

Resting Energy Expenditure in Athletes: Accuracy of Cunningham Prediction
Equation Using DXA-Derived Fat Free Mass

By
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Jessica Provost, RD
Bachelor of Science in Dietetics, Kansas State University, 2017

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Committee Chair: Aaron Carbuhn, Ph.D., RD, CSSD

Debra Sullivan, Ph.D., RD

Holly Hull, Ph.D.

Date Defended: April 21, 2020

The thesis committee for Jessica Provost certifies that this is the
approved version of the following thesis:

Resting Energy Expenditure in Athletes: Accuracy of Cunningham Prediction
Equation Using DXA-Derived Fat Free Mass

Chair: Aaron Carbuhn, Ph.D., RD, CSSD

Graduate Director: Heather Gibbs, Ph.D., RD, LD

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Abstract

Introduction: It is recommended to use the fat free mass (FFM) based Cunningham equation to accurately estimate resting energy expenditure (REE) in athletic populations. However, it is unknown if FFM derived from dual-energy x-ray absorptiometry (DXA) can accurately estimate REE in collegiate athletes. Therefore, our aim is to determine REE accuracy of Cunningham equation using DXA-derived FFM compared to measured REE in Division I collegiate athletes.

Methods: 15 (14F, 1M) NCAA Division I athletes from 7 collegiate sports completed the test protocol. Indirect calorimetry (Parvo Medics' TrueOne® 2400 metabolic cart with canopy system) was used to measure REE. A DXA (GE Lunar iDXA™ enCORE-based) measurement was completed within 14 days of measured REE to provide FFM. The DXA-derived FFM was inserted into Cunningham equation ($\text{REE [kcal/day]} = 500 + 22 \times \text{FFM[kg]}$) to predict REE. A Wilcoxon signed-rank test and Bland-Altman analysis were used to determine respective difference and bias between measured and predicted REE.

Results: Predicted REE by Cunningham equation using DXA-derived FFM was not statistically different from measured REE ($Z = -1.306$, $p = 0.191$). Average measured and predicted REE was 1448 kcal/day and 1587 kcal/day. Mean percent difference was $\pm 20.09\%$ with 47% of participants REE difference $> \pm 10\%$. Bland-Altman analysis suggested proportional bias, with overestimations among low measured REE values (~ 1200 kcal/day) and underestimations among high measured REE values (~ 1800 kcal/day).

Conclusions: The Cunningham equation, using DXA-derived FFM to predict REE, was not significantly different from measured REE in Division 1 collegiate athletes. However, reported bias and possible clinical significance (i.e. % difference) warrant further investigation to validate our preliminary findings.

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Chapter 1: Introduction

For athletic populations, the estimation of energy expenditure to determine appropriate energy requirements (i.e. energy balance) is often utilized by health practitioners to help optimize performance, enhance recovery, prevent injury, alter body composition, and support overall health. Estimating resting energy expenditure (REE) is a significant component of an athletes' daily energy requirements with previous research reporting REE makes up 45–75% of total energy expenditure ([1](#)). Thus, an accurate estimation of REE appears to be a logical starting point for health practitioners in determining and managing an athlete's energy balance.

The gold standard for measuring and estimating REE is indirect calorimetry, in which respiratory gas exchange is assessed as an indicator of energy production ([2](#)). Unfortunately, measured resting energy expenditure (mREE) via indirect calorimetry on athlete populations has been prohibitive and not generally practiced. Thus, predicted resting energy expenditure (pREE) equations derived from indirect calorimetry mREE data are utilized.

Referenced guidance on pREE equations use within athletic populations is provided by the *Nutrition and Athletics Performance* joint position paper by the *Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine* ([3](#)). They recommend using the Cunningham or Harris-Benedict pREE equation ([3](#)) with an understanding that these referenced equations only provide a “reasonable estimate” and may not accurately estimate REE for all athletes ([3](#)). This “reasonable estimate” and potential inaccuracies are due to these respective pREE equations being derived from mREE of general populations which did not include athletes and athletic populations of varying body size, competition level, and sport ([4](#), [5](#)).

There is a key difference between the Cunningham and Harris-Benedict pREE equations that may be important, especially relative to athletes. The Cunningham pREE equation includes fat-free mass (FFM) as a predictor variable. Inclusion of FFM improves the prediction of REE in both male and female athletes despite the equation's mREE population not including athletes. This suggests FFM, instead of total body weight, is an important predictor of REE ([6-8](#)). However, body composition methods used to determine FFM for the Cunningham pREE in athletes have primarily used techniques such as skinfolds ([9, 10](#)), bioelectrical impedance analysis (BIA) ([8](#)), or air displacement plethysmography (ADP) i.e. BOD POD ([6, 7](#)). Despite the increased popularity of dual-energy x-ray absorptiometry (DXA) in athletic populations to assess body composition and FFM, to the author's knowledge, little research has examined the accuracy of the Cunningham pREE in athletes using a DXA derived FFM value.

Therefore, the purpose of this study was to determine if there was a difference in REE in Division I collegiate athletes between the Cunningham equation using FFM measured by DXA compared to indirect calorimetry. In order to address this question, mREE by indirect calorimetry was compared to the Cunningham pREE equation using FFM from a total body DXA measurement.

Chapter 2: Review of Literature

2.1 Introduction

REE is a key component for athletic populations in supporting appropriate energy balance and health because these respective energy requirements are needed to sustain essential bodily functions ([1](#)). Determining mREE is different from Basal Metabolic Rate (BMR) which involves stricter indirect calorimetry testing conditions and seeks to determine an individual's true minimum (i.e. basal) energy requirement. Despite easier testing conditions compared to BMR, mREE has been validated in athletic populations and can provide a practical approach in accurately determining fundamental energy requirements ([1](#)). However, when mREE is not convenient or feasible, pREE is utilized to estimate REE. Unfortunately, the most commonly used pREE equations recommended for athletic populations were developed within samples of the general population, which did not include individuals with athletic backgrounds; thus, resulting in greater reported pREE inaccuracies compared to mREE ([4](#), [5](#)).

2.2 Athletes' Need for Accurate Resting Energy Estimates

Athletes require accurate estimates of energy needs in order to support performance, body composition, and general health goals. Inaccurate energy estimates, beginning with REE, can influence athletic performance and training adaptations due to undesired effects on physical work and exercise recovery. Energy intake provides the “fuel” to do work as well as the energy and building blocks to recover from physical training stressors. For example, when pREE underestimates energy requirements, athletes create a negative energy balance (EB) ([6](#)). A negative EB disrupts the energy needs for work and recovery and as a result, limits optimal athletic performance and prevents training

adaptations ([11](#)). Therefore, an accurate pREE is needed to promote optimal athletic performance gains.

The importance of accurately assessing REE is further illustrated by chronic states of negative EB. Athletes who consume inadequate energy to meet their REE in addition to the cost of exercise are at a greater risk of numerous negative health effects. These negative effects are reported to occur within general metabolic and reproductive function, bone density, immune function, muscle protein turnover, cardiovascular health, exercise training acclimatization, and overall psychological well-being ([12](#)). More specifically, when altered reproductive function and decreased bone density were observed with negative EB, this was described as the Female Athlete Triad (FAT) ([13](#)). However, these risks are not limited to female athletes and only three health indicators. Therefore, a new term was coined in 2014, Relative Energy Deficiency in Sport (RED-S), to describe this complex syndrome surrounding negative EB amongst all athletes ([12](#)). Enhancing our accuracy of REE estimations (pREE vs mREE) will improve awareness and prevention of RED-S. This is recognized by the governing bodies that recommended mREE by indirect calorimetry in their latest recommendations: *The IOC Consensus Statement: Beyond the Female Athlete Triad—Relative Energy Deficiency in Sport (RED-S)* ([12](#)).

2.3 Measured Resting Energy Expenditure and Athletes

An athlete's total energy expenditure (TEE) includes the sum of all energy costs: REE, thermic effect of food, and thermic effect of activity. TEE is assessed in athletes using doubly labeled water (DLW) to find a weekly average. This method is costly and requires specialized materials and equipment for analysis. TEE varies greatly day-to-day and

throughout an athletic season ([14](#)). Therefore, using DLW for frequent TEE measurements is impractical. REE, however, stays relatively stable for athletes throughout a week or athletic season as long as energy balance is achieved ([14](#)). REE does not provide a complete picture of TEE and is a lower percentage of TEE in athletes than non-athletes ([15](#)), nonetheless, it is still the largest and most stable contributor to energy expenditure ([14](#)). Of the studies reviewed, REE was estimated to contribute 45% to 75% of TEE in athletes ([1](#), [6](#), [8](#), [10](#), [15](#)).

Currently, the “gold standard” method for a mREE is indirect calorimetry ([7](#), [15](#)). While indirect calorimetry mREE is accepted and validated in athletes ([6](#), [8](#), [11](#), [16](#)), its integration is limited due to equipment costs, specialty training for operators, and time requirements for testing which includes a pre-test fast and exercise restriction that has further precluded testing feasibility in this population. Conversely, regression equations developed for pREE are well-established in health care and require no added expense or inconvenience to athletes. Thus, when mREE, using indirect calorimetry, is not available or practical, pREE equations are utilized to provide REE values similar to mREE.

2.4 Predicted Resting Energy Expenditure Equations and Athletes

Across various sample populations, two variables that can differ greatly between individuals – FFM and body weight – are reported to be the strongest predictors of REE ([11](#)). For example, FFM can account for up to 70% of mREE ([11](#)). Therefore, various pREE equations are generally divided into two categories: weight-based, and FFM-based. Weight-based equations use easily-obtained anthropometric data – height, weight, and age – while FFM-based equations require body composition assessments to estimate or measure fat mass (FM) and FFM. Equations typically used within athletic populations include the Harris-

Benedict, Mifflin, Owen, and DeLorenzo equations, which are weight-based, and the Cunningham and Nelson equations, which use FFM (7).

2.4.1 Body Composition Methods to Assess FFM for pREE

FFM is defined as the mass of muscle, bone, fluids, organs, and all other tissues when FM is subtracted from total body mass (17). When FFM is required for pREE equations, a variety of body composition techniques may be considered. FFM is more difficult to assess than other anthropometrics – requiring either costly equipment such as Dual-energy X-ray Absorptiometry (DXA), hydrodensitometry or underwater weighing (UWW), bioelectrical impedance analysis (BIA), and air-displacement plethysmography (ADP), or a skilled practitioner to perform skinfold thickness measurements.

All the techniques previously stated assess the body in two components (2C): FM and FFM (17). A difference between DXA and the other models mentioned is that DXA can differentiate FFM into bone and non-bone lean tissue. DXA uses low-energy, filtered x-ray energy to quantify FM, bone, and non-bone lean tissue (17).

In addition, DXA is considered more ideal than other body composition assessments to determine FFM because it is less burdensome than UWW and has a lower standard of error compared to other body composition assessment techniques that potentially utilized in an athletic setting – ADP, BIA, and skinfold measurements (3). Therefore, while DXA is a validated technique to assess body composition within athletic settings (18), differences between DXA and other FFM measurement methods can be significantly different. For example, when evaluating DXA compared to UWW the standard error of the estimate of DXA was 3.0%, indicating good accuracy of DXA vs. UWW (19). In a study comparing DXA to BIA measurements

of FFM in Division I collegiate football players, it was determined that BIA-derived FFM was significantly different from DXA with BIA overestimating FFM by 2.4 ± 4.3 kg ([18](#)). In female Division 1 collegiate athletes, further comparisons between DXA and BIA FFM reported BIA values were significantly greater (i.e. 2.1 ± 3.7 kg) than DXA; thus, stating significant “discrepancies between the two devices” ([20](#)). In that same study, DXA and BIA agreed very well on lean tissue measurements, which implies that BIA may assess muscle well, but in using a fixed assumption for bone density, will not match DXAs measurements of that individual value ([20](#)). A study sample evaluating percent body fat (BF%) of underweight, normal weight, and overweight adults found “good agreement” in normal weight subjects, but variation in underweight and overweight groups ([21](#)). In underweight, ADP was 6.79% higher than DXA and in overweight, ADP was 1.68% lower than DXA. to skinfold methods, DXA has been reported to have a low coefficient of variation for FFM of 0.8% ([22](#)) while skinfold FFM had up to a 5% coefficient of variation depending on practitioner skill ([17](#)).

The variation between the 2C devices reveals the limitations of their assumptions of constant body water and bone tissue density. Improved accuracy would necessitate a multi-component model of body composition which can have 1-2% accuracy and precision ([17](#)). The 4-component (4C) model uses multiple techniques to assess mass, total body water (TBW), total body volume (TBV), and bone mineral content (BMC) in order to calculate FM and FFM ([17](#), [23](#)). However, while the 4C model would provide additional accuracy and is the reference method for body composition ([17](#)), it requires multiple methods. For the 4C model, the requirement of additional equipment, and time and cost make it not feasible to perform in

athletes ([24](#), [25](#)). So, while a 4C model would be ideal, a 2C single-technique measure such as DXA is likely to be more feasible in a clinical sports setting.

DXA's clinical use for estimating FFM, but potential significant differences in comparison to the other 2C techniques and lower accuracy than a multi-component model warrant further considerations regarding its potential influence on FFM-based pREE. Since body composition derived FFM values are used in various sports settings to estimate athletes' energy needs, it is important to understand whether DXA-derived FFM will affect pREE equation accuracy.

2.4.2 Recommended pREE Equations for Athletes

Guidance on predictive equation use for athletes is provided by the joint position paper entitled *Nutrition and Athletic Performance* by the Academy of Nutrition and Dietetics (AND), Dietitians of Canada (DC), and the American College of Sports Medicine (ACSM) ([3](#)). The paper's broad consensus on pREE equations is to use either the Cunningham (FFM-based) equation or, for a weight-based alternative, the Harris-Benedict equation. This recommendation is made with the caveat that either equation may only provide a "reasonable estimate" that would be improved with more specific equations tailored to each athlete sub-type population ([3](#)). Without pREE equations specifically developed for each sport, training level, gender, or age, studies have investigated how these general equations apply to varying athletic populations. This review will consider the Cunningham equation in particular.

2.4.3 Cunningham Equation Accuracy in Athletes

The Cunningham equation ([4](#)), recommended by the AND/DC/ACSM position paper, ([3](#)) is an entirely FFM-based equation:

$$\text{REE (kcal/day)} = 500 + 22 \times \text{FFM (kg)}$$

FFM is the largest contributor to REE changes ([26](#), [27](#)). However, the FFM variable has not been assessed with a standardized body composition measure among the studies investigating the validity of the Cunningham equation in athletes ([6-10](#)). Furthermore, there is no FFM measurement standard for the Cunningham equation since it was developed from a regression equation estimate of FFM using the body weight and age data of 223 subjects from the 1918 Harris-Benedict study ([4](#), [28](#)).

With multiple methods used to assess FFM, studies investigating pREE equation accuracy in athletes have varied. For example, in a study of elite (German National Team) rowers and canoeists, using skinfold calipers to assess FFM, the Cunningham equation underestimated REE by an average of 133-202 kcal/day ([10](#)). In another study also using skinfold-derived FFM amongst female athletes in a wide variety of Division II collegiate sports, the Cunningham equation differed significantly from mREE, with a standard error of 128 kcal/day ([9](#)). However, a study of NCAA Division III collegiate athletes with FFM derived from ADP concluded that the Cunningham equation was the most accurate choice for females, but not for males ([29](#)). Yet, ADP-derived FFM in recreational Dutch athletes reported good accuracy with the Cunningham equation overall, in both male and female athletes ([7](#)). Using BIA-derived FFM, in Korean elite and recreational high school athletes, the Cunningham equation was found to only be reasonably accurate in males and not accurate in females ([8](#)).

Lastly, two recent studies have shown no significant difference between pREE and mREE when the Cunningham equation is used with female athletes, one using FFM from ADP ([6](#)), the other from DXA ([15](#)).

In addition to considering body composition technique FFM value variations and Cunningham accuracy, overall FFM *among* athletes may also contribute to Cunningham pREE accuracy. The Cunningham equation was developed using data from a “normal weight” population and specifically excluded 16 trained athletes from its sample ([4](#), [5](#)). Thus, with an average FFM of 46 kg used to develop the equation, the Cunningham sample did not represent physiques with greater FFM ([4](#), [10](#)). For example, in athletic populations, a study that included skinfold measurements of elite rowers showed FFM to exceed 90 kg, with averages of 56 kg for females and 81 kg for males ([10](#)). Furthermore, in a study of 49 Division III collegiate athletes from a diverse assortment of sports, the ADP assessed body fat values varied significantly from 9% to 26% ([6](#)). A study of 93 female athletes assessed by DXA had a FFM difference of up to 32 kg or 70 pounds between subjects ([27](#)). A companion study of 57 male athletes had upper and lower FFM values ranging 40 kg or 88 pounds ([26](#)). Therefore, these greater FFM values and wide variations highlight the diverse range of body types between and within sports as well as the likely difference from Cunningham sample population which may also influence Cunningham accuracy.

2.5 Discussion

With the variety of body composition methods used in athletic settings, differences in FFM-assessment values combined with the wide range of FFM in different athletes may significantly influence reported Cunningham pREE accuracies as described thus far.

DXA does have its own limitations, including the equipment cost and the radiation dose, but the scan is also simple to perform, fast, non-invasive, and less affected by hydration status than other methods, which can make it preferable for practical purposes ([3](#), [22](#)). While DXA use with athletes is increasing ([17](#)), there seems to be a paucity of literature evaluating this body composition method derived FFM validity in the Cunningham equation, particularly compared to mREE from indirect calorimetry for any population, particularly athletes. To the author's knowledge, only the Loureiro study looked at mREE by indirect calorimetry and pREE (i.e. Cunningham) using DXA-derived FFM in athletes ([15](#)). However, this study was on an adolescent population and not in a collegiate athlete population ([15](#)).

Based upon the wide variety of reported Cunningham pREE accuracy results described thus far using various body composition techniques, this review of literature indicates this equation's accuracy in athletes may be influenced by FFM methodological assessment. Thus, when different body composition techniques are used across existing studies to assess this FFM, it is problematic to understand the accuracy in athletes when using different body composition techniques. The validity of the Cunningham equation when DXA is used for FFM has not been investigated for collegiate athletes despite DXA usage becoming increasingly popular ([3](#)). Therefore, in order to determine whether the Cunningham equation is valid for some or all collegiate athletes, it is important to first understand whether the equation is accurate when FFM is derived by DXA.

Chapter 3: Methods

3.1 Overview

In order to determine whether the Cunningham equation using DXA-derived FFM can accurately predict mREE in a collegiate athlete population, Division 1 male and female student-athletes, predominantly age 18-23 years, on athletic teams at the University of Kansas eligible for DXA testing between August, 2019 and March, 2020 (Golf, Tennis, Track & Field, Baseball, Swim & Dive, Basketball, Cross Country, Soccer, Volleyball, and Football) were recruited. Within 14 days of their total body DXA measurement, an indirect calorimetry mREE was conducted to ensure DXA-derived FFM value was accurate for Cunningham pREE and mREE comparison.

Athlete DXA testing was already well-established within the athletic department's performance nutrition division, with the test offered 1-3 times per year to teams on a rotating schedule (Appendix A – DXA Schedule). All completed DXA measurements were part of an ongoing research study investigating the effects of sport and exercise on bone and body composition; thus, all DXA standard operating procedures for testing and data collection were approved by the University of Kansas Human Subjects Committee. When athletes were notified by email (from their team dietitian) of their upcoming DXA appointment window, they also received information on signing up for the indirect calorimetry mREE appointment on available dates within that two-week period. Once an athlete scheduled an appointment, they received an email with pre-testing instructions (lab location, fasting, apparel, etc.) and contact information for the technician in case of questions. The email procedures and templates are included in Appendix B – Athlete Scheduling Standard Operating Procedure (SOP).

3.2 Setting and Schedule

DXA and indirect calorimetry measurements took place at separate appointment times and locations, with the DXA in a Performance Nutrition office at the Anderson Family Strength Center, and indirect calorimetry in a procedure room within the Anderson Family Football Complex athletic training room. The DXA room was climate-controlled, and the door was closed during tests per IRB approved protocol. Indirect calorimetry testing required specific operating conditions – temperature 14-30°C (57-86°F), relative humidity 10-80% non-condensing, and barometric pressure 400-780mmHg – verified with a weather station device 30 minutes before all appointments. Appointments for DXA were offered during the team dietitians' office hours and indirect calorimetry appointments were offered on Tuesdays and Fridays, at 7:00AM, 8:00AM, and 9:00AM in the fall semester. During the spring semester, an additional test day (Wednesday) was added. The first indirect calorimetry test took place on September 24, 2019, and data collection continued through February 11, 2020.

3.3 Ethics

This project was conducted within an existing protocol for Dr. Aaron Carbuhn's Athlete's Resting Energy Expenditure study, which was approved by the Human Subjects committee at the University of Kansas. This research does not qualify for exempt status; thus, the indirect calorimetry research technician explained study procedures, risks, and benefits, and required each athlete to complete a separate consent form for DXA and indirect calorimetry testing (Appendix C – Informed Consent Statement). Athletes were informed that they could end a test or withdraw from the study at any point.

3.4 Procedures

Prior to mREE testing, athletes were instructed (by two separate emails) that they must have no alcohol 24 hours before test as well as no exercise or food 12 hours before test (30). The athletes were asked to confirm they had met these requirements prior to testing, and if they had not, their test would have been rescheduled. Also, if the athlete's respiratory quotient (RQ) was 0.91–1.3 during the measurement, this result was considered outside of the fasting range, and so the test was to be rescheduled (30). On testing days, the technician arrived to power on the indirect calorimeter's analyzer on one hour before the first appointment of the day. The procedures to then set up the equipment and perform the measurement are detailed in Appendix D-Technician SOP. There was a 10-minute supine rest before each test began, then the first 10 minutes of the measurement were discarded, to achieve the recommended minimum of 20 minutes rest before data collection (30). The technician reviewed the consent form with the athlete and obtained their signature prior to beginning the measurement (Appendix C-Informed Consent Statement). A participant history document was completed at this time, including verification that the athlete correctly restricted food, exercise, and alcohol per instructions (Appendix E – Participant History). The actual indirect calorimetry measurement took 30 minutes.

At the end of every testing morning, an alcohol burn measurement was performed for quality assurance and REE measurement accuracy (Appendix F – Alcohol Burn SOP). Data from the day's athlete REE testing and alcohol burn were exported to Excel for data correction (Appendix G – Alcohol Burn Analysis Template and Appendix H – Alcohol Burn Data Correction SOP). Data was then entered to REDCap (Appendix I – Data Transfer and Entry SOP).

DXA scans were completed with subjects wearing minimal clothing. Athletes were instructed to arrive for their appointment wearing compression spandex shorts and a t-shirt, with a sports bra (metal-free) for females, and all athletes were told to refrain from eating or exercise one hour before their appointment. Upon arrival, the trained technician had the athlete remove shoes, any clothing in other than the shorts and t-shirt specified, watches, and all jewelry and first assessed height and weight. The technician explained the consent form and obtained signatures for new participants. The technician had the athlete lie supine on the DXA table and helped align the athlete in the correct position. The scan took 7-12 minutes and the athlete was then free to leave. Results were entered into an Excel spreadsheet and added to REDCap.

3.5 Materials

The primary equipment used for in study was a DXA (GE Lunar iDXA™ enCORE-based X-ray Bone Densitometer) and an indirect calorimeter (Parvo Medics' TrueOne® 2400 metabolic cart with canopy system). Additional equipment included a Detecto® 6339 digital body weight scale with stadiometer for weight and height assessment in the DXA office and a digital body weight scale with stadiometer in the indirect calorimetry lab. For indirect calorimetry, the Davis Instruments® VantageVue® Weather Station confirmed environmental parameters, a 20" 3-speed fan was used at all times during operation, and the alcohol burn validation required a Digital lab scale and Fisher Chemical Methanol, 500mL.

3.6 Data Collected

Both indirect calorimetry and DXA testing had a Participant History Form which included first name, last name, middle initial, birth date, sport, position, academic classification, height (inches), and weight (pounds). All testing also required consent forms, with printed name, date, and signature. The technician recorded participant fat-free mass (pounds, from DXA), participant raw REE (kcal/day, from indirect calorimetry), and participant corrected REE (kcal/day, adjusted after alcohol burn) as well as Daily Alcohol Burn Analysis results.

3.7 Analysis

The DXA test provided the FFM value needed for the Cunningham predictive equation. The Cunningham pREE was calculated for all subjects using the DXA-derived total FFM value. In this sample size of 15, normal distribution could not be verified and non-parametric statistical analysis was required. To compare the pREE to the mREE values, a Wilcoxon signed-rank test was used to consider statistical differences between the two values at a significance of $p < 0.05$. Because the goal was to compare two different methods of estimating energy expenditure, a Bland-Altman analysis was appropriate to assess how much the results from the two methods agreed or differed within a 95% confidence interval ([31](#)). Statistical analysis was performed with SPSS (IBM SPSS Statistics Subscription for Mac OS, Version 26.0).

Chapter 4: Results

4.1 Athlete Characteristics

The final study sample for analysis was comprised of 15 Division I collegiate athletes (14 female, 1 male). Subject demographics are summarized in **Table 1**. With an average age of 20.7 ± 0.8 years, this sample of collegiate athletes represented a different developmental stage and competition level in comparison to one other study comparing pREE accuracy to mREE using DXA-derived FFM values in adolescent athletes with an average age of 15 ± 2 years ([15](#)). The average subject weight of 64.8 ± 6.9 kg and FFM of 49.5 ± 5.5 kg aligned closely with the female subjects of other collegiate and young adult athlete samples such as Division III collegiate athletes ([6](#)), Division II collegiate athletes ([9](#)), competitive recreational ([7](#)), and elite rowers ([10](#)), but this similarity was only seen when considering the all-female studies or female subgroups. Studies with more male subjects reported higher weights and FFM values ([6](#), [7](#), [10](#), [32](#)), which is logical given this study's 93% female composition.

Seven different sports were represented as well as two different events within the sport of track & field. The represented sports are reported in **Table 2**. Nine athletes represented more endurance-focused events (Cross Country, Soccer, Swimming, and Track & Field-Middle Distance) and 6 represented skill-based events (Golf, Rowing-Coxswain, Track & Field-Pole Vault, and Volleyball). This is similar to the sport distribution of other collegiate studies that assess a variety of events to begin to evaluate overall equation accuracy ([6](#), [9](#)), but different from others that focus on specific sport types in order to investigate more tailored predictions ([15](#)). However, our studied collegiate sample group is unique given it is Division 1 which has yet to be investigated and will contribute further insight into pREE accuracies.

Table 1. Subject Demographics (n=15)

| | |
|--------------------------|-----------------------|
| Gender | 14 Female, 1 Male |
| Student Classification | 11 Juniors, 4 Seniors |
| Age (years) | 20.67 ± 0.82 |
| Height (cm) | 171.62 ± 9.14 |
| Weight (kg) | 64.83 ± 6.89 |
| BMI (kg/m ²) | 22.29 ± 2.00 |
| Body Fat % | 23.1% ± 6.8% |
| FFM (kg) | 49.42 ± 5.66 |

BMI: Body Mass Index, FFM: Fat Free Mass

Table 2. Sports Represented

| | |
|---------------------------------|---|
| Cross Country | 2 |
| Golf | 2 |
| Rowing - Coxswain | 1 |
| Soccer | 3 |
| Swimming | 2 |
| Track & Field – Pole Vault | 2 |
| Track & Field – Middle Distance | 2 |
| Volleyball | 1 |

4.2 Comparison of REE Methods

The Wilcoxon signed-rank test showed that the difference between pREE and mREE was not statistically significant in this sample of athletes ($Z = -1.306$, $p = 0.191$). There were 9 positive ranks, or a greater pREE than mREE, indicating the prediction overestimated the measured value; and 6 negative ranks with a lower pREE than mREE, or an underestimation. There were no ties of equal mREE and pREE. Seven of the 15 subjects had a difference between

pREE and mREE that was greater than $\pm 10\%$, with the mean difference of 20.09% and a median difference of 6.71%. Individual athlete results of the signed-rank test are reported in **Table 3**.

Table 3. Ranked Differences in Measured and Predicted REE
(Wilcoxon Signed-Rank Test)

| Rank* | Weight (kg) | Body Fat % | mREE (kcal/day) | pREE (kcal/day) | kcal Difference (pREE-mREE) | Percent Difference | |
|-------------|--------------|--------------|-----------------|-----------------|-----------------------------|--------------------|----|
| 1 | 67.8 | 20.3% | 1696 | 1707 | + 11 | + 1% | |
| 2 | 58.6 | 26.2% | 1463 | 1446 | - 17 | - 1% | |
| 3 | 66.4 | 39.4% | 1406 | 1726 | - 36 | - 2% | |
| 4 | 66.1 | 27.9% | 1588 | 1551 | - 37 | - 2% | |
| 5 | 79.4 | 28.3% | 1690 | 1358 | + 48 | + 3% | |
| 6 | 59.5 | 22.5% | 1565 | 1496 | - 69 | - 4% | |
| 7 | 61.3 | 17.7% | 1494 | 1577 | + 83 | + 6% | |
| 8 | 63.8 | 16.9% | 1759 | 1641 | - 118 | - 7% | |
| 9 | 61.1 | 16.4% | 1444 | 1607 | + 163 | + 11% | ** |
| 10 | 62.0 | 17.8% | 1316 | 1609 | + 293 | + 22% | ** |
| 11 | 62.2 | 31.0% | 1048 | 1452 | + 404 | + 39% | ** |
| 12 | 80.2 | 26.9% | 2245 | 1815 | - 430 | - 19% | ** |
| 13 | 56.4 | 19.1% | 966 | 1486 | + 520 | + 54% | ** |
| 14 | 67.0 | 18.3% | 1114 | 1711 | + 597 | + 54% | ** |
| 15 | 60.6 | 17.7% | 923 | 1627 | + 704 | + 76% | ** |
| MEAN | 64.83 | 23.1% | 1448 | 1557 | +139.45 | 20.1% | |

| Summary of Ranks | | n | Mean Rank | Sum of Ranks |
|------------------|---------------|---|-----------|--------------|
| Negative | (pREE < mREE) | 6 | 6.17 | 37 |
| Positive | (pREE > mREE) | 9 | 9.22 | 83 |
| Ties | (pREE = mREE) | 0 | | |

* = Ranked by absolute difference, ** = difference > $\pm 10\%$. $Z = -1.306$, $p = 0.191$

The Bland-Altman assessment, plotting the agreement of the difference between each subject's pREE and mREE with the mean of their pREE and mREE values, did not report a significant bias ($p=0.070$). Shown in **Figure 2**.

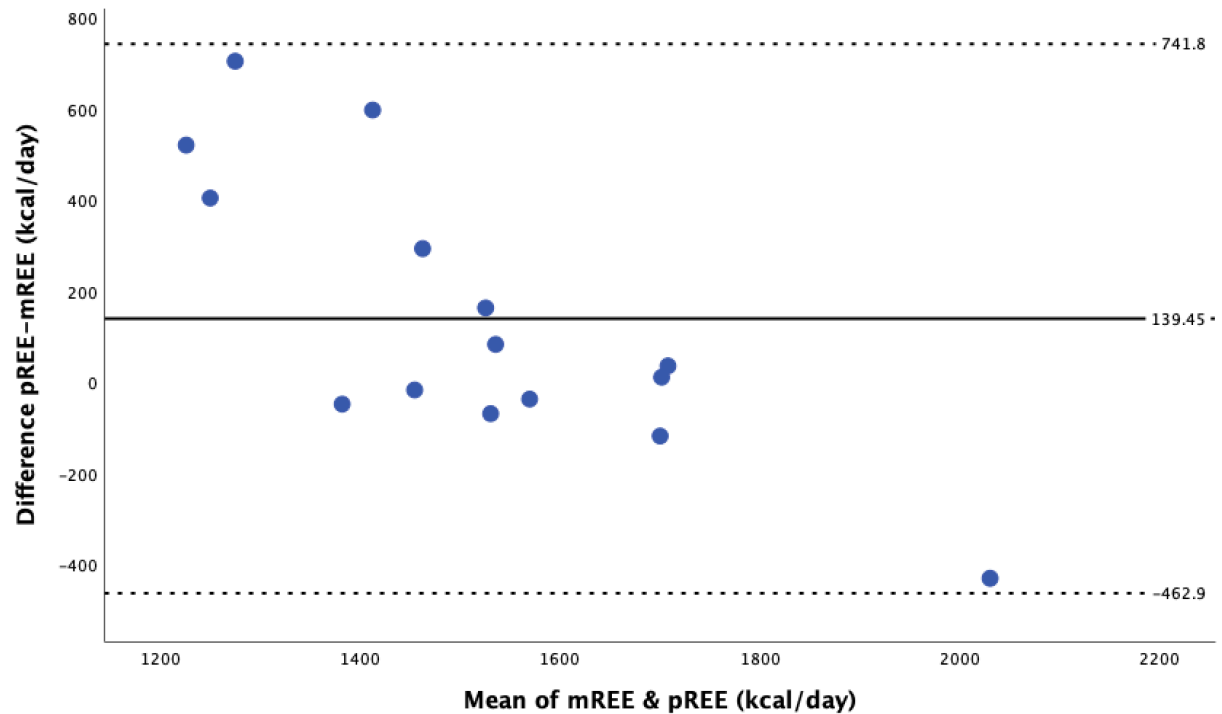


Figure 1. Bland-Altman agreement assessment. Solid line = mean of differences, Dotted lines = agreement lines at ± 1.96 standard deviations from mean. $r = 0.480$, $R^2 = 0.23$ ($p = 0.070$).

Chapter 5: Discussion

The purpose of this study was to determine whether DXA-derived FFM in the Cunningham equation was significantly different from mREE. In this sample of Division 1 collegiate athletes, the pREE was not statistically different from mREE as well as no significant bias.

5.1 Comparison of REE Methods

Our primary finding is in accordance to a previous report examining a similar question by Loureiro et al. in a sample of 28 elite adolescent pentathletes ([15](#)). Despite other previous studies using different methods of assessing FFM, such as ADP in the work of Jagim et al. ([6](#)) and skinfolds by Watson et al. ([9](#)), they also found a similar agreement between mREE and the Cunningham pREE in collegiate female athletes ([6](#), [9](#)).

However, from a clinical perspective, Haaf & Weijs et al. states accuracy of a pREE in an athletic setting must be less than $\pm 10\%$ kcal difference between mREE and pREE ([7](#)). Our study found only 53.3% were within $\pm 10\%$ clinical accuracy. These findings differ from previous reports that found the Cunningham equation were clinical accurate in 78.4% of female and 84.9% of male recreational athletes ([7](#)). Therefore, despite no reported statistically significant difference within our sample mean, 7 study participants were $>10\%$ kcal difference and clinically inaccurate warranting possible REE inaccuracies of Cunningham pREE when using DXA-derived FFM in Division 1 collegiate athletes.

The Bland-Altman analysis illustrated that while the differences were within the limits of agreement of -452.8 to 731.9 kcal, those differences may not be clinically accurate as previously discussed. However, the bias was not statistically significant in this sample ($p = 0.07$). Nonetheless, it was observed subjects with lower REE values (under ~ 1200 kcal) appeared to

have greater overestimations and those with higher REEs (over ~1800 kcal) had likely underestimations. This aligns with a similar trend reported by Carlsohn et al. who looked at elite rowers with particularly high FFM and concluded that for those higher metabolic rates, the predictive equations were more likely to underestimate calorie needs ([10](#)). Therefore, it is possible athletes with higher FFM (i.e. middle-distance runners, pole vaulters) would have pREE underestimated and smaller athletes or those with lower FFM (i.e. long-distance runners and swimmers) would have pREE overestimated. However, further research is needed to validate these simple observations and trends.

5.3 Limitations

While the sample size of 15 was small and lacked strength to make general recommendations for larger groups or teams, no prior literature was identified that assessed DXA-derived FFM for prediction of REE in Division I athletes. However, the sample size was in line with previous athlete REE studies whose samples were under 20 ([10](#), [11](#), [33](#), [34](#)).

Both male and female teams were recruited evenly, yet the sample was predominantly female. It is possible that this imbalance would not be seen with a larger sample size and a longer test period to include large men's teams that were not available or eligible for DXA testing during study recruitment. It is possible that increasing male participation would increase the proportional bias due to the higher FFM seen in these male athletes. Therefore, future recruitment practices should either be further modified to achieve more even representation of genders or for sufficient sample size to perform separate analysis by gender. The single male subject in this sample was not analyzed separately however as he was not an outlier in any data

category – all of his individual values were within the minimum and maximum range of the other 14 subjects.

A larger limitation of the DXA testing protocol was the short pre-test exercise and food restriction of one hour. Convenience for the athletes was an important consideration, as this test is performed under an existing protocol and takes place three times per year. In order to limit disruptions to training and fueling, a one-hour pre-test restriction was determined to be appropriate in this setting. However, other studies evaluating DXA in athletes typically use longer pre-test fasts and exercise restrictions of 8-12 hours ([18](#), [19](#), [25](#)).

Another consideration made to minimize athlete schedule disruptions was allowing a maximum of 14 days between the DXA and indirect calorimetry tests. With the testing equipment in separate buildings and the indirect calorimetry test not centrally located for most athletes, this protocol was chosen to limit major body composition changes between tests. However, hydration and fueling could certainly have introduced differences in measurements in that two-week period. Ideally, possible variation between the tests would be minimized with same-day testing, which would also consolidate the need for pre-test fasting or exercise restrictions to one test session.

5.4 Conclusions

The importance of improving individual athlete predictions is clear and this study's results highlight the need for accurate methods. The purpose was to determine whether the Cunningham equation using DXA-derived FFM would accurately predict mREE in a Division I collegiate athlete population. The agreement found between the indirect calorimetry mREE and the Cunningham pREE in this sample of athletes supports the use of DXA-derived FFM as a valid

body composition assessment for prediction of resting energy expenditure. However, while the analysis determined differences were not statistically significant, caution is indicated for health practitioners due to potential clinical inaccuracies using DXA-derived FFM in Cunningham pREE. Further research is warranted to validate these potential clinical inaccuracies.

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Appendix A – DXA Schedule

Baseball

- Pre-season: November 4, November 11
- Post season: May 4
- Mid-off season: September 3, September 9

Cross Country (M&W)

- Pre-season: August 19
- Post season: November 18
- Mid-off season: March 23, March 30

Football

- February 24-March 20, 2020
- June and July 2020

Golf (M&W)

- Pre-season: February 17
- Post season: April 27
- Mid-off season: September 16

Track and Field (M&W)

- Pre-season: January 13
- Post season: March 23, March 30
- Mid-off season: September 23, October 7

Women's Basketball

- Pre-season: October 28
- Post season: April 13
- Mid-off season: June 10, 2019 – in 2020 just in Summer

Coxswains

- Pre-season: February 10
- Post season: April 13
- Mid-off season: October 14

Soccer

- Pre-season: July 29, August 5
- Post season: December 9
- Mid-off season: April 6

Softball

- Pre-season: December 2
- Post season: April 27
- Mid-off season: August 26

Swim and Dive

- Pre-season: October 21
- Post season: April 20
- Mid-off season: February 3

Tennis

- Pre-season: December 2
- Post season: Possibly May 11 (depending on end of season play)
- Mid-off season: September 16

Volleyball

- Pre-season: August 12
- Post season: January 27
- Mid-off season: April 6

Appendix B – Athlete Scheduling SOP

Last Revised: 7/10/2019

Kansas Athletics Indirect Calorimetry REE Athlete Scheduling SOP

TEAM REE MEASUREMENT FORECASTING AND PLANNING:

1. Go to <https://outlook.office.com/owa/?path=/bookings/homepage> to access KAI Performance Nutrition scheduling webpage for DXA and Indirect Calorimeter.
 - a. Username: natasha.hansen@kuathletics.com
 - b. Password: Nutrition!1
2. Select “Calendar” on left side of page to see when Natasha has upcoming team(s) scheduled to complete their DXA scans.
3. Identify team(s) that are scheduled to complete DXA scans in the next 4-6 weeks and begin REE planning appointment schedules.

ATHLETE “EARLY NOTIFICATION” OF UPCOMING REE SCHEDULING:

1. Once you have identified the team(s) to complete REE measurements, begin to review each team roster and identify the juniors and seniors to complete a REE measurement.
2. Once these athletes are identified, select “Customers” on menu column of Office 365 booking webpage.
3. Each athlete’s e-mail should be available to select.
4. Two weeks before the athlete’s tentative REE measurement week, send a brief e-mail to athlete stating that in addition to their DXA they will also be asked to also signup for a resting energy expenditure measurement.
 - a. Please use the following e-mail template below in **Appendix A**.

ATHLETE REE SCHEDULING:

1. One week prior to DXA testing, Natasha Hansen will send each athlete from team an e-mail that provides a description and notification to sign up for their DXA measurement. In that same e-mail, Natasha will also send a short paragraph (**Appendix B**) addressed to the juniors and seniors of that team to also sign up for a REE measurement. They should be aware of this request from “Early Notification” procedures.
 - a. This e-mail will provide each athlete pre-measurement requirements to complete prior to REE testing.
2. “GRA” calendar times will be made available to these student athletes to see what days and times are available that week(s) to complete this measurement.
 - a. The GRA will have available times at 7:00am, 8:00am, and 9:00am. Available days will be determined on team needs for each week.
3. Once each athlete signs up for a time, their name will be added to the “GRA” calendar which is located within the “Calendar” link.
4. Review athlete REE appointments in calendar and make sure all targeted athletes have signed up. If they have yet to sign up, please send a second e-mail notifying them again to please sign up.

Appendix B – Athlete Scheduling SOP, continued

Last Revised: 7/10/2019

ATHLETE REE APPOINTMENT REMINDERS:

TWO DAYS before appointment

1. Send each scheduled athlete a reminder e-mail (**Appendix C**) of their upcoming appointment and location of appointment. This template e-mail will also restate pre-testing requirements for REE completion.

24 HOURS before appointment

1. Natasha will have a 24hr reminder notification sent to the athlete that will remind them of both upcoming appointments. This reminder e-mail will use same language as stated In Appendix C reminder template e-mail.

ATHLETE REE APPOINTMENT NO-SHOWS:

1. If an athlete fails to show up for their scheduled appointment please use template e-mail below (**Appendix D**) to notify athlete of missed appointment and request they sign up again.
2. Give each athlete one opportunity to make up missed appointment.
3. If athlete misses for a second scheduled appointment then do not take any further time to reschedule again.

Appendix B – Athlete Scheduling SOP, continued

Last Revised: 7/10/2019

Appendix A

Dear “Student-Athlete”,

You are receiving this e-mail to inform you that when you receive your e-mail request to sign up for your DEXA body composition measurement you will also be provided a notification and link to sign up for a resting energy expenditure measurement.

This resting energy expenditure measurement will assess how many calories you burn at rest and provide you a better understanding of your metabolism and energy calorie needs to support your training and performance.

Additional information will be provided once you sign up for your designated time to complete this measurement.

If you have any questions in the meantime, please reply to this e-mail.

Sincerely,

“GRA” contact information

Appendix B

Juniors and Seniors,

In addition to your body composition measurement, please use the link below to schedule an appointment to complete your resting energy expenditure measurement.

This appointment must be completed within two weeks of your body composition test.

Important reminders before scheduling your appointment:

1. No food 8-12 hours before measurement.
2. No exercise 12 hours before measurement.
3. No alcohol intake 24 hours before measurement.

Link:

Appendix B – Athlete Scheduling SOP, continued

Last Revised: 7/10/2019

Appendix C

This is a friendly reminder of your upcoming resting energy expenditure measurement tomorrow morning.

This measurement will be completed at the Anderson Family Football Complex in the Athletic Training Room located on the lower level of the facility next to the football weight room.

Finally, please remember to adhere to the following pre-measurement requirements. Failure to comply with these requirements will result in rescheduling your appointment.

1. No food 8-12 hours before measurement.
2. No exercise 12 hours before measurement.
3. No alcohol intake 24 hours before measurement.

If you have any further questions, please contact:

“GRA” contact information.

Appendix D

Dear “Student-Athlete”,

We missed seeing you this morning for your scheduled resting energy expenditure measurement at “time”.

Please use the link below to sign up again to complete your resting energy expenditure appointment.

If you have any questions regarding this second appointment request, please contact:

“GRA” contact information

Appendix C – Informed Consent Statement

Informed Consent Statement/HIPAA Authorization

Resting Metabolic Requirements of Male and Female Athletes

INTRODUCTION

The Department of Dietetics and Nutrition at the University of Kansas Medical Center supports the practice of protection for human subjects participating in research. The following information is provided for you to decide whether you wish to participate in the present study. You may refuse to sign this form and not participate in this study. You should be aware that even if you agree to participate, you are free to withdraw at any time. If you do withdraw from this study, it will not affect your relationship with this unit, the services it may provide to you, or the University of Kansas.

PURPOSE OF THE STUDY

Our goal is to measure resting metabolic rates in male and female athletes from various sports with the aim of developing sport specific resting metabolic prediction equations for these male and female athletes. The primary objective is to provide athletes and health practitioners equations that can accurately predict athlete resting metabolic requirements to assist in their performance nutrition recommendations.

PROCEDURES

You will be asked to report to the Anderson Family Football Complex for a total of one hour on a weekday between 6-9am following an overnight fast for 8-12 hours as well as no exercise for at least 12 hours and no alcohol for 24 hours. Once you arrive for testing we will obtain your current height and weight and then we will ask you to lie on a padded table with a pillow to relax. Next, a plastic hood will be placed over your head to begin resting metabolic rate measurement. The hood will form a tight seal preventing room air from entering to ensure measurement is correct. You will be required to stay still and awake while you lie down on the padded table. Total measurement time with hood will be between 30-45 minutes depending upon body movement. Once you complete the metabolic measurement you will have the opportunity to review your results with the research team member conducting the measurement.

This study will abide stringently to HIPAA and your personal health information. All of your bone and body composition measurements obtained through these DXA will be saved electronically on a password protected DXA computer that operates the machine as well as the Kansas Athletics Sports Medicine Electronic Medical Records (EMR).

RISKS

Only potential discomfort that may occur is anxiety of the plastic hood being placed over your head. If this occurs please notify research technician and the hood will be removed immediately and testing will stop. If you do not wish to continue the measurement we will remove you from the study.

Rev 7/13

Appendix C – Informed Consent Statement, continued

BENEFITS

The main benefit for participating in this study is to receive your specific resting metabolic rate to help better understand your individual dietary intake needs. Additional benefits include helping develop new resting metabolic prediction equations for your sport. This will help future athletes and health practitioners use these new prediction equations to accurately determine their appropriate daily energy intake needs in efforts to support the overall health and safety of collegiate athletes.

PAYMENT TO PARTICIPANTS

There is no payment for participation in this study. This is strictly voluntary.

INFORMATION TO BE COLLECTED

To perform this study, researchers will collect information about you. This information will be obtained from: a physical exam that involves collecting only height and current body weight as well as your date of birth. Also, information will be collected from the study activities that are listed in the Procedures section of this consent form.

Your name will not be associated in any way with the information collected about you or with the research findings from this study. The researcher(s) will use a study number or a pseudonym instead of your name.

The information collected about you will be used by: Aaron Carbuhn, Kansas Athletics sports medicine nutrition staff, and representatives of the Human Subjects Committee Lawrence Campus (HSCL) that review and monitor this research study.

The researchers will not share information about you with anyone not specified above unless (a) it is required by law or university policy, or (b) you give written permission.

Permission granted on this date to use and disclose your information remains in effect indefinitely. By signing this form you give permission for the use and disclosure of your information for purposes of this study at any time in the future.

REFUSAL TO SIGN CONSENT AND AUTHORIZATION

You are not required to sign this Consent and Authorization form and you may refuse to do so without affecting your right to any services you are receiving or may receive from the University of Kansas or to participate in any programs or events of the University of Kansas. However, if you refuse to sign, you cannot participate in this study.

CANCELLING THIS CONSENT AND AUTHORIZATION

You may withdraw your consent to participate in this study at any time. You also have the right to cancel your permission to use and disclose information collected about you, in writing, at any time, by sending your written request to: [Name and address of Researcher]. If you cancel permission to use your information, the researchers will stop collecting additional information about you. However, the research team may use and disclose information that was gathered before they received your cancellation, as described above.

Appendix C – Informed Consent Statement, continued

QUESTIONS ABOUT PARTICIPATION

Questions about procedures should be directed to the researcher(s) listed at the end of this consent form.

PARTICIPANT CERTIFICATION:

I have read this Consent and Authorization form. I have had the opportunity to ask, and I have received answers to, any questions I had regarding the study and the use and disclosure of information about me for the study. I understand that if I have any additional questions about my rights as a research participant, I may call (785) 864-7429 or write the Human Subjects Committee Lawrence Campus (HSCL), University of Kansas, 2385 Irving Hill Road, Lawrence, Kansas 66045-7563, email irb@ku.edu.

I agree to take part in this study as a research participant. I further agree to the uses and disclosures of my information as described above. By my signature I affirm that I am at least 18 years old and that I have received a copy of this Consent and Authorization form.

Type/Print Participant's Name

Date

Participant's Signature

Researcher Contact Information

Aaron Carbuhn, PhD, RD, CSSD
Principal Investigator
Department of Dietetics and Nutrition
3901 Rainbow Blvd
MS 4013
University of Kansas Medical Center
Kansas City, KS 66160
913.945.9601

Appendix D – Technician SOP

Last Revised: 1/27/2020

Kansas Athletics Indirect Calorimetry REE Measurement Technician Standard Operating Procedures

1 HOUR BEFORE FIRST APPOINTMENT:

POWER ON *[User's Manual 3-1]*

1. Turn on power strip to turn on analyzer, computer, monitor, & printer.
 - o Verify TrueOne® 2400 Analyzer Module is on.
 - o Must warm up **30 minutes** before alcohol burn procedure
 - If power strip does not work turn on analyzer, computer, monitor, printer, & weather station *[User's Manual 11-2]* individually
 - Analyzer on/off switch is on rear of TrueOne® 2400 module *[Fig. 2-4]*
2. Plug In circular fan and place at entry door of room. Turn on highest level (3) to circulate air throughout unit and room. Point fan between tables and wall towards the indirect calorimeter.
3. Make sure both tables in room are 1.5 tiles away from wall to ensure fan has enough room to circulate air.
4. Make sure dilution pump is OFF.
5. Place balance scale on lower counter left of sink and plug-in and turn on.

30 MINUTES BEFORE FIRST APPOINTMENT:

VERIFY APPROPRIATE OPERATING CONDITIONS

1. Weather station values must be within the following ranges:
 1. Temperature: 14°–30° C (57–86° F)
 2. Relative Humidity: 10–80%, non-condensing
 3. Barometric Pressure: 400–780mmHg
2. If temperature and/or humidity are out of referenced range cancel and reschedule participant appointment and contact Dr. Aaron Carbuhn.

10 MINUTES BEFORE PARTICIPANT ARRIVAL:

PARTICIPANT PREPARATIONS

1. Have table cleaned with disinfectant wipe.
2. Check pillow for any stains or dirt and replace with new pillow case if needed.
[Replacement pillow cases are located within white plastic drawer organizer.]
3. Turn on and tare digital body weight scale.
4. Prepare a participant folder that includes a blank consent form and participant history form. *[Blank consent and history forms are located in top drawer of wood cabinet in corner of room.]*

Appendix D – Technician SOP, continued

Last Revised: 1/27/2020

UPON PARTICIPANT ARRIVAL:

CONSENT FORM

1. Review consent form and obtain signature If athlete agrees to participate.
2. Document on participant history form participant has "completed Informed consent".
3. If participant is NEW to study Input their information on "NEW Participant IC Measurement Tracking" document. *[Located within Technician SOP manual]*
4. Also, document participant measurement on "ALL Participant IC Measurement Tracking" document. *[Located within Technician SOP manual]*

PARTICIPANT HISTORY FORM

1. Create and Document Unique Participant ID on top right of form.
 - a. How to create Unique ID
 - * First letter of first name, four-digit number from "New Participant IC Measurement Tracking" document, last letter of first name.
 - Example: Aaron Carbuhn, New Participant Tracking Number 0001.
"A0001N"
2. Obtain or verify participant's date of birth.
3. Verbally ask and confirm participant has not consumed food and/or water in the last 8-12 hours, has not exercised in the last 12 hours, and has not consumed any alcohol within the last 24 hours. If confirmed, document on participant history form.
4. If participant cannot confirm any of the three questions the technician must reschedule appointment and clarify pre-measurement requirements.
5. Finally, ask participant their sport playing position If applicable and current student classification (I.e. Jr, Sr, etc.). Add Information under "Additional Testing Notes".

ANTHROPOMETRIC MEASUREMENTS

1. Have participant remove their shoes as well as any other items in pockets. Obtain participants height and weight and document on participant history form.
2. Escort participant to SECA-BIA room and complete a body composition assessment. Use unique ID for SECA-BIA profile.
3. Ask participant If they need to use restroom facilities before moving to IC table for the next 40-45 minutes.
4. Direct participant to table next to Indirect calorimeter and instruct to lie on table with head rest fixed at 45° angle.
5. **Begin pre-measurement 10-minute supine rest.**
6. Have participant remove watch and silence phone. Place in bowl with items removed from pockets prior to weigh-in.

BEGIN CALIBRATION *[User's Manual 4-1]*

1. Flowmeter Calibration (Flow Cal) *[User's Manual 6-1]*
 - o Enter temperature, humidity, barometric pressure

Appendix D – Technician SOP, continued

Last Revised: 1/27/2020

- o Attach 3-liter calibration syringe to blue tubing. Blue breathing tube **must** be dry.

* Calibration cont'd.

- o Click Sample Baseline
 - i. One detection stroke
 - ii. Four flush strokes
 - iii. Five calibration strokes - Flow should be between 40–50 L/m peak flow
- o Error should be between +/- 3%
- 2. Gas Analyzer Calibration (Gas Cal) *[User's Manual 6-7]*
 - o Click gas calibration
 - o Click sampling line calibration
 - o When prompted, **remove** sampling line from back of mixing chamber.
Twist to remove.
 - o After room air calibration, when prompted, return sampling line to back of mixing chamber.
 - o Error should be +/- 3%
 - o Click OK on gas calibration screen
 - o Turn cal gas 90 degrees to ON position
 - o Error should be +/- 3%

TESTING: *[User's Manual 7-1]*

ROOM

1. Turn ON corner lamp next to Indirect calorimeter and turn OFF room lights.
2. Close the door nearly shut but keep fan on by the door and allow it to continue to circulate air.

DILUTION PUMP

1. Turn ON dilution pump located to the right of computer monitor.

PARTICIPANT SUPINE POSITION

1. Move head side of table back down flat to full supine position and position participant's head comfortably on pillow.

FINAL INSTRUCTIONS FOR PARTICIPANT

1. Remind participant they are to remain motionless throughout the measurement.
2. Remind participant they are not permitted to fall asleep during the measurement.
3. Remind participant If they feel any discomfort to notify you immediately and will stop the measurement.

10 MINUTE PRE-MEASUREMENT SUPINE REST VERIFICATION

Appendix D – Technician SOP, continued

Last Revised: 1/27/2020

1. Ensure participant has completed designated rest and document under "Additional Testing Notes" on participant history form.

METABOLIC MEASUREMENT

1. On Main Menu screen select "VO₂/Metabolic Testing".
 - a. **DO NOT input participant's name!**
 - b. Input only Unique ID within "Medical Record Number" for file identification.
* Unique ID is found on top right corner of Participant History Form.
2. Input current temperature, relative humidity, and barometric pressure.
3. Click Ok to create participant file and move to "Ready to Start Testing" window
4. DO NOT start testing. Keep on screen and move to next step. * Do not keep this screen on for > 10 minutes. May disrupt measurement values.

CANOPY HOOD (Located on top of cabinets above sink)

1. Position canopy hood
 - a. Place hood over participant's head
 - b. IMMEDIATELY connect blue tubing to hood
 - c. Position hood to eliminate leaks
 - o Top of hood should contact pillow, with no overhang
 - o Tuck drape around pillow and participant's shoulders and arms.
 - o Place blanket over participants chest to secure the seal.
2. While positioning hood, explain procedure to participant:
 - o This clear plastic dome (hood) will be positioned over head and shoulders to measure the gas you breathe in and out
 - o Fresh air will gently flow into the hood during the entire test
 - i. May be able to hear slight changes in the rate of air flow, but air is not restricted and will always flow
 - o Simply 1. be still, 2. breathe normally, 3. stay awake
 - o The only noticeable change throughout the test may be some condensation from your breath – this is normal
 - o Can withdraw at any point, for any reason

ADJUST DILUTION PUMP, BEGIN REE MEASUREMENT

1. At "Ready To Start Testing" window that is still open, adjust dilution pump rate until FE_{CO}₂ = 0.8–1.2%
 - a. Starting point to reach initial desired dilution rate is to divide participants weight (lbs)/7 in L/min.
 - b. Flow rates are typically between 15-30 L/min. Flow rates cannot be <10 L/min and > 40 L/min.
 - c. Click OK and begin REE measurement
2. In first 5 minutes of test, readjust the rate to keep FE_{CO}₂ = 0.8–1.2%

Appendix D – Technician SOP, continued

Last Revised: 1/27/2020

PARTICIPANT MOVEMENT CONFIRMATION

1. Monitor participant movement and alertness throughout the 30-minute measurement.
2. Confirm on participant history form if instructions were met.

PARTICIPANT HISTORY FORM "TESTING NOTES"

1. Document any additional information deemed important to include with each participant measurement within their respective participant history form.
2. Respiratory Quotient (RQ) and possible error detection.
 - A. Document RQ if values fall outside physiological range (< 0.67 or > 1.3) during measurement. If this occurs, technician should suspect an error and contact Dr. Aaron Carbuhn in addition to scheduling a repeat measurement.
 - B. Document RQ if value is between 0.91 and 1.3 and participant stated they are fasted. If this occurs, technician should suspect an error (equipment or invalid fasting statement) and contact Dr. Aaron Carbuhn in addition to scheduling a repeat measurement.

AFTER TEST

1. When test has run for 30 minutes, click "End Test"
2. Remove hood from participant and set aside
3. Turn OFF dilution pump
4. Check "Save Data" and "Print Report" and click "OK"

RESULTS REVIEW (OPTIONAL)

1. Ask participant if they would like to review the results of completed measurement.
2. If participant has additional questions please let them know they must discuss further with the Performance Nutrition Department for Kansas Athletics.

PARTICIPANT IS FREE TO LEAVE

1. Notify participant has completed all requirements for the measurement and is free to leave. Please make sure they do not leave any personal items behind.

BACK TO BACK STUDY PARTICIPANTS

1. If you have another participant measurement please refer to "BEFORE PARTICIPANT ARRIVAL" and follow as instructed.

AT END OF TESTING DAY:

ALCOHOL BURN

1. Complete an alcohol burn measurement for quality assurance of all REE participant measurements today.
2. Locate *Alcohol Burn Standard of Practice (SOP)* document located in "Technician SOP Manual" for specific instructions to complete measurement.

Appendix E – Participant History



Participant Number _____
IC- part history 2019

Indirect Calorimetry Participant History

| | | |
|-------------------------------|-------------------|-----------|
| Last Name, _____ | First Name, _____ | MI, _____ |
| Date of Birth: ____/____/____ | Sex: M or F | |

| Measurement Date | Performed by |
|------------------|--------------|
| | |
| | |
| | |
| | |
| | |
| | |

Resting Energy Expenditure Measurements Only

INDIRECT CALORIMETRY CHECKLIST

| | | |
|---|---|---|
| <p>Scan Date: _____</p> <p>Height: _____ inches</p> <p>Weight: _____ lbs</p> <p>Trained Operator: _____</p> <p>____ 1. completed informed consent</p> <p>____ 2. confirmed no food or water last 8-12 hours</p> <p>____ 3. confirmed no exercise or alcohol last 12 and 24 hours respectively</p> <p>____ 4. height and weight assessed</p> <p>____ 5. confirmed motionless and did not fall asleep</p> <p style="text-align: center;">□ □</p> <p>Additional Testing Notes:</p> | <p>Scan Date: _____</p> <p>Height: _____ inches</p> <p>Weight: _____ lbs</p> <p>Trained Operator: _____</p> <p>____ 1. completed informed consent</p> <p>____ 2. confirmed no food or water last 8-12 hours</p> <p>____ 3. confirmed no exercise or alcohol last 12 and 24 hours respectively</p> <p>____ 4. height and weight assessed</p> <p>____ 5. confirmed motionless and did not fall asleep</p> <p style="text-align: center;">□ □</p> <p>Additional Testing Notes:</p> | <p>Scan Date: _____</p> <p>Height: _____ inches</p> <p>Weight: _____ lbs</p> <p>Trained Operator: _____</p> <p>____ 1. completed informed consent</p> <p>____ 2. confirmed no food or water last 8-12 hours</p> <p>____ 3. confirmed no exercise or alcohol last 12 and 24 hours respectively</p> <p>____ 4. height and weight assessed</p> <p>____ 5. confirmed motionless and did not fall asleep</p> <p style="text-align: center;">□ □</p> <p>Additional Testing Notes:</p> |
|---|---|---|

Appendix F – Alcohol Burn SOP

Alcohol Burn SOP

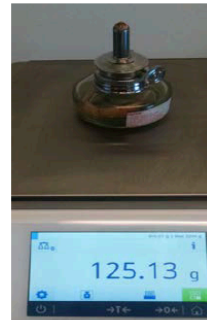
1. **Complete at conclusion of morning REE measurements.**
 - a. Machine must remain on. DO NOT turn off.
 - b. Keep circular fan ON HIGH and positioned in same place as instructed during REE measurements.
 - c. Check and measure wick height to ensure it is set at 0.5 centimeters prior to burn.
 - d. Check wick placement to ensure is laying on bottom of jar. If not, with a latex glove, adjust the wick to proper placement.
 - e. Calibrate scale as outlined in balance scale instruction manual located in back pocket of binder.
2. **Make sure dilution pump is turned OFF**
3. **Run flow calibration**
 - a. Enter *temperature, humidity, barometric pressure*
 - b. Attach 3-liter calibration syringe to blue tubing
 - c. Click Sample Baseline
 - i. One detection stroke
 - ii. Four flush strokes
 - iii. Five calibration strokes - should be between 40-50 L/min
 - d. Error should be +/- 3%
4. **Run gas calibration**
 - a. Click gas calibration
 - b. Click sampling line calibration
 - c. Prompted to remove sampling line from back of mixing chamber (black box on bottom right) (twist to remove)
 - d. After calibration, prompted to return sampling line
 - e. Error should be +/- 1 %
 - f. Click OK on gas calibration screen
 - g. Turn cal gas 90deg for on position
 - h. Error should be +/-3%
5. **Click VO2/metabolic testing**
 - a. Select **alcohol burn** for first and last name, NOT PATIENT
 - b. Attach blue tubing to methanol burning apparatus
 - c. With protective latex gloves, prepare alcohol (methanol) burning apparatus by filling liquid holder with ~ **7-8 ml**. If methanol encounters skin wash and clean immediately.
 - d. Weigh initial methanol content ASAP. Record date, pre-weight and humidity on *Alcohol Burn Measurement Tracking* document located within Technician SOP Manual.
* (Note: Methanol evaporates, so you want to do the pre-weight and then immediately move to the next step to minimize amount of time methanol is sitting before burn test begins).
 - e. Burn apparatus setup should be in accordance to picture located on next page.
 - f. Click OK and turn the dilution pump on
 - g. Place alcohol in holding apparatus
 - h. Flow should be between 15-17 lpm. (*Aim for 17 lpm*).
 - i. Start test and light the burner and place large cylindrical beaker over
 - j. First 30 seconds the RER will be low and should aim for 0.64-0.67
 - k. Ensure % FECO₂ stays between 1.0% - 1.2% throughout entire burn test.
 - l. If you need to adjust flow to get %FECO₂ within desired range complete within 1-3 minute of test.
 - m. Run test for 20 minutes
 - n. After 20 minutes, blow out flame, re-cover the burning apparatus with cylinder, and wait 1 min (*pick up cylinder cover from bottom, top is HOT).

Appendix F – Alcohol Burn SOP, continued

- o. **CLICK End Test** after 21 minutes have been completed on the test and weigh amount of methanol still in holder. **Record post-weight** on same *Alcohol Burn Measurement Track* doc located within Technician SOP Manual. * (*Note: Methanol evaporates, so you want to do the post-weight immediately after finishing the burn*).
- p. Turn off dilution pump
- q. Clean up when you're done =)



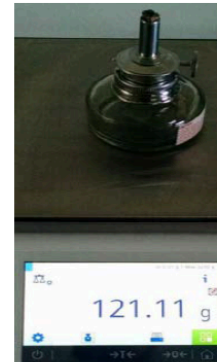
Methanol + chamber with wick attached



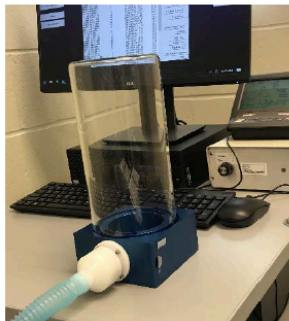
Methanol + chamber weighed prior to burn



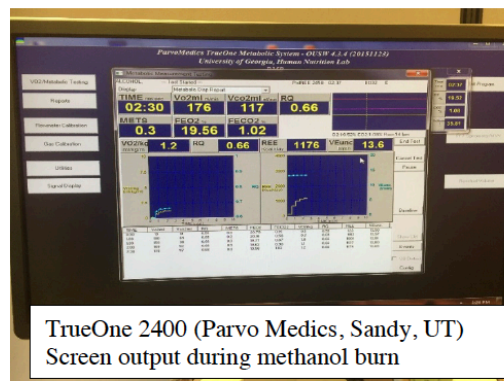
TrueOne 2400 (Parvo Medics, Sandy, UT)
Burn kit apparatus



Methanol + chamber weighed after burn



TrueOne 2400 (Parvo Medics, Sandy, UT)
Setup for methanol burn



TrueOne 2400 (Parvo Medics, Sandy, UT)
Screen output during methanol burn

Appendix G – Alcohol Burn Analysis Template

methburn template

| | Time data | | | |
|-------------|----------------------|-------------|--------------|---------|
| | dd/mm/yy hh:mm | O2 ml/min 1 | CO2 ml/min 1 | RQ |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
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| | | | | |
| | | | | |
| | | | | |
| | minutes | | | RER |
| totals | 20.0 | 0.000 | 0.000 | #DIV/0! |
| average | | #DIV/0! | #DIV/0! | #DIV/0! |
| st dev | | #DIV/0! | #DIV/0! | |
| | (mL) | #DIV/0! | #DIV/0! | #DIV/0! |
| theoretical | (moles) | 0.000 | 0.000 | |
| | (mL) | 0.000 | 0.000 | #DIV/0! |
| recovery | (% based on average) | #DIV/0! | #DIV/0! | |
| | (% based on actual) | #DIV/0! | #DIV/0! | |
| | | | | |

Appendix H – Data Correction SOP

Alcohol Burn REE Data Correction SOP

1. Use the "% based on actual" O₂ and CO₂ values from the completed excel alcohol analysis template document to correct the completed REE from that same day.
2. Open each excel participant REE measurement that was exported with the analyzed alcohol burn measurement. They should all be located within same "month_day" folder of current "Year" in the external hard drive.
3. Once each REE participant file is open complete the following steps for each file:
 - a. Insert a column between VO₂ (column B) and VO₂/kg (column C).
 - b. Label newly created column C beginning on line 26 to state "Correct", "VO₂" (line 27), "ml/min" (line 28).
 - c. Insert a column between VCO₂ (column F) and VE (column G).
 - d. Label newly created column G beginning on line 26 to state "Correct", "VCO₂" (line 27), "ml/min" (line 28).
 - e. Label column M beginning on line 26 to state "Correct", "REE" (line 27), "kcal/D" (line 28).
 - f. **DELETE Rows 29-39 to remove first 10 minutes of REE measurement.** First row for "time" column should be "10.99" minute measurement and end at "30.00" minute measurement.
 - g. Label column A on line after end of measurement to state "Corrected Mean".
 - h. Highlight, in green, the three newly column (C,G,M) cells beginning from line 26 to end of O₂ and CO₂ minute measurement of each REE test.
 - i. Highlight, in green, the "Corrected Mean" line from column A-M.
 - j. For reference please refer to page 3 of this document.
4. The REE file is now ready to insert correction factor from analysis of alcohol burn.
5. Steps to complete participant REE measurement correction:
 - a. Review your "% based on actual" O₂ and CO₂ values from alcohol analysis template.
 - i. **Example scenario: O₂, CO₂ values BELOW 1.0.**
 1. If your "% based on actual" value states "0.98" for O₂ and "0.97" for CO₂ then you must add ".02 or 2%" to the O₂ ml/min values and ".03 or 3%" to CO₂ ml/min values recorded each minute for participant REE measurement.
 - a. To correct O₂ values, would insert the following formula beginning on column C, line 30; $= (B29 * .02) + B30$
 - b. Copy and paste to all O₂ values on REE measurement file.
 - c. To correct CO₂ values, would insert the following formula beginning on column G, line 30; $= (F29 * .03) + F30$
 - d. Copy and paste to all CO₂ values on REE measurement file.
 - e. To correct REE, would insert the following Weir formula beginning on column M, line 30;
$$= ((3.941 * (C29 / 1000)) + (1.106 * (G29 / 1000))) * 1440$$
 - f. Copy and paste to all REE values on REE measurement file.
 - g. At "corrected mean" line at bottom of each corrected column average the corrected values.

Appendix H – Data Correction SOP, continued

- h. The average REE value calculated is your corrected REE for participant and will be Inserted Into masterdata set.
 - ii. Example scenario: O₂, CO₂ values ABOVE 1.0.
 1. If your "% based on actual" value states "1.02" for O₂ and "1.03" for CO₂ then you must subtract ".02 or 2%" from the O₂ ml/min values and ".03 or 3%" to CO₂ ml/min values recorded each minute for participant REE measurement.
 - a. To correct O₂ values, would insert the following formula beginning on column C, line 30; $= (B29 * .98)$
 - b. Copy and paste to all O₂ values on REE measurement file.
 - c. To correct CO₂ values, would Insert the following formula beginning on column G, line 30; $= (F29 * .97)$
 - d. Copy and paste to all CO₂ values on REE measurement file.
 - e. To correct REE, would Insert the following Weir formula beginning on column M, line 30;
$$= ((3.941 * (C29 / 1000)) + (1.106 * (G29 / 1000))) * 1440$$
 - f. Copy and paste to all REE values on REE measurement file.
 - g. At "corrected mean" line at bottom of each corrected column average the corrected values.
 - h. The average REE value calculated is your corrected REE for participant and will be Inserted Into masterdata set.
 6. Once data correction is complete within participant's REE excel measurement file, Save and rename file; "Corrected_Unique ID".
- * Should only need to Insert "Corrected" In front of file name when saving.
7. Locate "REE Data Transfer SOP" located in Technician SOP Manual to complete final step.

Appendix I – Data Transfer SOP

Athlete Resting Energy Expenditure Research Study Data Transfer and Entry SOP

** Data Transfer to REDCap must be completed at the end of each REE measurement day!*

1. Login to REDCap (<https://redcap.kumc.edu>) using your KUMC username and password credentials.
2. Select "My Projects". Located top left of home page.
3. Select "Athlete Resting Energy Expenditure" project.
4. Under the REDCap column located on left of page under "Data Collection" select "Add/Edit Records".
5. For new participant data, enter participant's unique ID in "Enter a new or existing Study ID" box and press enter or click outside of box.
6. Under "Visit 1" column for newly created "NEW Study ID 1" record Input all required Information for the following data collection Instruments:
 - * To complete the following Instrument entries, begin by clicking on "Incomplete" data circle (gray). Begin with participant Information data circle.
 - a. Participant Information
 - i. Scan and upload signature page of participant's consent form to this section.
 - b. REE Data
 - c. DXA Data
7. After Inputting all required Information and data for each Instrument must select "Save & Exit Form" or "Save & Go To Next Form" before leaving each respective data Instrument page.
 - a. You can track data entry status and location on left column of page.
8. "Participant Withdrew Information" - Only enter Information here If participant had to be withdrawn from study voluntarily or by research team.
9. Repeat all previous steps for each participant that completed REE measurement on same day.
10. DXA Data
 - a. It Is OK If you come back to enter the participant's DXA Data If not yet available.
 - b. Make a note and enter when available.
11. Any questions about participant data entry using REDCap contact Dr. Aaron Carbuhn for assistance.