquid-cooled heat exchanger. Convection system calorimeters consist of an insulated chamber ventilated with air flow at a known rate. Heat lost by a subject inside the chamber is calculated from the specific capacity of the air and increase in temperature as ventilation is leaving the chamber. Other ways of measuring energy expenditure involve both a rigid and flexible total collection system which is measured with a Douglas bag or a Tissot gasometer; however, these methods are limited by their inability to measure real-world scenarios outside of the laboratory under free-living conditions.

Collecting reliable data in free-living scenarios has evolved since Durnin and Brockway discussed the uncertainty of indirect calorimetry using the Douglas bag method in 1958 [17]. Uncertainties include errors in the technique of measuring different activities and taking measurements after the event rather than during the event [17]. Modern tools have been developed such as portable metabolic systems, expiratory collection, open-circuit, and indirect calorimeters (e.g., The Douglas bag as previously mentioned); these may facilitate short-term field-based measurements of oxygen consumption and expenditure at rest and during routine activities. Portable metabolic systems can be used in the field but have limitations due to terrain, electricity supply, and the duration of potential observation windows. When the limitations do not allow for the use of portable equipment, researchers have utilized the linear relationship between heart rate (HR) and  $VO_2$  which has been implemented in studies to estimate free-living TDEE [18]. The

combination of HR and activity level give a better estimate of TDEE [19] while eliminating the need for bulky equipment. The linear relationship between HR and oxygen consumption can provide prediction relationships for the estimation of energy expenditure [20]. However, this approach is vulnerable to a variety of confounding variables [20]. HR may dissociate from energy expenditure because of changes in emotion, posture, and environmental factors [21]. The relationship between HR and energy expenditure is reliably linear within a narrow range of approximately 90 – 150 beats<sup>-1</sup> min [22], [23]. Individualized equations developed through lab based trials (graded exercise VO<sub>2</sub> max testing) can be used to increase the accuracy of the HR – oxygen consumption relationship [22] within a single subject. Therefore, when attempting to estimate rates of TDEE in a variety of free-living individuals, commonplace laboratory equipment cannot be employed beyond short, steady-state collection periods.

The doubly labeled water (DLW) methodology is considered the gold standard for determining TDEE in free-living scenarios over multiple days, with 2% accuracy and 2-10% precision [24]–[27]. This method was developed by Lifson in the 1940's to study TDEE in small mammals over the course of multiple days and was later implemented in humans in the 1980's by Schoeller and colleagues [25]. In the years following, there has been a steady increase in the use of DLW across a range of human subject research [28]. Although DLW was developed to measure TDEE throughout multiple days, this method has proven effective in shorter time frames as well, provided that the water turnover rate and the EE is high enough to increase the effective rate of isotope loss [29] [4]. The DLW method has been used to determine TDEE in high performing occupational and athletic



scenarios, ranging from wildland firefighters to multi day cycling events [13], [16], [30]–[33]. Plasqui et al. [30] evaluated seven male cyclists during the 24-day Giro D'Italia professional road cycling race. The race traversed 3445 km and included 10 mountain stages [30]. The cyclists maintained an average TDEE  $32.3 \pm 3.4 \text{ MJ}^{-1} \text{ day}$  ( $7,719 \pm 812 \text{ kcal} \cdot \text{day}^{-1}$ ) [30]. Saris et al. [34] used the same approach and studied five cyclists during the 22 day Tour De France. The observed average TDEE was  $25.4 \pm 1.4 \text{ MJ} \cdot \text{day}^{-1}$  ( $6,070 \pm 334 \text{ kcal} \cdot \text{day}^{-1}$ ) which was accompanied with an average energy intake of  $24.7 \pm 2.4 \text{ MJ} \cdot \text{day}^{-1}$  ( $5,903 \pm 573 \text{ kcal} \cdot \text{day}^{-1}$ ) [34].

### Cycling Overview

Cycling is a complex sport with several physiological, mechanical, and environmental factors which influence power and speed [35]. A review by Atkinson et al. outlines the various components of successful road cycling performance including, bike design, inherent physiological ability (type 1 or type 2 fiber distribution), rider position, cycling velocities and nutrition strategies [36]. The dominating component which can separate a rider from the Peloton is peak power output and the ability to sustain relatively high power output for an increased duration [35], [36]. Researchers have shown a strong association between power output and  $\text{VO}_2$  [7], [37], [38]. The relationship proves to be curvilinear over long durations due to other physiological components such as blood lactate accumulation and the recruitment of less efficient muscle fibers [38]–[40]. Lucia et al. [37] compared elite and professional road cyclists by performing a ramp protocol where  $\text{VO}_2$ , pulmonary ventilation, respiratory exchange ratio, ventilatory threshold, blood lactate, and electromyographic activity were recorded. Significant differences were

observed at the absolute submaximal intensities whereby the elite athletes maintained a higher power output and  $\text{VO}_2$  with little change in other physiological measurements (lactate, ventilation) [37]. Tour De France riders commonly demonstrate sustained peak power outputs between 400 – 450 W with values higher than 500 W during the most competitive time trial performances [7]. The ability to sustain high power output is commensurate with high values for  $\text{VO}_{2\text{max}}$  (mean values: 70 – 80  $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) in professional cyclists [7]. The maintenance of these high-power outputs and  $\text{VO}_2$  in the Tour De France stages, which ranges from 16 mi to 136 mi in length, lead to elevated TDEE such as the 32.4 MJ (7,743 kcal) previously discussed [7], [34]. Road cycling is most often performed on evenly paved surfaces which allows athletes to draft off teammates or competitors, easily coast downhill and focus primarily on maintaining power output at high speeds. When cycling is performed on uneven terrain where the athlete needs to be aware of rocks, gravel, and other obstacles, the ability to draft off competitors, easily coast downhill, and maintain power output increases in difficulty.

Mountain biking (MTB) is a popular outdoor recreational activity that has progressed into a competitive sport [41]. MTB competitions include cross-country circuit races, regional, national, and international Olympic level events. MTB introduces varied levels of energy expenditure with climbing, sprinting, and pacing intervals as opposed to the more commonplace steady state patterns of road cycling. For these reasons, MTB has high intensity interval (HIIT) sections within the race [44] [45]. Cyclist are required to traverse varied terrain such as rocky paths, technical single-track, and open forestry roads, all of which often include jumps and different level drops [43]. The uneven terrain

inherent with MTB, increases upper body activity and therefore alters the energy expended during the duration of the activity [44]. Many studies show changes in HR,  $VO_2$ , skeletal muscle activity, peak power, lactate threshold, and onset of blood lactate (OBLA) during a MTB race [41]; however, no studies have attempted to quantify the TDEE over the course of a 24-hour ultra-endurance MTB race.

### Purpose & Problem

Many of the studies focused on TDEE, nutrition, and water turnover within athletes that maintain a high level of EEA in shorter timeframes are focused around road racing [7], [30]. **Road cycling** omits the intermittent tasks, difficult terrain and the increases in upper body activity unique to MTB. Although DLW has been used for other 24 hour ultra-endurance competitions [4], these results cannot be reliably extrapolated to ultra-endurance MTB events.

The purpose of this study was to measure the total energy expenditure and energy intake of a professional rider participating in a 24-hour MTB race. These findings can be used to more specifically inform training, fluid, and food intake plans, prior to and during participation in ultra-endurance cross-country cycling events.

### Hypothesis

We hypothesize that the TEE will be between 5 – 9 x RMR, similar to the findings of previous research including Ironman and cycling performances [16], [30] [4].

## Limitations and Delimitations

The DLW method was originally developed for research in small mammals before being used in sedentary humans. The use of DLW in high energy expenditure free-range humans introduces different methodological limitations due to background isotopic enrichment shifts caused by geographic variations and consumed water sources and accelerated isotopic elimination rates. Methodological limitations that can lead to inaccurate or abnormal readings are listed below.

- The variables that come with increased EEA include changes in sweat rate and ventilation.
  - Increases in sweat rate which eliminates the labeled oxygen and deuterium from the body at a higher rate.
  - Environmental effects on isotope enrichment [45], [33].
  - Increases in ambient temperature leads to a higher water turnover and an increase in fluid consumption [46] altering isotope dilution.
- Isotopic levels can vary between water sources from different geographic locations, altering a subject's baseline isotope levels if traveling during data collection.
- Normal baseline geographic isotopes can influence the subject's baseline biologic isotope levels.
- An increase in elimination rates will lead to a decrease in the accuracy of the DLW method if not accounted for.

- The measurements were only taken on one subject. This will limit the external validity of our data.

Limitations in DLW can be overcome by altering isotopic dose, sample collection interval, or adding a control group [45], [47], [48]. Many studies include the implementation .... these changes to ensure proper elimination rates in real world, high performance, scenarios. DLW has been used in many high EE activities including Ironman [16] [4], WS 100 [4], wildland firefighting [31], and military training [49], [50]. Chapter two outlines prior investigations that have utilized the DLW methodology and their respective findings. The DLW methodology only calculates the total energy expended during the measurement window, most often expressed as MJ·24 hours<sup>-1</sup> and cannot account for higher resolution (hourly variations). However, during extended competition or work, this limitation in measurement resolution is accepted since the technique does not inhibit the athlete's performance or the need for continuous sampling. The population of athletes that can perform a 24-hour cross-country MTB race at the highly trained level is limited and novel in the literature; therefore, the limitation of a case study is acceptable. Moreover, application of gold standard methodology has not been utilized to consider the 24-hour energy demands of an ultra-endurance MTB competitor.

## **Chapter two: Review of Literature**

Humans can sustain high levels of total daily energy expenditure (TDEE) for extended durations. TDEE is the sum of resting metabolic rate (RMR), the thermic effect of food (DIT), and energy expended during physical activity (EEA). When homeostasis is disrupted, TDEE will change in response to the level of activity [9], diseased state [51], or injury [52]. Musculoskeletal activity (physical activity) dominates the magnitude of TDEE and can be voluntarily changed. Studies on TDEE include a wide range of activity from a sedentary desk job as low as  $0.09 \times \text{RMR}$  [48], to climbing Mount Everest at  $3 \times \text{RMR}$  [53] and even as high as  $8.5 \pm 1.5 \times \text{RMR}$  during 24 hours of ultra-marathon trail running [4]. Modalities such as cycling, running, MTB, swimming, hiking, and walking have different characteristics that can alter short term measures of steady state  $\text{VO}_2$  and extended measures of TDEE. Scott et al. compared oxygen uptake and energy expenditure during uphill running and road cycling [54]. They concluded lactate threshold, oxygen uptake, and excess post oxygen consumption were similar for uphill running and road cycling when performed at high non-steady state intensities for one minute, then summed the total aerobic and anaerobic energy expenditure over the course of the one minute exercise (aerobic + anaerobic exercise energy expenditure) and found an absolute total energy expenditure of  $118.0 \pm 1.9 \text{ kJ}$  and  $125.4 \pm 19.2 \text{ kJ}$  for cycling and running respectively ( $p = 0.90$ ) [54]. Statistical significance occurred when separating aerobic and anaerobic energy expenditures. Anaerobic energy expenditure was significantly greater for cycling ( $32.7 \pm 8.9 \text{ kJ}$ ) versus running ( $22.5 \pm 11.1 \text{ kJ}$ ) ( $p = 0.009$ ). Scott et al. claims that the differences shown in the anaerobic state is due to the rapid glycolytic ATP re-synthesis component, which was 28% for cycling and 17% for running. Bijker et al. has

further described this discrepancy by comparing the delta efficiency between running and road cycling (mean efficiency during running (42%) and cycling (25%)) [55]. They attribute the differences to the mostly concentric nature of cycling, which differ from running which includes eccentric, isometric and concentric contraction characteristics [55]. Different characteristics of each movement pattern and/or mode of activity's alters the expected  $VO_2$  and EE during both short and extended measurement periods.

Off road cycling and MTB occurs across varied and often rugged terrain, in contrast to traditional road cycling. MacDermid et al. [56] measured  $VO_2$  (measured with a portable gas analyzer), cadence, speed, power, and geographical position throughout two MTB races. Their findings show a significant change in  $VO_2$ , cadence, speed, and power when geographical position changed from downhill (decreased variables) to uphill (increased variables). Their research also showed an all-out sprint in the first 500m of each race which led to a  $O_2$  deficit of  $\sim 1.58 \pm 0.67 \text{ L}^{-1} \text{ min}$ . MacDermid and colleagues' findings show a that MTB race is characterized by fast start sprints, numerous climbs, and long descents [56]. By favoring high intensity intervals and deviating from steady state power output, MTB can induce significant fluctuations in HR, systemic fatigue, local muscular fatigue, and altered fuel utilization [57], [58]. Schubert et al., demonstrates a 2 x RMR increase in TEE due to three 20-second all out cycling sprints performed in a lab. The increase in TEE indicates an increase in TEE due to HIIT [59]. Competitive MTB is a high intensity sport which may include a wide range of expected exercise intensities and a range of physiological measures (HR,  $VO_2$ ) [41], [56]. The nature of a High intensity work load is exaugurated when the whole body is engaged.

Hurst et al. [44] measured upper body muscle activity during MTB on man-made and natural terrain descents. They found a significant increase in isometric contraction of upper body muscle during downhill MTB on natural terrain [44]. Isometric contractions can lead to an increase in energy expenditure of  $312.2 \pm 125.7 \text{ kJ}\cdot\text{min}^{-1}$  ( $17.6 \pm 30.0 \text{ kcal}\cdot\text{min}^{-1}$  [60]. The increase in upper body isometric activity points to a potential increase in  $\text{VO}_2$  during technical sections, despite minimal power output while coasting downhill. The examples above represent an amalgamation of variables which separates MTB racing from other endurance counterparts. The variables that separate MTB from other modalities have not been observed within a highly trained, free-living athlete in a competitive race. The use of the DLW method during an ultra-endurance MTB race will improve the specificity for nutritional and fluid delivery recommendations in these events and broaden the overall understanding of the physiology of this unique sport.

### Carbohydrate Intake

Athletes are encouraged to consume a high carbohydrate (CHO) diet before, during, and after endurance races [61]. These elevated levels of CHO replenish CHO stores within the muscle (glycogen) and extend time to exhaustion [61]. The International Olympic Committee on nutrition for athletes states, "A high CHO diet in the days before competition will help enhance performance, particularly when exercise lasts longer than about 60 min" and "Athletes should aim to achieve CHO intakes that meet the fuel requirements of their training programs and adequately replace their CHO stores during the recovery between training sessions and competitions" [62]. In the early 1900's Krogh et al. identified the importance of CHO as a fuel source during exercise when they



compared high fat diets with high CHO diets [63]. They found that the subjects maintained a higher tolerance to exercise when provided a high CHO diet [63]. Levine et al. furthered the observation by measuring blood glucose concentration in athletes performing in the 1923 Boston Marathon [64]. They concluded fatigue brought on at the end of the marathon was due in part to the observed low blood glucose levels [64]. After identifying hypoglycemia in the runners, they encouraged athletes to consume CHO during the next Boston Marathon which led to a significant improvement in running performance [64]. These foundational descriptive studies serve as the building blocks which led to modern, commonly held sport nutrition and feeding strategies.

Ivy et al. demonstrated that CHO timing can influence post-exercise rates of glycogen resynthesis [65]. Subjects cycled for 70 min at ~ 80%  $\text{VO}_2\text{max}$  and were given oral CHO supplements equivalent to 2  $\text{g}\cdot\text{kg}^{-1}$  body weight directly following the bout of exercise. Muscle biopsies and blood draws were taken at 0, 2, and 4 hours post exercise to measure glycogen resynthesis, insulin, and glucose levels, respectively [65]. They concluded that the optimal time to consume CHO to maintain blood insulin and glucose while resynthesizing glycogen is directly following a bout of exercise. Research on CHO intake has shown its importance in fuel supply and regulation, endurance performance, rate of perceived exertion, the enhancement of tissue repair and augmentation of muscle protein synthesis [61], [62], [65]–[68]. However, the vast majority of CHO intake and recovery oriented investigations are limited to a few hours of activity. There are some studies which investigate CHO intake during extended durations.

Harger-Domitrovich et al. [69] studied men and women during two 10 hr trials using a combination of arm ergometer, cycle, and treadmill exercise. Subjects were given 0.6 g·kg<sup>-1</sup>·fat free mass<sup>-1</sup> of CHO during one trial and a placebo for the other. They observed the trial with the CHO drink maintained whole-body CHO oxidation when compared with the placebo group. Additionally, they measured 52% higher net glycogen usage within the placebo group [69]. These data demonstrate that ingestion of CHO during long-duration exercise ameliorates net muscle glycogen use and augments whole-body CHO oxidation. The positive adaptations that accompany CHO supplementation have focused additional research to delineate the recommended hourly intake of exogenous CHO sources during exercise.

Research has demonstrated the most advantageous amount of CHO intake in g that will yield better performances. Smith et al. [70] studied 12 cyclist during a 2 h constant-load ride ~ 77% peak O<sub>2</sub> uptake followed by a 20-km (12.4 mi) time trial. In their cross over design, 3 different hourly doses of CHO were evaluated relative to exercise performance. Their research model suggested an optimal dose 60 g·hr<sup>-1</sup> when compared to 15 and 30 g·hr<sup>-1</sup> [70]. Jeukendrup et al. [61] furthered the research by studying subjects performing 2-5 hr of cycling at 51 – 64% VO<sub>2max</sub> and concluded when events last longer than 2 hr, a CHO dose of 60 – 90 g·hr<sup>-1</sup> will yield the best results [61], [71]. Although these studies have shown increases in performance proportionate to the hourly CHO dose, there is a lack of consistency regarding the recommended rates CHO during endurance activities and competitions.

Smith et al. [72] attempted to identify a refined relationship between the rate of CHO ingestion and performance during a 2 hr cycling bout at 95% of the power output associated with a 4 mmol·L<sup>-1</sup> blood lactate concentration. After the trials they developed a mean regression formula which correlated the optimal performance and rate of CHO ingestion. They concluded CHO ingestion and endurance performance (~160min) have a curvilinear dose response relationship and that the optimal ingestion for 2 hours of riding at the above noted intensity approximates 78 g·hr<sup>-1</sup>. The volatility of CHO (g·hr<sup>-1</sup>) recommendations within lab based, shorter, exercise trials could lead to feeding strategy errors when performing ultra-endurance races. More real world, free living, studies within the ultra-endurance community are required to identify the validity and effectiveness of these lab-based CHO recommendations.

### Doubly Labeled Water

The DLW method is a stable isotope-based technique which estimates respiratory CO<sub>2</sub> production in free-living humans. DLW is based on the measured rates of differential isotope loss from the total body water. The calculated difference in elimination rates between the two isotopes (<sup>2</sup>H and <sup>18</sup>O) provides for the measurement of CO<sub>2</sub> production (rCO<sub>2</sub>).

An oral dose of DLW increases the baseline isotope enrichment within the body through ingestion of predetermined levels of labeled water including (<sup>18</sup>O) and (deuterium, <sup>2</sup>H). <sup>18</sup>O can be lost through routes of fluid (H<sub>2</sub><sup>18</sup>O) loss (sweat, urine) and via expired breath as C<sup>18</sup>O<sub>2</sub>. The deuterium is lost through body water as bodily fluid loss (<sup>2</sup>H<sub>2</sub>O)

alone. The majority of DLW studies performed on humans collect urine at specific times, due to its noninvasive, quick, and easily storable collection process [16], [30], [47]–[49]. Sample collections at specific times throughout the measurement window can account for the changes in isotopic enrichment and the subsequent rate of isotope elimination [25], [27]. The overall technique estimates CO<sub>2</sub> production and total water flux throughout the period of data collection. The calculated rates of CO<sub>2</sub> production is paired with an estimate of the respiratory quotient, (from dietary intake) [25] to enable the calculation of the TEE for the desired measurement period. DLW is considered the gold standard methodology when assessing the total energy demands of free-living individuals and athletes. The calculations used in this study are shown and explained in chapter three.

When using the DLW method, athletes are not encumbered by lab equipment and instrumentation during the measurement period. The athletes are allowed to participate in the event without having to perform any other tasks for the researcher. Due to the price of DLW still being relatively high (~\$1,000), alternative measurement strategies have also been developed. Sequential measures of HR can be used during free-living scenarios to estimate TDEE. During steady-state exercise, there is a close relationship between HR and oxygen consumption. Because the HR/VO<sub>2</sub> relationship is linear across a wide range of intensities, unknown values of VO<sub>2</sub> can be estimated from measured HR. However, the slope is greatly altered by the mode of activity and is individual specific which weakens the approach for measures of TDEE. The HR vs VO<sub>2</sub> relationship has been shown to develop errors of 30% when the duration of the study is extended to 24 hours [73]. The

ease of collection, accuracy, and lack of need for cumbersome lab equipment during the measurement period makes DLW the gold standard for measures of TDEE in free-living humans.

DLW dosing is calculated for each individual with a ratio of 0.09 to 0.12 g  $^2\text{H}$ /kg total body water (TBW) and 0.18 to 0.23 g  $^{18}\text{O}$ /kg TBW [74]. TBW can be calculated by taking the average of  $^2\text{H}$  and  $^{18}\text{O}$  dilution space divided by 1.041 and 1.007 respectively to correct for *in vivo* isotopic exchange [75]. There are two ways to collect and measure the elimination rate of the isotope: the “two point method” (TP) [25][76] and the “multipoint method” (MP)[77]. Cole and Coward reveal in their DLW review that the TP is more accurate, while the MP is more precise [78]. The TP plateau collection process is predominantly used in the literature to capture TEE over the course of multiple days [78]. The TP method consists of five overall collections of urine: one base line sample before the dose of DLW, two samples post dose, and two samples at the end of the desired time frame [45]. The TP method also assumes a linear rate of isotope loss which is in contrast with the MP method. The MP method is similar to the TP method; however, there are added collections throughout the desired time frame. These added collections are preferably taken at the same time of day within the desired window.

### Water Turnover and Comparison of Total Energy Expenditure/Intake

The isotope dosing and tracking provides a measure of water turnover that is more direct than the traditional dietary recall and survey [46]. By equilibrating the deuterium within the total body of water before the start of the event and analyzing its loss (as measured from changes in isotopic enrichment measured in urine samples), relative to changes in measures of nude body weight enables the calculation of water flux [46]. A comparison of this flux rate relative to body weight changes, provides an estimate of the total daily fluid needs across the measurement period [4]. Ruby et al. [4] demonstrated a water turnover rate of  $230 \pm 60 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  ( $16 \pm 4.2 \text{ L}\cdot\text{day}^{-1}$ ) during the Western States 100 ultra-endurance running race and  $250 \pm 60 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  ( $19.1 \pm 4.6 \text{ L}\cdot 24\text{hr}^{-1}$ ) in the Badwater ultra. The Kona ironman showed a water turnover rate of  $152 \pm 25 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$  ( $0.8 \pm .25 \text{ L}\cdot\text{hr}^{-1}$ ) with the data being normalized to 12 hours. These data can provide specific recommendations regarding the expectant hydration demands throughout a range of activities and environmental stressors [46].

The DLW method has been used in numerous studies to observe TDEE of many different modalities. Hulton et al. used this method to observe the TDEE of a four-person team racing in the Race Across America, a multi-day cycling team event. They measured total mean energy expenditure of  $181 \pm 13.2 \text{ MJ}\cdot\text{day}^{-1}$  ( $43,401 \pm 3,175 \text{ kcal}\cdot\text{day}^{-1}$ ) with a mean daily energy expenditure of  $26.8 \pm 1.9 \text{ MJ}\cdot\text{day}^{-1}$  ( $6,420 \pm 470 \text{ kcal}\cdot\text{day}^{-1}$ ) over the course of six days, ten hours, and 51 min [79]. These results are comparable to a four-person team of septugenarians which showed a TDEE of  $24.7 \pm 4.2 \text{ MJ}\cdot\text{day}^{-1}$  ( $5,900 \pm 1,015 \text{ kcal}\cdot\text{day}^{-1}$ ) and a water turnover of  $10.2 \pm 0.8 \text{ L}\cdot\text{day}^{-1}$  [80]. Cuddy et al. [16] used the DLW methodology in the Ironman world championship. They observed a TEE of 37.3

MJ (8,914 kcals) for the event with a concomitant water turnover of 16.6 L and a decrease in body weight of 5.9 kg. The use of DLW was extended with additional subjects by Ruby et al. [4] including 5 males and 1 female. The average TEE was  $37.8 \pm 1.3$  MJ ( $9,040 \pm 1,390$  kcals) across the event (finish time =  $12.7 \pm 1.6$  hr). Deuterium was delivered to additional subjects for a total sample size of  $N=13$ ,  $n=2$  F,  $n=11$  M) to evaluate water flux. Water turnover was expressed relative to average body weight loss to calculate water in ( $9.3 \pm 1.8$  L) vs. water out ( $10.0 \pm 1.9$  L) normalized for 12 hours and associated with a commensurate change in body weight of 2.3 kg.

Rehrer et al. utilized DLW to study the energy expenditure during a 6-day 10-stage road cycling event. Rehrer et al. observed an average TDEE of  $27.4 \pm 2.0$  MJ·day<sup>-1</sup> ( $6,546 \pm 478$  kcals·day<sup>-1</sup>) [81]. They precisely developed nutrition plans for all riders to eat *ad libitum* and observed the energy deficit throughout the trials which was accompanied by a body weight loss of  $0.3 \pm 2.4$  kg. The average total daily energy intake (TDEI) was 62% of the measured TDEE. The nutrition strategies that were laid out for the athletes and/or the *ad libitum* habits of the riders were insufficient to match the energy demands of the event. The limited weight loss suggests serious under-reporting of TEI. This is common for certain.

To capture the relationship between TDEE and TDEI in a free-living individual accurately, the DLW methodology should be utilized. All studies listed above gave insight into the respective modality and increased metabolic knowledge base to improve trainers,

coaches, and athlete performance. These studies have led to the manipulation of feeding strategies to increase TDEI and attenuate fatigue within high performing individuals.

### Total Daily Energy Intake (TDEI)

TDEI reported during endurance events such as the Ironman and ultra-endurance mountain runs range from 30-45% of measured TDEE [79], [82]–[84]. Cycling events, on average, have a higher TDEI percentage of TDEE when compared to running. The single mode of activity (cycling) would translate to transportability and ease of repeated access. Ironman athletes tend to consume the majority of their food during the cycling portion of the event [82]. The ability to consume a greater number of calories and fluids can attenuate maladaptive weight changes. Ultra-endurance events (15 – 24 hours in duration) range from 1 – 5.7 % weight loss which can be attributed to decreases in glycogen and fat stores and/or changes in hydration/total body water status [82]. The current recommendations of 60-90 g·hr<sup>-1</sup> for events >2.5 hours in a lab based setting show favorable changes in performance and carbohydrate absorption [61], [71]. Due to the guidelines being developed in lab based studies lasting 2 hours at ~70% VO<sub>2</sub> max [71], [85], [86], these results may not be transferrable to MTB events lasting 24 hours at a 40 - 60% VO<sub>2</sub> max.

DLW has been used to further the understanding of humans' ability to sustain elevated energy expenditures [4], [13], [30], [34]. DLW has aided in the advancement of feeding and hydration strategies across multiple modalities (shown in table 1). In consequence, trained athletes adapted preferred nutrition strategies which lead to better



performance; however, the DLW methodology has yet to be applied to an ultra-endurance MTB athlete during a 24-hour competitive race.

Table 1. Measures of Total Daily Energy Intake (TDEE), Body weight (BW) changes, and Water Turnover I endurance events using the Doubly Labeled Water methodology.

Author	Modality	TDEE (MJ, kcal)	BW change (kg)	Water turnover (L)
Saris et al. [34]	Tour de France	25.4 ± 1.4 (6,070 ± 334)	-0.3	X
Plasqui et al. [30]	The Giro d'Italia	32.3 ± 3.4 (7,719 ± 812)	0	X
Rosales et al. [80]	Race Across America	24.7 ± 4.2 (5903 ± 1,003)	-0.7 ± 1.1	10.2 <sup>A</sup>
Hulton et al. [79]	Race Across America	181.5 ± 13.3 (43,401 ± 3,175)	X	X
Rehrer et al. [81]	Tour of Southland	27.4 ± 2.0 (6,548 ± 478)	+0.3	X
Cuddy et al. [16]	Ironman	37.3 (8,926)	-5.9	16.6 <sup>B</sup>
Ruby et al. [4]	Ironman	9.8 ± 1.3 (2,342 ± 310)	-2.5 ± 0.4	10.8 <sup>C</sup>
Ruby et al. [4]	Western states 100	68.2 ± 12.4 (16,300 ± 2,963)	-1.5 ± 0.6	18.9 <sup>D</sup>
Ruby et al. [4]	Badwater Ultra	X	+1.6 ± 0.3	54.8 <sup>E</sup>

<sup>A</sup> average L·24 hours<sup>-1</sup> for the race duration (6 days 13 hours 13 min); <sup>B</sup> total L for the race duration (10 hours 40 min 16 seconds); <sup>C</sup> average total L for the race duration (12.7 ± 1.6 hours) <sup>D</sup> total L for the race duration (25.9 ± 3 hours) <sup>E</sup> total L for the race duration (42.5 ± 5.6).

## **Chapter three: Methodology**

### *PARTICIPANT*

A trained male cyclist (41 yr, 74.8 kg, 172.4 cm) competing in the Mudslinger-24 mountain bike race (Bend, Oregon, USA) served as the subject and signed a University Institutional Review Board approved informed consent form.

### *RACE DESCRIPTION*

The Mudslinger 24 took place in Bend, Oregon at 1104 m elevation. The race consisted of a 11.1-mile loop with an average of 8.8% incline which gave the subject ~828 ft of climbing and coasting per loop. The subject had 24 hours to complete this loop as many times as possible. This race was part of the National 24hour solo MTB overall Championship series.

The Mudslinger-24 is a time capped cross-country, single track cycling event (17.9 km, ~368 m (GPS) vertical each lap). Each rider has 24 hours to accumulate as many complete laps as possible. Sections of the course contain loose gravel and large rocks, which require athletes to periodically dismount to navigate the terrain.

### *STABLE ISOTOPE ADMINISTRATION*

Administration of DLW followed the methodology done previously during shorter measurement periods [4], [16], [80]. Following collection of a background urine sample, an oral dose of  $^2\text{H}_2\text{O}$  and  $\text{H}_2^{18}\text{O}$ ,  $0.12 \text{ g}\cdot\text{kg}^{-1} \text{ } ^2\text{H}_2\text{O}$  and  $2.0 \text{ g}\cdot\text{kg}^{-1} \text{ H}_2^{18}\text{O}$  of estimated initial total body water (TBW) was administered approximately 34 hours prior to the start of the race. Dose vials were rinsed 3 times with tap water ( $25 \text{ ml}\cdot\text{rinse}^{-1}$ ) and consumed to ensure total isotope delivery. The subject consumed 94 ml of water overnight before the morning void collection. All overnight voids were collected and added to the overall morning first void volume to account for tracer loss and correct the initial calculation of TBW.

### *BODY WEIGHT AND URINE COLLECTION*

Nude body weight and urine were collected before isotope delivery (34 hours prior to the start of the race), the morning after isotope delivery (28 hours before the start of the race), immediately prior to the race, halfway (12 hours), and directly after the race (24 hours). An additional urine sample was collected the night before the race (5:29 pm, 16 hours prior to the start of the race). All urine samples were immediately separated into 5 ml cryogenic vials and wrapped in parafilm, then stored in a cooler until subsequent analyses.

### *ENERGY EXPENDITURE CALCULATIONS*

Total energy expenditure was calculated from the rates of isotopic elimination using equations 1 and 2:

$$\text{Equation 1: } rCO_2 = 0.455 \times N \times \frac{1000}{18.012} [(1.007 \times K_o) - (1.042 \times K_d)]$$

$$\text{Equation 2: } TEE = rCO_2 \times (1.1 + 3.9 \div FQ) \times 22.4 \times 4.18 \div N_o$$

Where  $rCO_2$  is the rate of  $CO_2$  produced,  $K_o$  is the rate of labeled O lost,  $K_d$  is the rate of deuterium lost,  $N$  is the average TBW,  $FQ$  is the estimated food quotient (estimated at 0.86), and  $N_o$  is the dilution space of the labeled O [76], [87].

An alternative hourly estimate of TEE was calculated using the ACSM  $VO_2$  prediction equation from measures of average hourly cycling power output (Watts). An assumed RER of .88 was used to convert hourly averages of  $VO_2$  ( $L \cdot min^{-1}$ ) to  $kcal \cdot hr^{-1}$  and MJ for the entire event.

$$\text{Equation 3: } 1.8 \times \frac{Kgm}{min} \times \frac{1}{BW (Kg)} + 7 \frac{ml}{Kg} / min$$

### *CYCLING METRICS*

Cycling metrics included horizontal and vertical distance and speed from a Lezyne model super pro TTR GPS system (Reno, NV) integrated with a chest strap HR monitor (Wahoo TICKR, WFBTHR04X, Atlanta, GA) and power meter (Stages Cycling, SHIMANO ULTEGRA R8000 left single leg power/cadence, Portland, OR). All performance metrics were recorded continuously throughout the event (1 second EPOC).

#### *NUTRITION, FLUID, AND WEATHER DATA COLLECTION*

Nutrient and fluid intake was evaluated on a 2-lap interval schedule when the subject returned to the pit staging area for maintenance and resupply. Consumed items were recorded, and each bottle was weighed pre and post segment to calculate total segment fluid consumption (ml). Nutrition (Base Nutrition, Boulder, CO) consisted of carbohydrate/electrolyte powder (80 kcal, 290 mg sodium, 21 g carbohydrate, 141 mg potassium, 67 mg magnesium, and 2 mg calcium), food bars (160-190 kcal, 45-55 mg sodium, 21-28 g carbohydrate, 70-220 mg potassium, 10-60 mg calcium, 5-7 g protein), and gels (120 kcal, 270 mg sodium, 29 g carbohydrate, 4 mg potassium, 5 mg calcium). Other forms of nutrient intake included Coca-Cola (140 kcal, 45 mg sodium, 39 g carbohydrate), Heed Hammer Drink (110 kcal, 60 mg sodium, 27 g carbohydrate, 25 mg potassium, 31 mg magnesium, 57 mg calcium), Red Bull (155 kcal, 129 mg sodium, 37 g carbohydrate, 10 mg potassium, .8 g protein, .3 g fat), and Ramen Noodle (290 kcal, 1180 mg sodium, 39 g carbohydrate, 170 mg potassium, 6 g protein, 11 g fat). All nutrition intake was consumed ad libitum throughout the duration of the race.

Ambient weather data was recorded at a single location (course starting area) every hour with a Kestrel Fire Weather Meter Pro, 5500W (Downingtown, PA).

#### Experimental design

This was a non-experimental observational case study.

## **Chapter Four: Manuscript for International Journal for Sports Nutrition and Exercise**

### **Metabolism**

#### **ABSTRACT**

Previous studies have used the doubly labelled water (DLW) method to evaluate the total energy expenditure (TEE) during Ironman, ultra-marathon trail runs and competitive road cycling. However, the technique has not been applied to a 24-hour cross-country mountain bike event. This case study examined the TEE, cycling metrics and *ad libitum* nutrient/fluid intake in a trained male cyclist during a 24-hour cross-country mountain bike race. A trained male cyclist (41 y, 74.1 kg, 172.4 cm) received an oral dose of DLW prior to the 24-hour event for the calculations of TEE and water turnover ( $rH_2O$ ). Nude body weight and urine samples were collected pre, during, and post-race. Total nutrient (TNI) and fluid intake (TFI) and cycling metrics (speed, power output, cadence, HR) were continuously quantified during the event. The rider completed 383 km coupled with a vertical gain of ~7,737 m (GPS) during the 24-hour event. Average speed, power and HR were  $16.3 \pm 2$  km·hr<sup>-1</sup>,  $122 \pm 29$  W, and  $134 \pm 18$  bpm, respectively. TEE and TNI were 41 and 23.5 MJ, respectively. Total carbohydrate intake was 1192 g with an average hourly intake of  $58 \pm 22$  g·hr<sup>-1</sup>. Total body weight was 75.3 and 72.3 kg pre- and post-race, respectively with an *ad libitum* total fluid intake of 13.3 L. These data provide foundational measures of TEE, TEI and TFI during an ultra-endurance cross-country mountain biking event which can provide for future race/training strategies.

#### **ABSTRACT**

Previous studies have used the doubly labelled water (DLW) method to evaluate the total energy expenditure (TEE) during Ironman, ultra-marathon trail runs and competitive road cycling. However, the technique has not been applied to a 24-hour cross-country mountain bike event. This case study aimed to measure the TEE, cycling

metrics and nutrient/fluid intake in a trained male cyclist during a 24-hour cross country mountain bike race *ad libitum*. A trained male cyclist (41 y, 74.1 kg, 172.4 cm) received an oral dose of DLW prior to the 24-hour event for the calculations of TEE and water turnover ( $rH_2O$ ). Nude body weight and urine samples were collected pre, during, and post-race. Total nutrient (TNI) and fluid intake (TFI) in addition to cycling metrics (speed, power output, cadence, heart rate) were continuously recorded during the event. The rider completed 383 km coupled with a vertical gain of 7,737 m during the 24-hour event. Average speed, power and heart rate were  $16.3 \pm 2$  km·hr<sup>-1</sup>,  $122 \pm 29$  W, and  $134 \pm 18$  bpm, respectively. TEE and TNI were 41 and 23.5 MJ, respectively. Total carbohydrate intake was 1192 g with an average hourly intake of  $58 \pm 22$  g·hr<sup>-1</sup>. Total body weight was 75.3 and 72.3 kg pre- and post-race, respectively with a measured *ad libitum* TFI of 13.3 L. These data provide novel insights for measures of TEE, TEI and TFI during an ultra-endurance cross country mountain biking event and provide a foundation for future race/training needs.

Key words: Doubly labelled water, total energy expenditure, ultra-endurance, carbohydrate intake

## INTRODUCTION

The measurement of total energy expenditure (TEE) outside of the laboratory enables a more comprehensive understanding of the human ceiling for extended work and competition. However, the dominating limitations to obtaining valid measures of TEE in field studies requires a balance of accuracy, portability, and cost. Doubly labeled water (DLW) remains the gold standard for quantifying the TEE for humans in free-living environments without the need for additional body worn sensors or expired air collection (Schulz et al. 1989) thereby optimizing both accuracy and portability concerns.

DLW is an essential technique for measuring TEE during ultra-endurance exercise when aspects of physical activity cannot be accurately estimated using alternative methods (e.g. prediction equations for  $\text{VO}_2$  and TEE). For example, high rates of TEE have been demonstrated during multi-stage/day cycling events including the Giro d'Italia ( $32.3 \pm 3.4 \text{ MJ}\cdot\text{day}^{-1}$ ,  $7,719 \pm 813 \text{ kcals}\cdot\text{day}^{-1}$ ) (Plasqui et al., 2019) and the Tour de France ( $24.8 \pm 1.7 \text{ MJ}\cdot\text{day}^{-1}$ ,  $5,927 \pm 411 \text{ kcals}\cdot\text{day}^{-1}$ ) (Saris et al. 1989). DLW has also been used for shorter measurements (12-24hr) including the Kona Ironman triathlon ( $9.8 \pm 1.3 \text{ MJ}$ ,  $9,040 \pm 1,390 \text{ kcals}$ ) and the Western States 100 Ultramarathon ( $68.2 \pm 12.4 \text{ MJ}$ ,  $16,310 \pm 2,960 \text{ kcals}$ ) (Ruby et al. 2015).

Cross-country mountain biking requires additional muscle activation in the upper extremities, intermittent segments of varied power output, advanced bike handling and periodic dismount. Each of these each are likely to alter the relationships between  $\text{VO}_2$ , power output and heart rate compared to traditional road cycling (Lee et al., 2002, Hurst et al., 2012, Miller et al., 2018). Moreover, the physiological profile and self-selected feeding strategies of cross-country ultra-endurance mountain bikers have not been explored simultaneously with measures of TEE. The purpose of this case study was to quantify the *ad libitum* nutrient and fluid intake in parallel with stable isotopic turnover and derived measures of TEE in a competitive male cross-country cyclist during a sanctioned 24-hour event.

## **METHODS**

### *ATHLETE*

A trained competitive male cyclist (41 yr, 74.8 kg, 172.4 cm) racing in the Mudslinger-24 mountain bike race (Bend, Oregon, USA) served as the subject for this observational study and signed a University of Montana Institutional Review Board approved informed consent form in accordance with the Declaration of Helsinki.

### *RACE DESCRIPTION*



The Mudslinger-24 is a time capped cross-country, single track cycling event (17.9 km, ~368 m (GPS) vertical each lap). Each rider has 24 hours to accumulate as many complete laps as possible. Sections of the course consist of dominantly soft dirt, single track and loose gravel, fire roads with occasional large rocks that required periodic rider dismount to navigate the difficult terrain.

### *STABLE ISOTOPE ADMINISTRATION*

Administration of DLW followed the methodology deployed previously during shorter measurement periods (Cuddy et al. 2010; Rosales et al. 2022; Ruby et al. 2015). Following the collection of a background urine sample, an oral dose of tracer;  $^2\text{H}_2\text{O}$  and  $\text{H}_2^{18}\text{O}$ ,  $0.12 \text{ g}\cdot\text{kg}^{-1} \text{ } ^2\text{H}_2\text{O}$  and  $2.0 \text{ g}\cdot\text{kg}^{-1} \text{ H}_2^{18}\text{O}$  of estimated initial total body water (TBW) was administrated approximately 34 hours prior to the start of the race. Dose vials were rinsed three times with tap water ( $25 \text{ ml}\cdot\text{rinse}^{-1}$ ) and consumed to ensure total isotope delivery. The subject consumed 94 ml of water overnight before the morning void collection. All overnight voids were collected and added to the overall morning first void volume to account for tracer loss and correct the initial calculation of TBW. Timeline of isotope delivery, urine, and nude weight collections is depicted in Figure 1.

### *BODY WEIGHT AND URINE COLLECTION*

Nude body weight (Ohaus CW-11, Ohaus Corp, Pinebrook, NJ), and a background urine sample were collected before isotope delivery (34 hours prior to the start of the race), the morning after isotope delivery (28 hours before the start of the race), 16 hours prior, immediately prior to the race, halfway (12 hours), and directly after the race (24 hours). All urine samples were immediately separated into 5 ml cryogenic vials and wrapped in parafilm, then stored frozen until subsequent analyses.

### *ENERGY EXPENDITURE CALCULATIONS*

Total energy expenditure was calculated from the rates of isotopic elimination using equations 1 and 2:

$$\text{Equation 1: } r\text{CO}_2 = 0.455 \times N \times \frac{1000}{18.012} [(1.007 \times K_o) - (1.042 \times K_d)]$$

Equation 2:  $TEE = rCO_2 \times (1.1 + 3.9 \div FQ) \times 22.4 \times 4.18 \div N_o$

Where  $rCO_2$  is the rate of  $CO_2$  produced,  $K_o$  is the rate of labeled O lost,  $K_d$  is the rate of deuterium lost,  $N$  is the average TBW,  $FQ$  is the estimated food quotient (estimated at 0.86), and  $N_o$  is the dilution space of the labeled Oxygen (Schoeller et al., 1986, Speakman et al., 2021).

### *CYCLING METRICS*

Cycling metrics included horizontal and vertical distance and speed from a Lezyne model super pro TTR GPS system (Reno, NV) integrated with a chest strap HR monitor (Wahoo TICKR, WFBTHR04X, Atlanta, GA) and power meter (Stages Cycling, SHIMANO ULTEGRA R8000 left single leg power/cadence, Portland, OR). All performance metrics were recorded continuously throughout the event (1 second EPOC).

### *NUTRITION AND FLUID INTAKE, AND WEATHER DATA COLLECTION*

Nutrient and fluid intake was evaluated on a 2-lap interval schedule when the subject returned to the pit staging area for bike maintenance and provision resupply. Consumed items were recorded (based on empty wrappers), and each bottle was weighed pre and post segment to calculate total segment fluid consumption (ml). The comprehensive dietary and fluid derived provisions are outlined in Table 2. Ambient weather data was recorded at a single location (course starting area) every hour throughout the event with a Kestrel Fire Weather Meter Pro, 5500W (Downingtown, PA). All data is represented as mean  $\pm$  SD.

## **RESULTS**

The subject completed 21 laps of the course (383 km) including ~7,737 m (GPS) of climbing during the 24-hr race (GPS). Body weight decreased during the first 12 hours of the race (75.3 kg and 72 kg from pre-race to 12-hours, respectively). Body weight was maintained during the second half of the event (72 kg and 72.3 kg from 12-hours to post, respectively).

The calculated TEE derived from DLW for the entire 24-hour event was 41 MJ (9,775 kcals). The nutrient and fluid intake inventory demonstrated a TEI of 23.5 MJ (5,616 kcals), 57% of the calculated TEE.

The macronutrient and electrolyte intake profile are described in Table 1 for each measured race segment. Recorded fluid intake amounted to a total of 13.3 L with an intake response proportional to measured ambient air temperature throughout the race (Figure 2 A). Heart rate throughout the event ranged from 82% to 65% age predicted maximal HR for the first and second half of the event, respectively. Measures of power, speed, HR, and exogenous carbohydrate intake per lap are depicted in Figure 2 B. Hourly carbohydrate intake averaged  $58 \pm 22 \text{ g}\cdot\text{hr}^{-1}$  for the entire event but varied from a low of  $34 \text{ g}\cdot\text{hr}^{-1}$  (lap 17) to a high of  $97 \text{ g}\cdot\text{hr}^{-1}$  (lap 7), (Figure 2). The subject spent over 20% of the time at 200W during the day and 150W at night. There was a 10% increase in coasting time (0W) at night (Figure 3).

Average hourly fluid intake was  $660 \pm 272 \text{ ml}\cdot\text{hr}^{-1}$  with  $828 \pm 212 \text{ ml}\cdot\text{hr}^{-1}$  consumed during the daylight laps and  $284 \pm 98 \text{ ml}\cdot\text{hr}^{-1}$  consumed during the night laps, which paralleled fluctuations in ambient temperatures. Ambient temperature and relative humidity (RH) averaged  $18.8 \pm 10.5 \text{ }^{\circ}\text{C}$  and  $42.8 \pm 21.9 \text{ \%RH}$  for the entire event, respectively (low =  $3.1 \text{ }^{\circ}\text{C}$  and  $17 \text{ \%RH}$ , high =  $33.6 \text{ }^{\circ}\text{C}$  and  $84 \text{ \%RH}$ , day =  $28.6 \pm 3.0 \text{ }^{\circ}\text{C}$  and  $24.1 \pm 4.2 \text{ \%RH}$ , night =  $11.7 \pm 7.5 \text{ }^{\circ}\text{C}$  and  $57.6 \pm 18.6 \text{ \%RH}$ ). The changes in fluid intake and ambient temperature are shown in Figure 2.

## DISCUSSION

The purpose of this case study was to describe the TEE and cycling metrics in parallel with *ad libitum* nutrient and fluid intake patterns of a competitive ultra-endurance cross-country male cyclist when performing a solo 24-hour mountain bike race. We observed a TEI of 23.5 MJ (5,616 kcals) for the entire event which was approximately 57% of the measured TEE, 41 MJ (9,799 kcals). Measured TEI in the present data is considerably higher compared to the TEI reported during ultra-endurance events such as the Ironman and mountain trail runs, which ranges from 30-45 % of measured TEE (Barrero et al 2015; Hulton et al. 2010; Kimber et al. 2002; Ramos-

Campo et al. 2016). Ironman athletes tend to consume most of their food during the cycling portion of the event (Barrero et al. 2015). The current results could be partly explained by the single mode of activity (cycling), transportability and ease of repeated access to provisions. Despite maintaining high levels of fluid and nutritional intake, the athlete still lost 3.9% of initial body weight, which is comparable to other ultra-endurance events (15 – 24 hours duration) which range from 1 – 5.7 % (Barrero, et al. 2015; Cuddy et al. 2010; Ruby et al., 2015). This is most likely attributed to decreases in glycogen and fat stores and or changes in hydration/total body water status (Barrero et al., 2015).

The athlete maintained an average exogenous CHO intake of 58 g·hr<sup>-1</sup> which is comparable to the lower range of general lab-based recommendations of 60-90 g·hr<sup>-1</sup> for events >2.5 hours (Jeukendrup 2013). The overall average of 58 g·hr<sup>-1</sup> and range of 25-97 g·hr<sup>-1</sup> is similar to other studies using trained athletes during ultra-endurance cycling events (Geesmann, et al. 2014; Havemann et al. 2008). These data suggest that the exogenous CHO recommendations (60 – 90 g·hr<sup>-1</sup>) established at 51 – 64 % VO<sub>2max</sub> for durations of 2-5 hours may be less applicable or less sustainable during 24-hour ultra-endurance races performed at 40 – 45 % VO<sub>2max</sub> (Geesmann, et al. 2014; 2006; Van Loon et al. 1999).

The subject experienced moderate lower GI distress around 0300 hours (~18 hours into the race). The subject consumed 81 mg of caffeine over segments 3-6 and 11 g of fat after segment 7. Although, fat and caffeine intake are correlated with GI distress during ultra-endurance events (Jeukendrup et al. 2011), these quantities are considerably low compared to the overall total nutrient intake. Ultra-endurance events can cause upper and lower GI distress regardless of feeding strategy (Peters et al. 1999). The cumulative effects of high hourly exogenous carbohydrate intake during ultra-events (60 – 90 g·hr<sup>-1</sup>) may exceed absorption capabilities, potentially contributing to GI symptoms (Jeukendrup et al., 2011).

Although the subject averaged an hourly CHO intake commensurate with the literature (Jeukendrup et al. 2013), it did not prevent the decrease in segment power throughout the second half of the event. The most pronounced decrease in power output occurred between segment 6 and 7 (lap 12, 13) during dusk conditions at

approximately 2000 hours. CHO intake also dropped by 21 g·hr<sup>-1</sup> (50 – 29 g·hr<sup>-1</sup>, laps 14 – 15 respectively) at this time and remained low throughout the rest of the event (Figure 2). Changes in the light/dark cycle may contribute to overall central fatigue, which could be due to elevated cortisol and catecholamines (Grego et al., 2004). The effects of accumulated GI distress may also explain the measured decrease in power output. The ambient conditions also shifted at this time with a temperature decrease from 26.8 to 14.7 C°.

When performing an ultra-endurance event, the accumulation of sleep deprivation and duration of work output can decrease cognitive performance, reaction time, time to exhaustion, and rate of perceived exertion (Van Helder et al 1989; Zhong et al. 2005). Current nutrition and fluid guidelines should likely be adjusted accordingly for individuals performing ultra-endurance events at lower average power outputs in fluctuating ambient weather and light conditions.

## **APPLICATION / CONCLUSION**

This case study provides a baseline understanding of the energy, fluid, and nutrient demands of ultra-endurance mountain bike racing. Although regimented CHO intake is capable of prolonging performance and reducing fatigue, the ergogenic benefits cannot fully overcome the other factors contributing to fatigue over 24 hours (i.e. onset of sleep deprivation).

Despite diligent nutrient and fluid intake practices, these data demonstrate a gradual decline in maintained exercise intensity (HR, power output, lap times). The decay of power output appears more prominent compared to the HR response, especially for the later 1/3 of the event; however, it is likely that time of day and ambient light conditions were a factor. This physiological profile can serve as a baseline towards understanding the demands of ultra-endurance cross-country cycling when compared to road cycling. These data suggest the need for further investigation to refine current lab-based feeding/hydration recommendations to counter the onset of fatigue and reduce other complications that accompany the extended duration of ultra-endurance events.

## Tables and Figures

Table 1: Inventory of electrolyte and macronutrient composition of all nutrition supplementation.

Nutrient Supplement	Calories	Na+ (mg)	CHO (g)	K+ (mg)	Magnesium (mg)	Ca+ (mg)	Protein (g)	Fat (g)
One Base bottle (2 scoops) <sup>A</sup>	160	580	42	282	134	4	0	0
Heed Hammer bottle <sup>B</sup>	110	60	27	25	31	57	0	0
Coca Cola <sup>C</sup>	140	45	39	0	0	0	0	0
Salted Watermelon <sup>D</sup>	120	270	29	4	0	5	0	0
Strawberry cream <sup>D</sup>	120	270	29	4	0	5	0	0
Cola Gel <sup>E</sup>	100	60	22	0	0	20	0	0
Apple <sup>F</sup>	160	45	28	220	0	40	5	0
Cranberry lime <sup>F</sup>	190	45	25	70	0	60	5	0
Peanut Butter <sup>F</sup>	160	55	27	220	0	10	5	0
Peanut Almond Crisp <sup>F</sup>	210	50	21	170	0	30	7	0
Cocoa <sup>F</sup>	160	45	27	250	0	40	5	0
Ramen noodle <sup>G</sup>	290	1180	39	170	0	0	6	11
Red Bull <sup>H</sup>	155	129	37	10	0	0	0.8	0.3

<sup>A</sup> Nutrition drink (Base Nutrition, Boulder, CO); <sup>B</sup> Nutrition drink (Hammer nutrition, Whitefish, MT); <sup>C</sup> Soda pop (Coca-Cola, Atlanta, GA); <sup>D</sup> Nutrition Gel (Base Nutrition, Boulder, CO); <sup>F</sup> Nutrition food bar (Base Nutrition, Boulder, CO); <sup>G</sup> Ramen Noodles (Maruchan, Irvine, CA); <sup>H</sup> Energy Drink (Red Bull, Salzburg, AT)

**Table 2:** Macronutrient and electrolyte intake during the 24-hour MTB race. Energy intake (Kj), sodium (mg), carbohydrate (CHO) (g), potassium (mg), magnesium (mg), Calcium (mg), protein (g), and fat (g).

Lap number	Energy (kJ)	CHO (g)	Protein (g)	Fat (g)	Na <sup>+</sup> (mg)	K <sup>+</sup> (mg)	Magnesium (mg)	Ca <sup>+</sup> (mg)
1 & 2	2547	127	10	0	704	715	112	83
3 & 4	1897	90	10	0	583	675	111	53
5 & 6	2939	147	6	0	1184	508	214	147
7 & 8	3295	190	5	0	1363	703	246	78
9 & 10	2492	141	5	0	996	563	147	49
11&12	2483	142	5	0	988	529	145	49
13 & 14	2880	127	11	11	1924	602	99	48
15 & 16	1780	75	7	0	666	342	80	37
17 & 18	1617	72	10	0	331	587	55	81
19, 20, 21	1568	81	5	0	658	421	79	47
<b>Total</b>	<b>23500</b>	<b>1192</b>	<b>75</b>	<b>11</b>	<b>9401</b>	<b>5649</b>	<b>1291</b>	<b>675</b>

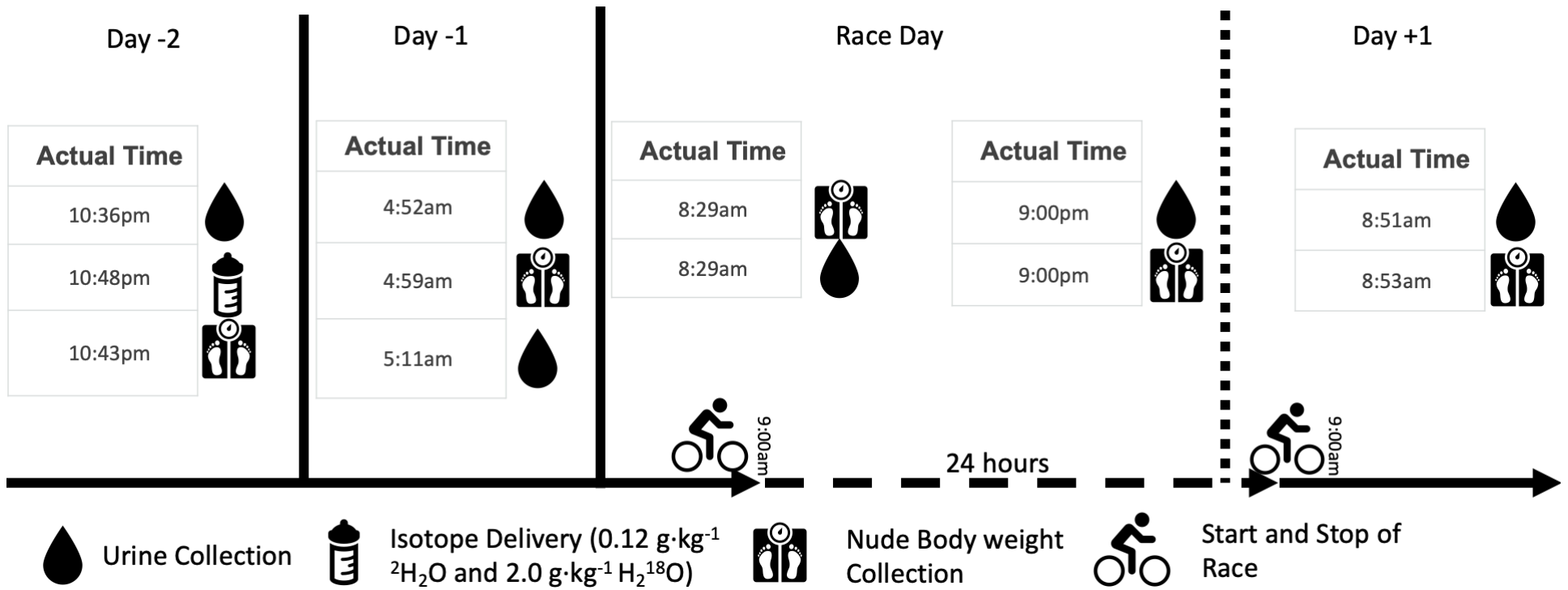
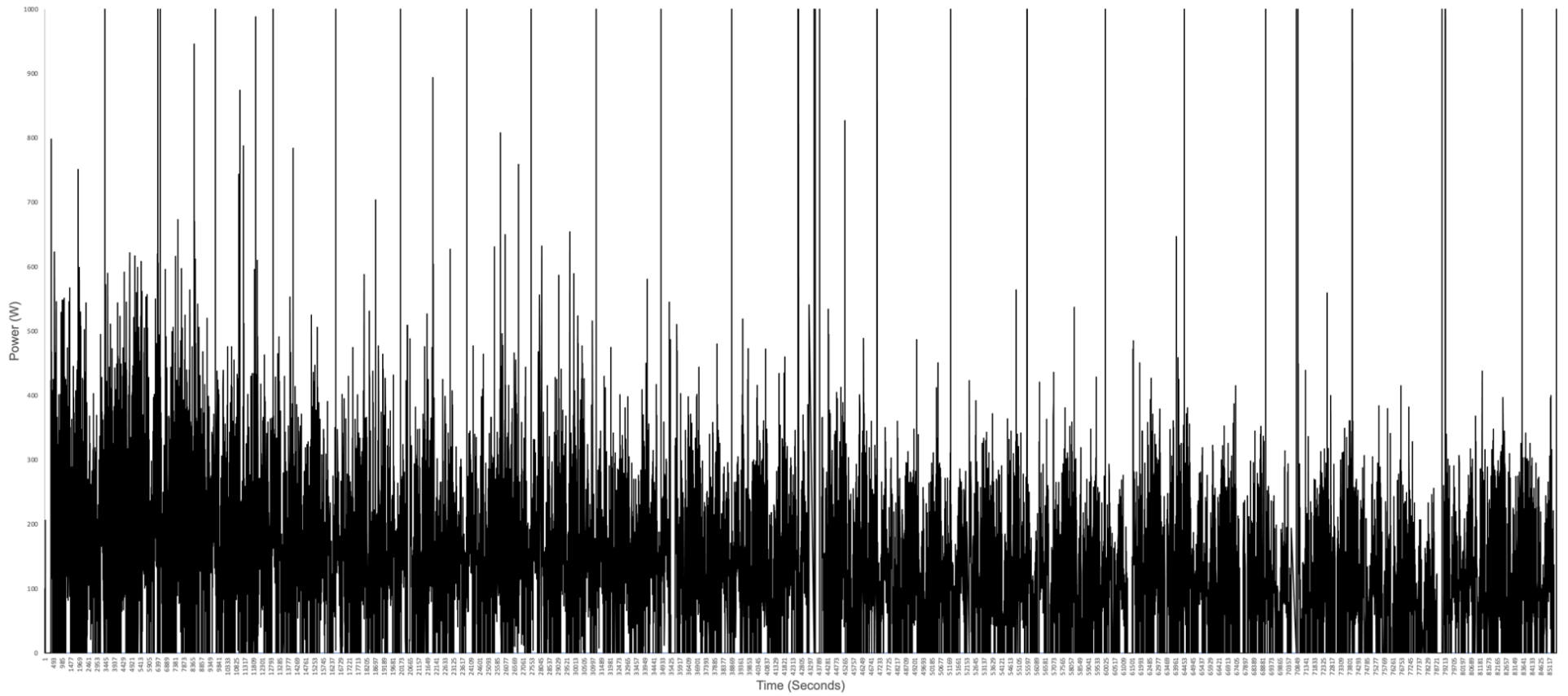
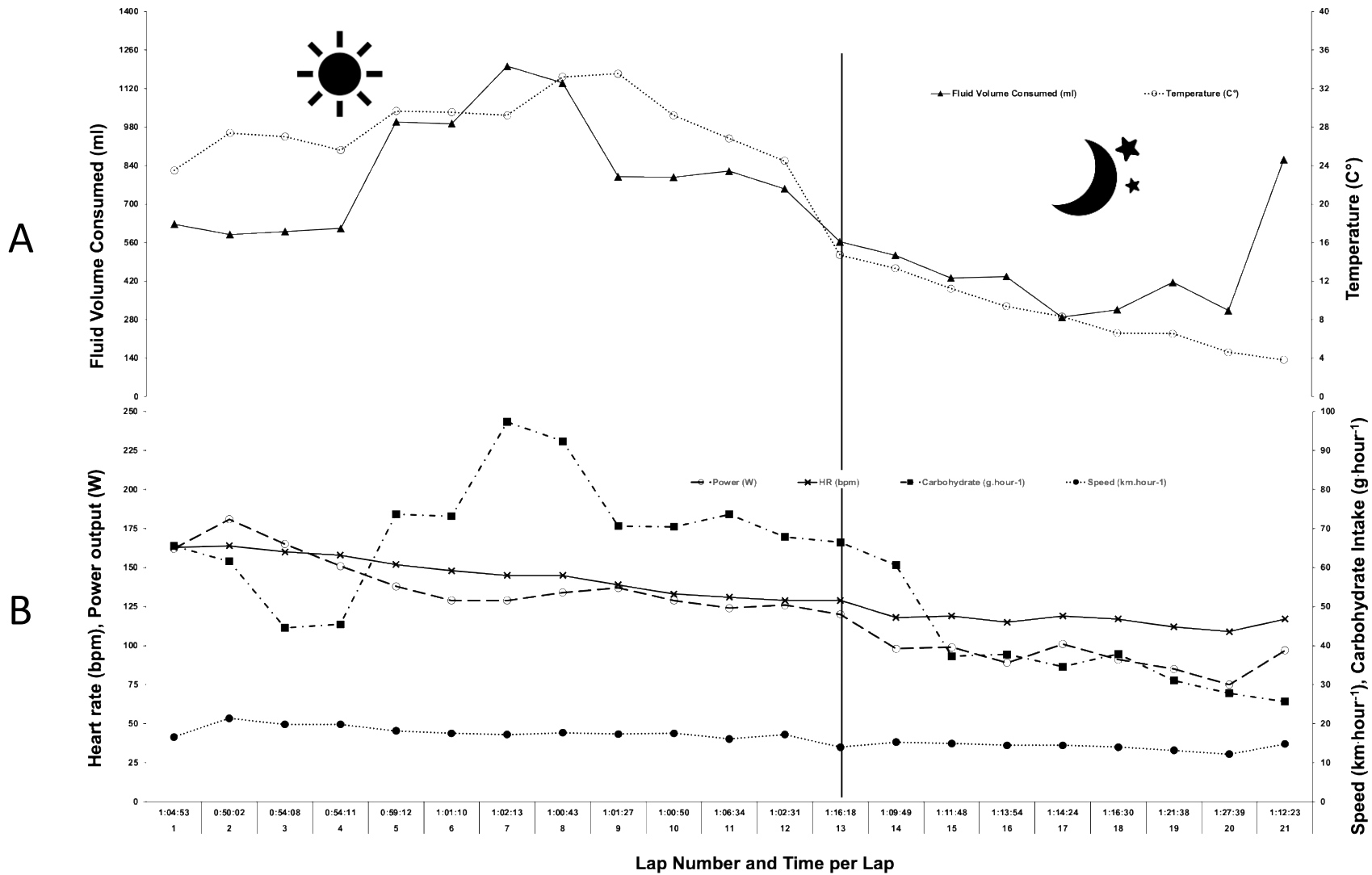


Figure 1: The timeline and collection process for DLW.

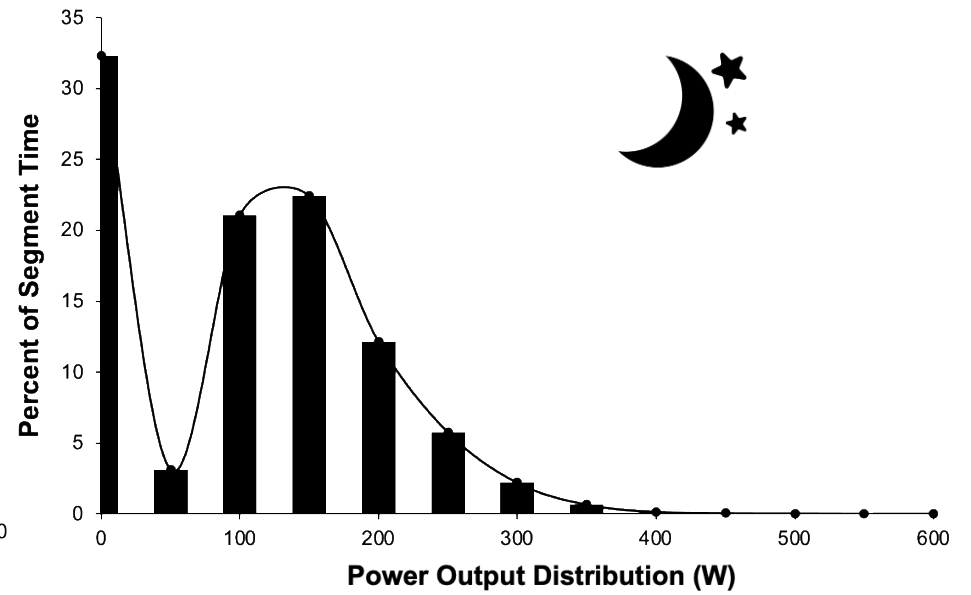
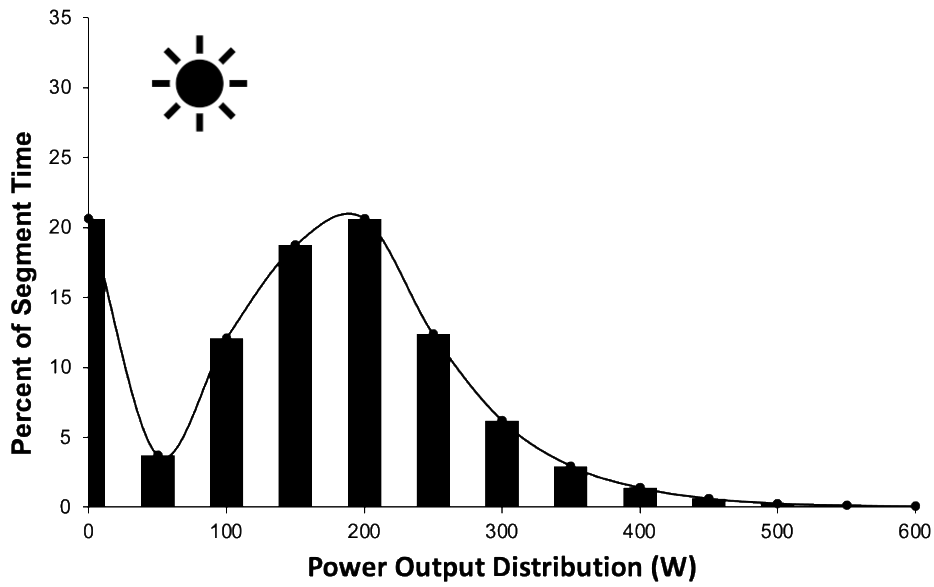




**Figure 2: Power output every second over the whole 24-hour race.**



**Figure 3:** **A** - Volume of fluid consumed (ml) and ambient temperature (C°) during each lap of the 24-hour event; **B** - Power output (watts), heart rate (bpm), cycling speed (km·hr<sup>-1</sup>), carbohydrate intake (g·hour<sup>-1</sup>), and lap count/split times throughout the 24-hour event.



**Figure 4:** Distribution of power output (W) for day and nighttime riding of the 24-hour, day riding laps 1 – 13, night riding laps 14 – 21.

## Manuscript references

- Barrero, Anna, Pau Erola, and Raúl Bescós. 2015. "Energy Balance of Triathletes during an Ultra-Endurance Event." *Nutrients* 7(1): 209–22.
- Cuddy, John S. et al. 2010. "Metabolic Profile of the Ironman World Championships: A Case Study." *International Journal of Sports Physiology and Performance* 5(4): 570–76.
- Geesmann, Bjoern, Joachim Mester, and Karsten Koehler. 2014. "Energy Balance, Macronutrient Intake, and Hydration Status during a 1,230 Km Ultra-Endurance Bike Marathon." *International Journal of Sport Nutrition and Exercise Metabolism* 24(5): 497–506.
- Grego, Fabien et al. 2004. "Effects of Long Duration Exercise on Cognitive Function, Blood Glucose, and Counterregulatory Hormones in Male Cyclists." *Neuroscience Letters* 364(2): 76–80.
- Havemann, Lize, and Julia H. Goedecke. 2008. "Nutritional Practices of Male Cyclists before and during an Ultraendurance Event." *International Journal of Sport Nutrition and Exercise Metabolism* 18(6): 551–66.
- Van Helder, T., and M. W. Radoki. 1989. "Sleep Deprivation and the Effect on Exercise Performance." *Sports Medicine* 7(4): 235–47.
- Hurst, Howard et al. 2012. "Influence of Course Type on Upper Body Muscle Activity in Elite Cross-Country and Downhill Mountain Bikers during off Road Downhill Cycling." *Journal of Science and Cycling* 1(2): 2–9. [http://www.jsc-journal.com/ojs/index.php?journal=JSC&page=article&op=view&path\[\]=20](http://www.jsc-journal.com/ojs/index.php?journal=JSC&page=article&op=view&path[]=20).
- Impellizzeri, Franco M., and Samuele M. Marcora. 2007. "The Physiology of Mountain

- Biking." *Sports Medicine* 37(1): 59–71.
- Jeukendrup, Asker E 2013. "The New Carbohydrate Intake Recommendations." *Nutritional Coaching Strategy to Modulate Training Efficiency*. 75: 63–71.  
<https://www.nestlenutrition-institute.org/sites/default/files/documents-library/publications/secured/9b554fc8b27955fbf3fb2435380f1fcc.pdf#page=77>.
- Jeukendrup, Asker E. 2006. "Exogenous Carbohydrate Oxidation during Ultraendurance Exercise." *Journal of applied physiology (Bethesda, Md. : 1985)* 100(4): 1134–41.
- Jeukendrup, Asker E. 2011. "Nutrition for Endurance Sports: Marathon, Triathlon, and Road Cycling." *Journal of Sports Sciences* 29(SUPPL. 1).
- Kimber, Nicholas E., Jenny J. Ross, Sue L. Mason, and Dale B. Speedy. 2002. "Energy Balance during an Ironman Triathlon in Male and Female Triathletes." *International Journal of Sport Nutrition* 12(1): 47–62.
- Lee, Hamilton et al. 2002. "Physiological Characteristics of Successful Mountain Bikers and Professional Road Cyclists." *Journal of Sports Sciences* 20(12): 1001–8.
- Van Loon, Luc J.C., Asker E. Jeukendrup, Wim H.M. Saris, and Anton J.M. Wagenmakers. 1999. "Effect of Training Status on Fuel Selection during Submaximal Exercise with Glucose Ingestion." *Journal of Applied Physiology* 87(4): 1413–20.
- Miller, Matthew C. et al. 2018. "Braking and Performance Characteristics of Experienced and Inexperienced Mountain Bikers Navigating an Isolated Off-Road Turn Using a Brake Power Meter." *International Journal of Performance Analysis in Sport* 18(4): 495–506. <https://doi.org/10.1080/24748668.2018.1496383>.
- De Oliveira, Erick Prado, and Roberto Carlos Burini. 2009. "The Impact of Physical

- Exercise on the Gastrointestinal Tract.” *Current Opinion in Clinical Nutrition and Metabolic Care* 12(5): 533–38.
- Plasqui, Guy et al. 2019. “Energy Expenditure during Extreme Endurance Exercise: The Giro d’Italia.” *Medicine and Science in Sports and Exercise* 51(3): 568–74.
- Ramos-Campo, D. J., Ávila-Gandía, V., Alacid, F., Soto-Méndez, F., Alcaraz, P. E., López-Román, F. J., & Rubio-Arias, J. Á. 2016. “Muscle Damage, Physiological Changes, and Energy Balance in Ultra-Endurance Mountain-Event Athletes.” *Applied Physiology, Nutrition, and Metabolism* 41(8): 872–78.
- Rosales, Alejandro M et al. 2022. “Septuagenarians Approach 4 Times the Basal Metabolic Rate During Race Across America.” *International Journal of Sports Physiology and Performance*: 8–11.
- Ruby BC, Cuddy JS, Hailes WS, Dumke CL, Slivka DR, Shriver TC, Schoeller DA. 2015. “Extreme Endurance and the Metabolic Range of Sustained Activity Is Uniquely Available for Every Human Not Just the Elite Few.” *Comparative Exercise Physiology* 11(1): 1–7.
- Saris, W. H.M. et al. 1989. “Study on Food Intake and Energy Expenditure during Extreme Sustained Exercise: The Tour de France.” *International Journal of Sports Medicine* 10(SUPPL. 1).
- Schoeller, D. A. et al. 1986. “Energy Expenditure by Doubly Labeled Water: Validation in Humans and Proposed Calculation.” *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* 250(5 (19/5)).
- Schulz, S., K. R. Westerterp, and K. Bruck. 1989. “Comparison of Energy Expenditure by the Doubly Labeled Water Technique with Energy Intake, Heart Rate, and

Activity Recording in Man.” *American Journal of Clinical Nutrition* 49(6): 1146–54.

Speakman, John R. et al. 2021. “A Standard Calculation Methodology for Human Doubly Labeled Water Studies.” *Cell Reports Medicine* 2(2

## Citations

- [1] D. R. Carrier *et al.*, “The Energetic Paradox of Human Running and Hominid Evolution [and Comments and Reply],” *Curr. Anthropol.*, vol. 25, no. 4, pp. 483–495, 1984, doi: 10.1086/203165.
- [2] D. E. Lieberman, M. Mahaffey, S. C. Quimare, N. B. Holowka, I. J. Wallace, and A. L. Baggish, “Running in tarahumara (Rarámuri) culture: Persistence hunting, footracing, dancing, work, and the fallacy of the athletic savage,” *Curr. Anthropol.*, vol. 61, no. 3, pp. 356–379, 2020, doi: 10.1086/708810.
- [3] H. Kaplan and K. I. M. Hill, “Hunting Ability and Reproductive Success Among Male Ache Foragers : Preliminary Results Author ( s ): Hillard Kaplan and Kim Hill Published by : The University of Chicago Press on behalf of Wenner-Gren Foundation for Anthropological Research Stable URL :,” vol. 26, no. 1, pp. 131–133, 1985.
- [4] S. D. Ruby BC, Cuddy JS, Hailes WS, Dumke CL, Slivka DR, Shriver TC, “Extreme endurance and the metabolic range of sustained activity is uniquely available for every human not just the elite few.,” *Comp. Exerc. Physiol.*, vol. 11, no. 1, pp. 1–7, 2015.
- [5] F. W. Booth *et al.*, “invited review,” pp. 3–30, 2022.
- [6] F. W. Booth, C. K. Roberts, J. P. Thyfault, G. N. Ruegsegger, and R. G. Toedebusch, “Role of inactivity in chronic diseases: Evolutionary insight and pathophysiological mechanisms,” *Physiol. Rev.*, vol. 97, no. 4, pp. 1351–1402, 2017, doi: 10.1152/physrev.00019.2016.
- [7] A. Lucia, J. Hoyos, and J. L. Chicharro, “Physiology of professional road cycling,”



- Sport. Med.*, vol. 31, no. 5, pp. 325–337, 2001, doi: 10.2165/00007256-200131050-00004.
- [8] J. L. Kinabo and J. V. G. A. Durnin, “Thermic effect of food in man: Effect of meal composition, and energy content,” *Br. J. Nutr.*, vol. 64, no. 1, pp. 37–44, 1990, doi: 10.1079/bjn19900007.
- [9] J. A. Levine, “Measurement of energy expenditure,” *Public Health Nutr.*, vol. 8, no. 7a, pp. 1123–1132, 2005, doi: 10.1079/phn2005800.
- [10] J. R. Speakman and C. Selman, “Physical activity and resting metabolic rate,” *Proc. Nutr. Soc.*, vol. 62, no. 3, pp. 621–634, 2003, doi: 10.1079/pns2003282.
- [11] E. J. Baker and T. T. Gleeson, “EPOC and the energetics of brief locomotor activity in *Mus domesticus*,” *Journal of Experimental Zoology*, vol. 280, no. 2, pp. 114–120, 1998, doi: 10.1002/(SICI)1097-010X(19980201)280:2<114::AID-JEZ2>3.0.CO;2-R.
- [12] E. B. Edwards and T. T. Gleeson, “Can energetic expenditure be minimized by performing activity intermittently?,” *J. Exp. Biol.*, vol. 204, no. 3, pp. 599–605, 2001.
- [13] J. A. Cooper, D. D. Nguyen, B. C. Ruby, and D. A. Schoeller, “Maximal sustained levels of energy expenditure in humans during exercise,” *Med. Sci. Sports Exerc.*, vol. 43, no. 12, pp. 2359–2367, 2011, doi: 10.1249/MSS.0b013e31822430ed.
- [14] K. R. Westerterp, W. H. M. Saris, M. V. A. N. Es, and F. T. E. N. Hoor, “Use of the doubly labeled water technique in humans during heavy sustained exercise,” *J. Appl. Physiol.*, no. 16, 2021.
- [15] L. R. Dugas *et al.*, “Energy expenditure in adults living in developing compared

- with industrialized countries: A meta-analysis of doubly labeled water studies,” *Am. J. Clin. Nutr.*, vol. 93, no. 2, pp. 427–441, 2011, doi: 10.3945/ajcn.110.007278.
- [16] J. S. Cuddy, D. R. Slivka, W. S. Hailes, C. L. Dumke, and B. C. Ruby, “Metabolic profile of the Ironman World Championships: A case study,” *Int. J. Sports Physiol. Perform.*, vol. 5, no. 4, pp. 570–576, 2010, doi: 10.1123/ijsp.5.4.570.
- [17] J. V. G. A. Durnin and J. M. Brockway, “Determination of the total daily energy expenditure in man by indirect calorimetry: assessment of the accuracy of a modern technique,” *Br. J. Nutr.*, vol. 13, no. 1, pp. 41–53, 1959, doi: 10.1079/bjn19590007.
- [18] S. J. Strath, J. Bassett, A. M. Swartz, and D. L. Thompson, “Simultaneous heart rate-motion sensor technique to estimate energy expenditure,” *Med. Sci. Sports Exerc.*, vol. 33, no. 12, pp. 2118–2123, 2001, doi: 10.1097/00005768-200112000-00022.
- [19] J. K. Moon and N. F. Butte, “Combined heart rate and activity improve estimates of oxygen consumption and carbon dioxide production rates,” *J. Appl. Physiol.*, vol. 81, no. 4, pp. 1754–1761, 1996, doi: 10.1152/jappl.1996.81.4.1754.
- [20] L. R. Keytel *et al.*, “Prediction of energy expenditure from heart rate monitoring during submaximal exercise,” *J. Sports Sci.*, vol. 23, no. 3, pp. 289–297, 2005, doi: 10.1080/02640410470001730089.
- [21] H. H. O. Bar-or, “Influence of climate on heart rate in children : comparison between intermittent and continuous exercise,” no. 1998, pp. 7–12, 2000.
- [22] G. B. Spurr, A. M. Prentice, P. R. Murgatroyd, G. R. Goldberg, J. C. Reina, and N.

- T. Christman, "Energy expenditure from minute-by-minute heart-rate recording: Comparison with indirect calorimetry," *Am. J. Clin. Nutr.*, vol. 48, no. 3, pp. 552–559, 1988, doi: 10.1093/ajcn/48.3.552.
- [23] M. Ceesay, M. Prentice, C. Day, and W. Scott, "+ 10.6 %).," pp. 175–186, 1989.
- [24] P. Krumbiegel, "Assessment of body composition and total energy expenditure in humans using stable isotope techniques; IAEA Human Health Series No. 3," *Isotopes Environ. Health Stud.*, vol. 46, no. 4, pp. 508–509, 2010, doi: 10.1080/10256016.2010.521823.
- [25] D. A. Schoeller, "Measurement of energy expenditure in free-living humans by using doubly labeled water," *J. Nutr.*, vol. 118, no. 11, pp. 1278–1289, 1988, doi: 10.1093/jn/118.11.1278.
- [26] H. Murakami *et al.*, "Accuracy of 12 wearable devices for estimating physical activity energy expenditure using a metabolic chamber and the doubly labeled water method: Validation study," *JMIR mHealth uHealth*, vol. 7, no. 8, 2019, doi: 10.2196/13938.
- [27] N. Lifson and R. McClintock, "Theory of use of the turnover rates of body water for measuring energy and material balance," *J. Theor. Biol.*, vol. 12, no. 1, pp. 46–74, 1966, doi: 10.1016/0022-5193(66)90185-8.
- [28] J. R. Speakman, "The history and theory of the doubly labeled water technique," *Am. J. Clin. Nutr.*, vol. 68, no. 4, pp. 932–938, 1998, doi: 10.1093/ajcn/68.4.932S.
- [29] T. P. Stein, R. W. Hoyt, R. G. Settle, M. O'Toole, and W. D. Hiller, "Determination of energy expenditure during heavy exercise, normal daily activity, and sleep using the doubly-labelled-water (2H218O) method," *Am. J. Clin. Nutr.*, vol. 45, no.

- 3, pp. 534–539, 1987, doi: 10.1093/ajcn/45.3.534.
- [30] G. Plasqui, G. Rietjens, L. Lambriks, L. Wouters, and W. H. M. Saris, “Energy Expenditure during Extreme Endurance Exercise: The Giro d’Italia,” *Med. Sci. Sports Exerc.*, vol. 51, no. 3, pp. 568–574, 2019, doi: 10.1249/MSS.0000000000001814.
- [31] B. C. Ruby, T. C. Shriver, T. W. Zderic, B. J. Sharkey, C. Burks, and S. Tysk, “Total energy expenditure during arduous wildfire suppression,” *Med. Sci. Sports Exerc.*, vol. 34, no. 6, pp. 1048–1054, 2002, doi: 10.1097/00005768-200206000-00023.
- [32] J. W. Castellani, J. P. Delany, C. O’Brien, R. W. Hoyt, W. R. Santee, and A. J. Young, “Energy expenditure in men and women during 54 h of exercise and caloric deprivation,” *Med. Sci. Sports Exerc.*, vol. 38, no. 5, pp. 894–900, 2006, doi: 10.1249/01.mss.0000218122.59968.eb.
- [33] M. R. Burstein *et al.*, “Energy expenditure variations in soldiers performing military activities under cold and hot climate conditions,” *Mil. Med.*, vol. 161, no. 12, pp. 750–754, 1996, doi: 10.1093/milmed/161.12.750.
- [34] W. H. M. Saris, M. A. Van Erp-Baart, F. Brouns, K. R. Westerterp, and F. Ten Hoor, “Study on food intake and energy expenditure during extreme sustained exercise: The Tour de France,” *Int. J. Sports Med.*, vol. 10, no. SUPPL. 1, 1989, doi: 10.1055/s-2007-1024951.
- [35] G. Atkinson, O. Peacock, A. S. C. Gibson, and R. Tucker, “Distribution of power output during cycling: Impact and mechanisms,” *Sport. Med.*, vol. 37, no. 8, pp. 647–667, 2007, doi: 10.2165/00007256-200737080-00001.

- [36] G. Atkinson, R. Davison, A. Jeukendrup, and L. Passfield, "Science and cycling: Current knowledge and future directions for research," *J. Sports Sci.*, vol. 21, no. 9, pp. 767–787, 2003, doi: 10.1080/0264041031000102097.
- [37] C. J. Lucía A, Pardo J, Durántez A, Hoyos J, "Physiological differences between professional and elite road cyclists," *Int. J. Sports Med.*, vol. 19, no. 5, pp. 342–348, 1998.
- [38] J. A. Zoladz, K. Duda, and J. Majerczak, "VO<sub>2</sub>/power output relationship and the slow component of oxygen uptake kinetics during cycling at different pedalling rates: Relationship to venous lactate accumulation and blood acid-base balance," *Physiological Research*, vol. 47, no. 6. pp. 427–438, 1998.
- [39] J. A. Zoladz, A. C. Rademaker, and A. J. Sargeant, "Non-linear relationship between O<sub>2</sub> uptake and power output at high intensities of exercise in humans.," *J. Physiol.*, vol. 488, no. 1, pp. 211–217, 1995, doi: 10.1113/jphysiol.1995.sp020959.
- [40] A. Lucía, J. Hoyos, A. Santalla, M. Pérez, and J. L. Chicharro, "Kinetics of  $\dot{V}O_2$  in professional cyclists," *Med. Sci. Sports Exerc.*, vol. 34, no. 2, pp. 320–325, 2002, doi: 10.1097/00005768-200202000-00021.
- [41] F. M. Impellizzeri and S. M. Marcora, "The physiology of mountain biking," *Sport. Med.*, vol. 37, no. 1, pp. 59–71, 2007, doi: 10.2165/00007256-200737010-00005.
- [42] U. Kingdom, "Exercise intensity during off-road cycling competitions," *J. Text. Inst. Proc.*, vol. 30, no. 12, p. P443, 1939, doi: 10.1080/19447013908687554.
- [43] H. Lee, D. T. Martin, J. M. Anson, D. Grundy, and A. G. Hahn, "Physiological characteristics of successful mountain bikers and professional road cyclists," *J.*

- Sports Sci.*, vol. 20, no. 12, pp. 1001–1008, 2002, doi:  
10.1080/026404102321011760.
- [44] H. Hurst *et al.*, “Influence of course type on upper body muscle activity in elite Cross-Country and Downhill mountain bikers during off Road Downhill Cycling,” *J. Sci. Cycl.*, vol. 1, no. 2, pp. 2–9, 2012, [Online]. Available: [http://www.jsc-journal.com/ojs/index.php?journal=JSC&page=article&op=view&path\[\]=20](http://www.jsc-journal.com/ojs/index.php?journal=JSC&page=article&op=view&path[]=20).
- [45] S. Bhutani, “Special Considerations for Measuring Energy Expenditure with Doubly Labeled Water under Atypical Conditions,” *J. Obes. Weight Loss Ther.*, vol. s5, no. Suppl 5, pp. 1–20, 2015, doi: 10.4172/2165-7904.s5-002.
- [46] Z. S. Swanson and H. Pontzer, “Water turnover among human populations: Effects of environment and lifestyle,” *Am. J. Hum. Biol.*, vol. 32, no. 1, pp. 1–13, 2020, doi: 10.1002/ajhb.23365.
- [47] J. E. Harbison *et al.*, “Seasonal variation in natural abundance of  $2\text{H}$  and  $18\text{O}$  in urine samples from rural Nigeria,” *J. Appl. Physiol.*, vol. 119, no. 1, pp. 55–60, 2015, doi: 10.1152/jappphysiol.00894.2014.
- [48] K. R. Westerterp, F. Brouns, W. H. M. Saris, and F. Ten Hoor, “Comparison of doubly labeled water with respirometry at low- and high-activity levels,” *J. Appl. Physiol.*, vol. 65, no. 1, pp. 53–56, 1988, doi: 10.1152/jappl.1988.65.1.53.
- [49] J. P. DeLany, D. A. Schoeller, R. W. Hoyt, E. W. Askew, and M. A. Sharp, “Field use of  $\text{D}218\text{O}$  to measure energy expenditure of soldiers at different energy intakes,” *J. Appl. Physiol.*, vol. 67, no. 5, pp. 1922–1929, 1989, doi: 10.1152/jappl.1989.67.5.1922.
- [50] P. J. H. Jones, I. Jacobs, A. Morris, and M. B. Ducharme, “Adequacy of food

- rations in soldiers during an arctic exercise measured by doubly labeled water,” *J. Appl. Physiol.*, vol. 75, no. 4, pp. 1790–1797, 1993, doi: 10.1152/jappl.1993.75.4.1790.
- [51] L. Kosmiski, “Energy expenditure in HIV infection,” *Am. J. Clin. Nutr.*, vol. 94, no. 6, pp. 1677–1682, 2011, doi: 10.3945/ajcn.111.012625.
- [52] M. I. Goran, E. J. Peters, D. N. Herndon, and R. R. Wolfe, “Total energy expenditure in burned children using the doubly labeled water technique,” *Am. J. Physiol. - Endocrinol. Metab.*, vol. 259, no. 4 22-4, 1990, doi: 10.1152/ajpendo.1990.259.4.e576.
- [53] K. R. Westerterp, B. Kayser, F. Brouns, J. P. Herry, and W. H. M. Saris, “Energy expenditure climbing Mt. Everest,” *J. Appl. Physiol.*, vol. 73, no. 5, pp. 1815–1819, 1992, doi: 10.1152/jappl.1992.73.5.1815.
- [54] C. B. Scott, N. D. Littlefield, J. D. Chason, M. P. Bunker, and E. M. Asselin, “Differences in oxygen uptake but equivalent energy expenditure between a brief bout of cycling and running,” *Nutr. Metab.*, vol. 3, pp. 1–5, 2006, doi: 10.1186/1743-7075-3-1.
- [55] K. E. Bijker, G. de Groot, and A. P. Hollander, “Differences in leg muscle activity during running and cycling in humans,” *Eur. J. Appl. Physiol.*, vol. 87, no. 6, pp. 556–561, 2002, doi: 10.1007/s00421-002-0663-8.
- [56] P. W. Macdermid and S. Stannard, “Mechanical work and physiological responses to simulated cross country mountain bike racing,” *J. Sports Sci.*, vol. 30, no. 14, pp. 1491–1501, 2012, doi: 10.1080/02640414.2012.711487.
- [57] M. A. Febbraio, D. L. Lambert, R. L. Starkie, J. Proietto, and M. Hargreaves,

- “Effect of epinephrine on muscle glycogenolysis during exercise in trained men,” *J. Appl. Physiol.*, vol. 84, no. 2, pp. 465–470, 1998, doi: 10.1152/jappl.1998.84.2.465.
- [58] R. H. Coker and M. Kjaer, “Glucoregulation during exercise: The role of the neuroendocrine system,” *Sport. Med.*, vol. 35, no. 7, pp. 575–583, 2005, doi: 10.2165/00007256-200535070-00003.
- [59] M. M. Schubert, H. E. Clarke, R. F. Seay, and K. K. Spain, “Impact of 4 weeks of interval training on resting metabolic rate, fitness, and health-related outcomes,” *Appl. Physiol. Nutr. Metab.*, vol. 42, no. 10, pp. 1073–1081, 2017, doi: 10.1139/apnm-2017-0268.
- [60] V. Seliger, L. Dolejš, and V. Karas, “A dynamometric comparison of maximum eccentric, concentric, and isometric contractions using EMG and energy expenditure measurements,” *Eur. J. Appl. Physiol. Occup. Physiol.*, vol. 45, no. 2–3, pp. 235–244, 1980, doi: 10.1007/BF00421331.
- [61] Asker Jeukendrup, “The New Carbohydrate Intake Recommendations,” *Nutr. Coach. Strateg. to Modul. Train. Effic.*, vol. 75, pp. 63–71, 2013, [Online]. Available: <https://www.nestlenutrition-institute.org/sites/default/files/documents-library/publications/secured/9b554fc8b27955fbf3fb2435380f1fcc.pdf#page=77>.
- [62] E. F. Coyle, “Fluid and fuel intake during exercise,” *J. Sports Sci.*, vol. 22, no. 1, pp. 39–55, 2004, doi: 10.1080/0264041031000140545.
- [63] A. Krogh and J. Lindhard, “The Relative Value of Fat and Carbohydrate as Sources of Muscular Energy,” *Biochem. J.*, vol. 14, no. 3–4, pp. 290–363, 1920, doi: 10.1042/bj0140290.



- [64] R. Cade *et al.*, “Marathon running: physiological and chemical changes accompanying late-race functional deterioration,” *Eur. J. Appl. Physiol. Occup. Physiol.*, vol. 65, no. 6, pp. 485–491, 1992, doi: 10.1007/BF00602353.
- [65] J. L. Ivy, A. L. Katz, C. L. Cutler, W. M. Sherman, and E. F. Coyle, “Muscle glycogen synthesis after exercise: Effect of time of carbohydrate ingestion,” *J. Appl. Physiol.*, vol. 64, no. 4, pp. 1480–1485, 1988, doi: 10.1152/jappl.1988.64.4.1480.
- [66] E. F. Coyle, A. R. Coggan, M. K. Hemmert, and J. L. Ivy, “Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate,” *J. Appl. Physiol.*, vol. 61, no. 1, pp. 165–172, 1986, doi: 10.1152/jappl.1986.61.1.165.
- [67] L. J. C. Van Loon, A. E. Jeukendrup, W. H. M. Saris, and A. J. M. Wagenmakers, “Effect of training status on fuel selection during submaximal exercise with glucose ingestion,” *J. Appl. Physiol.*, vol. 87, no. 4, pp. 1413–1420, 1999, doi: 10.1152/jappl.1999.87.4.1413.
- [68] C. M. Kerksick *et al.*, “International society of sports nutrition position stand: Nutrient timing,” *J. Int. Soc. Sports Nutr.*, vol. 14, no. 1, pp. 1–21, 2017, doi: 10.1186/s12970-017-0189-4.
- [69] S. G. Harger-Domitrovich, A. E. McClaughry, S. E. Gaskill, and B. C. Ruby, “Exogenous carbohydrate spares muscle glycogen in men and women during 10 h of exercise,” *Med. Sci. Sports Exerc.*, vol. 39, no. 12, pp. 2171–2179, 2007, doi: 10.1249/mss.0b013e318157a650.
- [70] J. W. Smith *et al.*, “Fuel selection and cycling endurance performance with ingestion of [ <sup>13</sup>C]glucose: Evidence for a carbohydrate dose response,” *J. Appl.*

- Physiol.*, vol. 108, no. 6, pp. 1520–1529, 2010, doi:  
10.1152/jappphysiol.91394.2008.
- [71] A. E. Jeukendrup, “Carbohydrate intake during exercise and performance,” *Nutrition*, vol. 20, no. 7–8, pp. 669–677, 2004, doi: 10.1016/j.nut.2004.04.017.
- [72] J. W. Smith *et al.*, “Curvilinear dose-response relationship of carbohydrate (0-120 g·h<sup>-1</sup>) and performance,” *Med. Sci. Sports Exerc.*, vol. 45, no. 2, pp. 336–341, 2013, doi: 10.1249/MSS.0b013e31827205d1.
- [73] P. N. Ainslie, T. Reilly, and K. R. Westerterp, “Estimating Human Energy Expenditure,” *Sport. Med.*, vol. 33, no. 9, pp. 683–698, 2003, doi:  
10.2165/00007256-200333090-00004.
- [74] J. Trabulsi *et al.*, “Precision of the doubly labeled water method in a large-scale application: Evaluation of a streamlined-dosing protocol in the Observing Protein and Energy Nutrition (OPEN) study,” *Eur. J. Clin. Nutr.*, vol. 57, no. 11, pp. 1370–1377, 2003, doi: 10.1038/sj.ejcn.1601698.
- [75] S. B. Racette, D. A. Schoeller, A. H. Luke, K. Shay, J. Hnilicka, and R. F. Kushner, “Relative dilution spaces of 2H- and 18O-labeled water in humans,” *Am. J. Physiol. - Endocrinol. Metab.*, vol. 267, no. 4 30-4, pp. 6–11, 1994, doi:  
10.1152/ajpendo.1994.267.4.e585.
- [76] D. A. Schoeller, E. Ravussin, Y. Schutz, K. J. Acheson, P. Baertschi, and E. Jéquier, “Energy expenditure by doubly labeled water: Validation in humans and proposed calculation,” *Am. J. Physiol. - Regul. Integr. Comp. Physiol.*, vol. 250, no. 5 (19/5), 1986, doi: 10.1152/ajpregu.1986.250.5.r823.
- [77] W. W. Wong, N. F. Butte, C. Garza, and P. D. Klein, “Comparison of the doubly

- labeled water (2H<sub>2</sub> 18O) method with indirect calorimetry and a nutrient-balance study for simultaneous determination of energy expenditure, water intake, and metabolizable energy intake in preterm infants.,” *Am. J. Clin. Nutr.*, vol. 45, no. 6, pp. 1543–1548, 1987, doi: 10.1093/ajcn/45.6.1543.
- [78] T. J. Cole and W. A. Coward, “Precision and accuracy of doubly labeled water energy expenditure by multipoint and two-point methods,” *Am. J. Physiol. - Endocrinol. Metab.*, vol. 263, no. 5 26-5, 1992, doi: 10.1152/ajpendo.1992.263.5.e965.
- [79] A. T. Hulton *et al.*, “Energy expenditure in the race across America (RAAM),” *Int. J. Sports Med.*, vol. 31, no. 7, pp. 463–467, 2010, doi: 10.1055/s-0030-1251992.
- [80] A. M. Rosales, N. A. Keck, T. C. Shriver, D. A. Schoeller, and B. C. Ruby, “Septuagenarians Approach 4 Times the Basal Metabolic Rate During Race Across America,” *Int. J. Sports Physiol. Perform.*, pp. 8–11, 2022.
- [81] N. J. Rehrer, I. J. Hellemans, A. K. Rolleston, E. Rush, and B. F. Miller, “Energy intake and expenditure during a 6-day cycling stage race,” *Scand. J. Med. Sci. Sport.*, vol. 20, no. 4, pp. 609–618, 2010, doi: 10.1111/j.1600-0838.2009.00974.x.
- [82] A. Barrero, P. Erola, and R. Bescós, “Energy balance of triathletes during an ultra-endurance event,” *Nutrients*, vol. 7, no. 1, pp. 209–222, 2015, doi: 10.3390/nu7010209.
- [83] N. E. Kimber, J. J. Ross, S. L. Mason, and D. B. Speedy, “Energy balance during an Ironman triathlon in male and female triathletes,” *Int. J. Sport Nutr.*, vol. 12, no. 1, pp. 47–62, 2002, doi: 10.1123/ijsnem.12.1.47.
- [84] J. Á. Ramos-Campo, D. J., Ávila-Gandía, V., Alacid, F., Soto-Méndez, F., Alcaraz,

- P. E., López-Román, F. J., & Rubio-Arias, “Muscle damage, physiological changes, and energy balance in ultra-endurance mountain-event athletes.,” *Appl. Physiol. Nutr. Metab.*, vol. 41, no. 8, pp. 872–878, 2016.
- [85] A. E. Jeukendrup, “Nutrition for endurance sports: Marathon, triathlon, and road cycling,” *J. Sports Sci.*, vol. 29, no. SUPPL. 1, 2011, doi: 10.1080/02640414.2011.610348.
- [86] A. E. Jeukendrup, L. Moseley, G. I. Mainwaring, S. Samuels, S. Perry, and C. H. Mann, “Exogenous carbohydrate oxidation during ultraendurance exercise.,” *J. Appl. Physiol.*, vol. 100, no. 4, pp. 1134–1141, 2006, doi: 10.1152/jappphysiol.00981.2004.
- [87] J. R. Speakman *et al.*, “A standard calculation methodology for human doubly labeled water studies,” *Cell Reports Med.*, vol. 2, no. 2, 2021, doi: 10.1016/j.xcrm.2021.100203.
- [88] S. Schulz, K. R. Westerterp, and K. Bruck, “Comparison of energy expenditure by the doubly labeled water technique with energy intake, heart rate, and activity recording in man,” *Am. J. Clin. Nutr.*, vol. 49, no. 6, pp. 1146–1154, 1989, doi: 10.1093/ajcn/49.6.1146.
- [89] M. C. Miller, P. W. Fink, P. W. Macdermid, D. Allen, and S. R. Stannard, “Braking and performance characteristics of experienced and inexperienced mountain bikers navigating an isolated off-road turn using a brake power meter,” *Int. J. Perform. Anal. Sport*, vol. 18, no. 4, pp. 495–506, 2018, doi: 10.1080/24748668.2018.1496383.
- [90] L. Havemann and J. H. Goedecke, “Nutritional practices of male cyclists before

- and during an ultraendurance event,” *Int. J. Sport Nutr. Exerc. Metab.*, vol. 18, no. 6, pp. 551–566, 2008, doi: 10.1123/ijsnem.18.6.551.
- [91] B. Geesmann, J. Mester, and K. Koehler, “Energy balance, macronutrient intake, and hydration status during a 1,230 km ultra-endurance bike marathon,” *Int. J. Sport Nutr. Exerc. Metab.*, vol. 24, no. 5, pp. 497–506, 2014, doi: 10.1123/ijsnem.2013-0169.
- [92] A. E. Jeukendrup and J. McLaughlin, “Carbohydrate ingestion during exercise: Effects on performance, training adaptations and trainability of the gut,” *Nestle Nutr. Inst. Workshop Ser.*, vol. 69, pp. 1–17, 2011, doi: 10.1159/000329268.
- [93] F. Grego *et al.*, “Effects of long duration exercise on cognitive function, blood glucose, and counterregulatory hormones in male cyclists,” *Neurosci. Lett.*, vol. 364, no. 2, pp. 76–80, 2004, doi: 10.1016/j.neulet.2004.03.085.
- [94] T. Van Helder and M. W. Radoki, “Sleep Deprivation and the Effect on Exercise Performance,” *Sport. Med.*, vol. 7, no. 4, pp. 235–247, 1989, doi: 10.2165/00007256-198907040-00002.
- [95] X. Zhong *et al.*, “Increased sympathetic and decreased parasympathetic cardiovascular modulation in normal humans with acute sleep deprivation,” *J. Appl. Physiol.*, vol. 98, no. 6, pp. 2024–2032, 2005, doi: 10.1152/jappphysiol.00620.2004.