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The Relationship Between Within-day Energy Balance and Protein Distribution on Body Composition in Collegiate Female Basketball Players

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ABSTRACT

THE RELATIONSHIP BETWEEN WITHIN-DAY ENERGY BALANCE AND PROTEIN DISTRIBUTION ON BODY COMPOSITION IN COLLEGIATE FEMALE BASKETBALL PLAYERS

Robert Bergia, Dan Benardot, Anita Nucci, Walter Thompson

Background: Previous research suggests associations between energy balance, eating frequency, macronutrient content, and macronutrient distribution with body composition. In particular, energy balance and protein intake have been conventionally evaluated in 24-hr time blocks, consistent with dietary recommendations and general public understanding. However, there is a potential benefit to investigating energy balance and protein intake in smaller increments of time to account for dynamic changes that occur within-day.

Objective: The purpose of this study was to evaluate protein intake/distribution relative to energy balance fluctuations during the day and body composition in collegiate female basketball players.

Methods: Subjects provided information on dietary intake and expenditure. Body composition was assessed by multi-current bioelectrical impedance. Energy balance (EB) and related protein distribution variables were determined with a Computerized Time-Line Energy Analysis procedure. Data were analyzed for associations between energy balance, protein intake and distribution, and body composition. Data are displayed as either traditional 24-hr EB and total protein intake or dynamic protein variables in relation to real-time EB (ingestion within ± 400 kcal EB or > 0 kcal EB).

Results: There was no relationship between net 24-hr energy balance and percentage body fat. A statistically significant positive relationship was observed between total protein intake and body fat mass ($R = .597$; $p = .031$). No relationship was observed between protein distribution variables (g in ± 400 kcal EB, g in > 0 kcal EB) and percentage body fat. Protein eating occurrences (>10g, ± 400 kcal EB) was inversely correlated with BMI ($R = -.650$; $p = .016$). Subjects with the greatest energy deficits presented with lower lean body mass ($R = -.736$; $p = .004$).

Conclusion: These data suggest that within-day protein distribution relative to energy balance are associated with BMI, but not with percentage body fat. Those with the highest protein intake had the highest body fat mass, with no correlation between protein intake and total energy intake detected. In this group, no association between 24hr intake net values or within-day intake values were found to be related to body fat percentage. However, the greatest energy balance deficit during the day was strongly inversely associated with lean body mass, indicative of potentially deleterious effects of energy restriction.

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TABLE OF CONTENTS

List of Tables iv

List of Appendices v

List of Abbreviations vi

Chapter

I. INTRODUCTION 1

II. LITERATURE REVIEW 4
Eating Frequency 4
Protein Intake and Distribution 6
Protein and Energy Restriction 13
Energy Availability 16

III. METHODS……………………………………………………………… .20

IV. RESULTS……………………………………………………………… 25

V. DISCUSSION AND CONCLUSIONS…………………………………….. .30

REFERENCES 35

APPENDICES…………………………………………………………...………41

LIST OF TABLES

Table Page

1. Descriptive Statistics of Collegiate Female Basketball Players 25

2. Energy Balance Descriptive Statistics of Collegiate Female Basketball Players 26

3. Protein Intake and Distribution Descriptive Statistics of Collegiate Female Basketball Players 27

4. Correlations Between Total Protein Intake, Body Fat Mass, and Total Kcal Intake of Collegiate Female Basketball Players 28

LIST OF APPENDICES

Appendix Page

A. Informed Consent Form 41

B. NutriTiming Data Entry Form 44

C. NutriTiming Data Output (ex.) 45

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CHO	Carbohydrate
CTLEA	Computerized Time-Line Energy Analysis
EO	Eating Occurrence
EB	Energy Balance
FSR	Fractional Synthetic Rate
hr/hrs	Hour/s
kcal	Kilocalories
kg	Kilograms
LBM	Lean Body mass
MPS	Muscle Protein Synthesis
NHANES	National Health and Nutrition Examination Survey
RED-S	Relative Energy Deficiency in Sport
RDA	Recommended Daily Allowance
SPSS	Statistical Package for the Social Science

CHAPTER I

THE RELATIONSHIP BETWEEN WITHIN-DAY ENERGY BALANCE AND PROTEIN DISTRIBUTION ON BODY COMPOSITION IN COLLEGIATE FEMALE BASKETBALL PLAYERS

INTRODUCTION

Discussions about how to best optimize diet for body composition benefits usually center on total daily distribution of energy substrates (protein, fat, and carbohydrate) and total energy intake. Predictive equations are performed that will give an energy goal for the day, and it is assumed by many that if you meet these requirements you will attain weight stability. Unfortunately, bioenergetics is much more complex than a “calories in”, “calories out” model. Energy availability is a concept that recognizes that dietary energy expended in one process (cellular maintenance, thermoregulation, growth, reproduction, locomotion, etc.) is not available for others (Loucks et al. 2011). Bioenergeticists define energy availability as dietary energy intake minus the energy expended in a particular metabolic demand of interest. For example, in exercise physiology, energy availability is defined as dietary energy intake minus the energy expended in exercise (EA = EI-EEE) (Loucks et al. 2011).

The International Olympic Committee has even recognized the importance of energy availability. Recently, the term RED-S (Relative Energy Deficiency in Sport) has supplanted the Female Athlete Triad as it was recognized that the phenomenon is not a triad of EA, menstrual function and bone health, but rather a syndrome resulting from relative energy deficiency that affects other physiological aspects (Mountjoy et al. 2014). In this case, energy requirements for locomotion may double or even quadruple, which will result in less available energy for other processes such as reproduction and cellular maintenance. For this reason, it is important to look at energy balance relatively. It is less important that an athlete meets total 24-hr energy requirements than that they have energy available when they need it throughout the course of the day.

"The Recommended Dietary Allowance (RDA) is an estimate of the minimum daily average dietary intake level that meets the nutrient requirements of nearly all (97 to 98 percent) healthy individuals."(Trumbo et al. 2002) The RDA for protein is set at 0.8 g protein/(kg x d) to meet needs and prevent deficiency for most of the population. Recently, there is evidence that the maximal rate of protein synthesis can be achieved for most with 20-30g of high quality protein per meal (to achieve 0.8g/kg per day), a level that is not met by most Americans (Paddon-Jones & Rasmussen, 2009; Symons et al. 2009). Energy and protein consumption is typically skewed toward the evening meal (38g protein) as opposed to the morning meal, which has a relatively low protein content (13g protein) (NHANES, USDA Agr Research Service, 2012). This 3-fold difference can explain how Americans easily meet the daily protein requirement yet may still be deficient for much of the day.

One of the prime reasons that real-time energy balance analysis is being examined instead of twenty-four hour total energy balance is that energy expenditure and hormone activity can be reliable factors. Twenty-four hour energy balance is just a description of calories in, and calories out. Analyzing dietary intake in real time accounts for periods of energy surplus and energy deficit as opposed to one net value. This is particularly important when looking at body composition because dietary factors influence numerous hormones with substantial impact on fat and fat-free mass. For example, a prolonged period in an energy deficit will increase concentrations of cortisol, which has particularly deleterious effects on fat-free mass. On the other hand, a notable energy deficit followed by a large meal will exponentially raise insulin which will result in marked increase in fat storage. Therefore, this study will examine protein intake and distribution relative to current energy balance to determine if a relationship exists between body composition and energy status when protein is ingested.

STUDY PURPOSE

The purpose of this study is to determine if protein intake and distribution, relative to current energy balance, is related to body composition in collegiate female basketball players.

HYPOTHESES

Hypothesis 1: Subjects with greater protein distribution (< 30g) while in positive EB (> 0 kcal) will have a lower percentage body fat than those with less protein distributed in EB.

Hypothesis 2: Subjects with greater protein distribution (< 30g) while in ± 400 kcal EB will have a lower percentage body fat than those with less protein distributed in relative EB.

Hypothesis 3: Subjects with a greater number of eating occurrences containing >10 g protein intake in a positive energy balance will have lower percentage body fat than those with less protein eating occurrences.

CHAPTER II

REVIEW OF LITERATURE

INTRODUCTION

Body composition has been examined as a phenomenon of net energy balance, or 'calories in, calories out' for decades. Macronutrient content and distribution, meal frequency and timing, and relative within-day energy balance has more recently gained traction in an effort to explain proportions of body composition variance. The following review covers a body of knowledge spanning the major sub-categories of eating frequency, protein intake and distribution, differential metabolic properties according to energy state, and energy availability. The purpose is to provide a solid foundation to justify the need to examine new indices to explain body composition differences in individuals.

EATING FREQUENCY

Research investigating eating frequency and body composition are a good base to build theories pertaining to energy balance upon. After all, it can be assumed that those who are frequent eaters spent a greater proportion of the day in relative energy balance compared to those who eat infrequently and therefore experience large energy deficits and surpluses. In fact, the 24h energy balance theory is often challenged by findings of eating frequency studies. Typically, frequent eating is associated with an increase in total daily energy intake. Those who had ≥ 5 eating occurrences (EO)/day consumed 800 kcal per day more than those who had ≤2 EO/day (Kerver et al. 2006). However, meals that stimulate a rapid increase (and subsequent decline) in blood glucose, such as large meals typical in infrequent eating patterns, have been implicated in promoting increased hunger and energy dysregulation (Roberts, 2000). The mechanism behind these findings appears to be a prolonged elevation of ghrelin as a result of large meals inducing a hyperinsulinemic response which will not inhibit ghrelin release (Saad et al. 2002).

Frequent eating (≥3 EO/day) is related to decreased visceral fat and triglycerides despite an increase in total daily calories consumed (House et al. 2014). Building upon this finding in an expanded replication study, House et al. (2015) found that frequent eaters had lower BMIs, waist circumferences, fasting insulin values, insulin resistance, and triglycerides than infrequent eaters, despite consuming more calories per day. A potential gender difference has been reported by Drummond et al. (1998), where in a cross-sectional study men exhibited a significant negative correlation between eating frequency and body weight/BMI, while women subjects displayed no such relationship.

Ramadan, an Islamic holiday involving fasting from sunrise to sunset for 29-30 days, presents as a unique opportunity to study differential meal frequency with reliability in a free-living setting. Results of Ramadan feeding (and hence a reduced meal frequency) on body composition have been inconclusive. A classic Ramadan pilot study detected an increase in caloric intake and body weight during Ramadan (Frost & Pirani, 1987). Al-Hourani and Atoum (2007) observed a significant reduction in body weight, BMI, body fat percentage, and total body water in young women after Ramadan. Muscle mass was unaffected despite a reduction in eating frequency. Recently, Nourouzy et al. (2013) observed differential reductions to body composition depending on age and gender. All subjects lost a significant amount of lean body mass as a result of reduced eating frequency. Men and subjects <35 years old lost the most weight and body fat. Women >35 years old were the only group to experience no reduction in body fat despite a significant decrease in lean body mass. More research is needed to investigate the potential deleterious effects of reduced eating frequency on adiposity in general, and visceral adiposity in particular.

Protein Intake and Distribution

The RDA for protein was established from studies that estimated minimum protein intake necessary to prevent a progressive loss of LBM as reflected by nitrogen balance (Wolfe et al. 2008). This methodology has numerous drawbacks, including the potential for low-protein diets to induce adaptions to spare nitrogen; thus confounding results (Morse et al. 2001). In fact, the Food and Nutrition Board acknowledged the limitations to basing the RDA upon nitrogen balance studies due to there being no relevant physiological end point (Wolfe et al. 2008). The problem that has arisen in recent years is that the RDA is minimalist (by design), yet it is often considered indicative of optimal intake. The RDA certainly does not address what the ideal amount of protein for optimal function is (Volpi et al. 2003). The key distinction between optimal functioning and the prevention of wasting cannot be overlooked. Athletes are not seeking to simply prevent deficiency or replete amino acids lost to catabolic pathways. Rather, they are seeking protein accretion and growth. Thus, the RDA may not be the best point of reference for athletes, as suggested in the ACSM guidelines which recommend intakes for athletes ranging from 1.2 to 1.7 g/kg.

National Health and Nutrition Examination Survey (NHANES) data indicate that protein consumption is skewed towards the evening (NHANES, USDA Agr. Research Service. 2012). This suggests that the majority of our population is protein deficient for much of the day followed by back-loading in the evening. The mean protein intake at breakfast is 13g, and the mean protein intake in the evening meal is 38 grams (NHANES. 2012). Using a secondary analysis of NHANES data, this indicates that the typical protein distribution pattern is dispersed as 10%, 20%, and 60% across breakfast, lunch, and dinner, respectively (10% snacking) (Krebs-Smith et al. 2010).

The question of how much protein we can utilize for muscle protein synthesis (MPS) if ~60% is consumed in one meal is being investigated. Symons et al. (2009) sought to answer that question by having one pair of groups (young, elderly) consume a moderately sized protein meal (113g lean beef, 30g protein, 10g EAAs, 220 kcal) and another pair consume a threefold larger meal (340g lean beef, 90g protein, 30g EAAs, 660kcal) and measuring protein synthesis responses. The study found that post-absorptive mixed muscle fractional synthetic rate (FSR) were similar across all groups. In essence, this study found that participants who consumed 90g protein gained no further protein synthetic advantage when compared to the smaller 30g meal. Moore et al. (2009) conducted a similar dose response study following resistance training exercise. Drinks contained 0, 5, 10, 20, or 40g whole egg protein. Results indicate that MPS was maximally stimulated at 20g; anything over this amount was irreversibly oxidized.

One recent study specifically investigated within-day dietary protein distribution on 24-h MPS (Mamerow et al. 2014). This crossover study spanned 7 days and included a normally distributed protein group (30g protein for breakfast, lunch, and dinner) and a skewed protein group (10g protein for breakfast, 15g for lunch, 65g for dinner). Results indicated that 24-h MPS was ~25% greater when protein intake was evenly distributed, compared with the skewed diet. Most studies suggest 20-30g protein to be the anabolic maximum in the normal healthy population, although it is worth noting that a blunted anabolic response to dietary protein intake has been reported with aging. One such study found that protein pulse feeding (72% of daily protein consumed in one meal at noon) was superior to normally distributed protein intake in conferring lean body mass index (Bouillanne et al. 2013).

Attention has been drawn to specific amino acids (particularly leucine and essential amino acids) being primarily responsible for the stimulation of muscle protein synthesis. One study assessed whether nonessential amino acids are required to stimulate muscle protein anabolism (Volpi et al. 2003). Groups were given either 18g EAAs or 40g balanced amino acids (18g EAA + 22g nonessential amino acids) in small boluses every 10 min for 3h. Results indicate that there was no difference between groups in degree of MPS. This implies that EAAs are primarily responsible for amino-acid stimulation of muscle protein anabolism and that EAAs are more anabolically efficient. An important consideration is that the 18g EAA could have been enough to attain maximal muscle protein synthesis and the additional nonessential amino acids would confer no further benefit for that reason.

Just as the threshold for maximal MPS has been postulated as ~30g, a similar threshold is suggested for EAAs. Research indicates that ~15g of EAAs are required to maximally stimulate MPS (Paddon-Jones et al. 2004). EAA-only supplementation has been shown to increase muscle protein anabolism to a similar degree as mixed amino acid solutions (Tipton et al. 1999; Volpi et al. 2003).

Indeed, the necessity of certain substrates for muscle protein synthesis can be refined even further. Leucine has been suggested as a prime activator of anabolic processes in muscle. Aside from leucine's role as a constituent of protein, leucine exhibits potent translational control of protein synthesis and glycemic regulation (Norton et al. 2006). Leucine stimulates MPS through the protein kinase mammalian target of rapamycin (mTOR), as well as through mTOR-independent mechanisms, which are outside the scope of this review (Norton et al. 2006). Leucine alone has been shown to be capable of stimulating MPS to a similar degree as complete protein or mixtures of amino acids, albeit the effects were acute (Crozier et al. 2005; Norton et al. 2009). The authors posited that leucine stimulatory effects on MPS were transient in nature due to prolonged increases in synthesis requiring a full complement of amino acids to act as substrate (Crozier et al. 2005). It can be inferred that as leucine serves as a signaling molecule to initiate protein synthesis, a threshold must be passed to maximally stimulate these processes. Norton et al. (2009) posited that a specific threshold of leucine intake is required to initiate mRNA translation and muscle protein synthesis, and that a low intake of some protein sources may not reach this 'initiating' threshold. This non-linear threshold response is evinced by MPS being 80% greater in egg protein feeding compared to soy protein feeding despite the actual leucine content differing only 10%

(Norton et al. 2012). The precise leucine initiating threshold in humans has not been elucidated. This general finding of an 'initiating' threshold being required is supported in the literature (Areta et al. 2013). Researchers examined MPS in response to 80g whey protein distributed as 2x40g (BOLUS), 4x20g (INT), and 8x10g (PULSE). Results indicated that the INT group had the overall highest rates of MPS (Areta et al. 2013). Thus, a PULSE (grazing) meal pattern does not produce enough of a plasma rise in amino acids or leucine to initiate muscle protein synthesis, while the BOLUS feeding pattern does not stimulate MPS often enough. West et al. (2011) assessed MPS in response to BOLUS and PULSE feeding as well. Results indicate that despite an identical net area under the EAA curve, MPS was elevated to a greater extent after BOLUS than after PULSE at time points 60 and 180 minutes after exercise. PULSE protein ingestion resulted in a smaller but sustained increase in aminoacidemia, but the spike (supporting the initiating threshold hypothesis) in EAA concentrations (162% in BOLUS vs 53% in PULSE) is posited as a primary trigger for MPS (West et al., 2011).

The saturating dose of leucine appears to be 2.5-3g, in which further increases would not likely promote further muscle protein synthesis (Churchward-Venne et al. 2012). Various protein sources have different proportions of leucine, hence it will require a larger serving of a protein source under-represented in leucine to reach the saturating dose. This has implications for consideration of protein quality and source in determining dietary adequacy of protein intake. Thus, there appears to be a 'Goldilocks principle' for protein and leucine ingestion, where it is ineffective to consume too little and inefficient to consume too much (and ineffective if displacing protein intake from other more dispersed time points).

If 20-30g protein intake is required for maximal MPS, a 75kg person would need to eat 20g at each meal (breakfast, lunch, dinner) to meet the 0.8 g PRO/kg/d RDA, an amount that can be met relatively easily. However, imagine a 100kg athlete seeking to efficiently ingest 1.5g PRO/kg/d (to optimize muscle protein synthesis and not simply prevent deficiency). This athlete would require 5 meals containing 30g protein to prevent wasteful oxidation above the threshold. An eating pattern to efficiently accommodate 150g of protein would require much more planning.

Protein synthesis as stimulated by leucine feeding or EAA ingestion has been shown to be elevated for approximately two hours (Bohe et al. 2001; Anthony et al. 2002). Different considerations, such as insulin rise, gastric emptying, and elevated fatty acid levels must be taken into account when considering a mixed-meal, however. Norton et al. (2009) determined the duration of protein synthesis to a complete meal of carbohydrate, fatty acids, and protein to be approximately 3 hours. Protein and amino acid concentrations do not fully explain muscle protein synthesis, it seems. Synthetic response fell off after the aforementioned 3 hours despite plasma leucine being elevated 3-fold over baseline (Norton et al., 2009). A similar finding by Bohe et al. (2001) has been reported where duration of elevated muscle protein synthesis in response to EAA infusion was only two hours long despite the infusion lasting six hours. Hence, it appears that muscle protein synthesis becomes ‘refractory’ to elevated plasma amino acid concentrations alone (Norton et al. 2009). This finding has been described as the ‘muscle-full effect’ (Atherton et al. 2010), where amino acid concentrations no longer correlate with rates of MPS. A cyclical pattern of rapid increases in amino acids followed by hypoaminoacidemia may superior to grazing or constant AA infusions which cause refractory periods to MPS in muscle (West et al. 2011). This could potentially explain a proportion of variance as to why more is not always better in eating frequency trials.

The incongruity between initiation signals and postprandial duration of MPS has not been fully elucidated. Potential explanations include a refractory response to external stimuli (previously mentioned), reduced availability of amino acids, or reduced signaling from insulin or other key signals (Wilson et al. 2011). Research by Wilson et al. (2011) supported the findings of significant correlation between translation initiation and MPS in the first 90 minutes, and a subsequent drop-off in the postprandial period despite elevated amino acid levels and mTORC1 signaling compounds. So, leucine and translation initiation signaling are required to facilitate an initial rise in MPS, but how can the response be sustained? It appears that insulin is not the sole critical factor in extending MPS (Wilson et al. 2011), due to leucine supplementation post-meal extending MPS to a similar degree as CHO despite decreased insulin concentration. The ratio of AMP/ATP and AMPKα phosphorylation in the muscle (and thus the energy status of muscle) was determined to be the prime limiting factor for MPS at 180 minutes after a meal (Wilson et al. 2011). In essence, amino acids do not always present themselves as the limiting factor in MPS, often an energy deficit is the prime culprit. This finding further supports the importance of examining protein intake and distribution relative to current energy balance, as neither component can adequately explain body composition variance by itself.

As it is established that there is a threshold for protein synthesis, the next logical step is to determine how often one can attain that threshold and still gain the synthetic advantages from a practical standpoint. The findings by Norton et al. (2009) on typical mixed meals invoking 3 hours of elevated protein synthesis is a good starting point to estimate required meals per day. As previously mentioned, a larger, more physically active person may require more protein feeding opportunities during the day to satisfy optimal intake recommendations. Loenneke et al. (2012) investigated the relationship between the number of times an individual hits the EAA threshold (~10g) and central adiposity. Results indicate that individuals who hit the EAA threshold more times present with lower central adiposity (Lonneke et al. 2012); which is significant because a physiological end-point is now associated with research which has been acute in nature.

To summarize, the literature points to anything above 20-30g protein per meal (~15g EAAs, 3g leucine) as potentially being energetically inefficient for most people. There is no great inherent danger to excessive oxidation of amino acids in the context of energy balance considerations, however, excess protein intake means the displacement of other important macronutrients. On the other end of the spectrum, it may not be prudent to follow a grazing pattern, as evidence of an 'initiation threshold' (Norton et al. 2009) is mounting. This narrow 'Goldilocks zone' (in which the optimal intake lies between extremes of both amount and frequency) warrants closer investigation. Daily distribution of protein is an important topic that is now being explored, but the ratio of real-time energy balance in relation to protein intake is a new frontier.

Protein and Energy Restriction

A review of literature concerning metabolic and body composition matters in the context of energy restriction is particularly important for the present study given the population investigated. The female basketball players presently studied are at risk for RED-S. Athletes commonly consume an inadequate amount of energy in relation to estimated needs, and female athletes appear to be more vulnerable to eating disorders. (Sundgot-Borgen, 1996). In fact, in comparison to male athletes (even when normalized for body weight) female athletes consume only ~70% of estimated energy and carbohydrate requirements (Loucks, 2004). Three distinct origins of energy deficiency threaten the athlete: 1.) Obsessive eating disorders in conjunction with mental illness, 2.) Misguided effort to reduce body size and fatness to succeed in competition, 3.) Failure to increase energy intake to meet the increase in energy expenditure (Loucks, 2011). In past research utilizing Computerized Time Line Energy Analysis, athletes presented with a far greater proportion of the day spent in a relative energy deficit as opposed to an energy surplus (Deutz et al. 2000). Furthermore, even when psychosocial factors are not considered, energy deficits caused by increased exercise energy expenditure do not stimulate concomitant increase in energy intake to the degree of food deprivation-induced hunger (Hubert et al. 1998). Therefore, examination of metabolic function during energy restriction/deficit is warranted.

Protein intake above the RDA has been proposed to attenuate loss of lean body mass during periods of energy deficiency by inducing alterations in protein turnover (Phillips, 2008). One study of interest was performed by Pasiakos et al. (2013) who sought to explore body composition and muscle anabolic responses to varying levels of protein intake. Participants were placed on isoenergetic diets containing either 1x PRO RDA (0.8g PRO/kg/d), 2x PRO RDA (1.6g PRO/kg/d), or 3x PRO RDA (2.4g PRO/kg/d) and then underwent a 10d weight maintenance diet followed by a 21d 40% energy deficient diet. Results indicate that consuming dietary protein at levels above the RDA spared fat-free mass while still promoting loss of body fat. Fat-free mass comprised

58% of weight lost in the RDA group, while the 2x- and 3x-RDA groups lost 30 and 36%, respectively, of fat-free mass as a proportion of total weight lost. Notably, a threshold effect was detected as the group consuming 3x the RDA for protein experienced no greater protection of fat-free mass than the group consuming 2x the RDA.

Proportion of energy from protein may be even more critical in periods of energy deficit. Pikosky et al. (2008) observed a decline in nitrogen balance in subjects consuming 0.9g PRO/kg/d when placed on a 7d diet producing a 1000-kcal energy deficit. However, nitrogen balance was maintained in the group consuming 1.8g PRO/kg/day throughout the same 7d 1000-kcal energy deficit diet, suggesting a protective effect.

The question of how protein influences mechanisms to improve retention of lean body mass is of central importance to helping many attain a healthy body composition. Likely, lean body mass is lost during periods of caloric restriction due to an increase in muscle cell proteolysis as opposed to muscle protein synthesis being downregulated. Research by Villareal et al. (2012) and Campbell et al. (2009) suggest that protein synthetic response can be maintained during energy restriction, but high rates of proteolysis are observed. Of interest to the present study population, Campbell et al. (2009) detected salient metabolic protective effects of resistance training on lean body mass. Additionally, Villareal et al. (2012) identified an increased anabolic response to feeding in subjects in an acute energy deficit, but did not detect the same effect after weight loss had occurred (suggesting an adaptive response).

The adaptive response to increased protein intake could explain some of the shortcomings of chronically high-protein diets. Protein intake is the main determinant in whole-body protein turnover rate (Pannemans et al. 1995). When protein intake notably surpasses maximal postprandial protein synthesis capacity, amino acid oxidation has been observed to increase 63-95% (Pannemans et al. 1998). Thus, increasing protein intake could simply increase the protein turnover rate, which would increase the protein requirement to maintain nitrogen balance (Gaine et al. 2006). In essence, the supply produces the demand to ultimately create a balance. However, the most potent modulator aside to the whole body protein turnover equation is exercise, which in tandem with increased protein intake can maintain anabolic sensitivity and result in a net positive nitrogen balance (Gaine et al. 2006; Campbell et al. 2009).

The means by which dietary protein can induce an energy deficit are also of interest. The effects of macronutrient proportions on diet-induced energy expenditure (DEE) is one potential contributor to the equation. DEE concerns the energy-requiring reactions in the post-prandial period: including intestinal absorption of nutrients, initiation of metabolism, and storage of those nutrients not immediately oxidized (Tappy, 1996). DEE for each specific nutrient varies, with fat having the lowest DEE value at 0-3%, carbohydrate at 5-10%, and protein at 20-30% (Tappy, 1996). Typically, DEE represents 10% of daily energy expenditure when in energy balance (Westerterp, 2004). Thus, a high-proportionate protein diet can induce a small but significant change in long-term energy balance.

Energy Availability

Relative energy availability is particularly important to athletes as energy expenditure for locomotion is notably increased. The impact of relative energy deficiency goes beyond effects on just body composition. Low EA can result in inhibition of any/all of the other four major metabolic activities (cellular maintenance, thermoregulation, growth, and reproduction) from energy deficits around the time of exercise, even if complete energy balance by the end of the day is achieved. The effect of low energy availability is particularly salient on reproduction in mammalian females (Loucks, 2003). Issues concerning this are outside the scope of this review, but it should be noted that secondary amenorrhea and oligomenorrhea are typical warning signs of low EA and disordered eating. This is dependent upon suspicion of athlete at risk and self-reporting, however, as there is currently no standardized guidelines to determine EA (Mountjoy et al. 2014).

Many athletes engage in energy restriction in an effort to attain a physique that is expected of them. This pressure is particularly pronounced on women and athletes in aesthetic sports. The practice of energy restriction in combination with exercise-related energy expenditure to reduce body fat is ineffective at achieving its goal. In response to energy restriction, the human adaptive response results in a reduction in resting metabolic rate. One study examined this phenomenon by having endurance runners consume either a low-energy diet or adequate energy diet and measuring resting metabolic rate (Thompson et al. 1993). The resting metabolic rate was significantly down-regulated in the group consuming the low-energy diet. A similar homeostatic response in regards to energy expenditure in runners compared to non-runners has also been observed (Mulligan & Butterfield, 1990). The finding relevant to the present study from the Mulligan & Butterfield (1990) research being that runners, in spite of greater energy expenditure than non-runners, maintained weight despite equivalent energy intakes.

By this mechanism the body adapts to energy restriction by lowering daily energy expenditure, often by shedding metabolically active tissue (muscle). Whereas glucose has glycogen and fatty acids have triglycerides, there is no inactive storage compound for amino acids (Volpi et al. 2003). This makes skeletal muscle protein particularly susceptible to significant losses in periods of fasting (Biolo et al. 2002). Indeed, it is counterproductive for an athlete who is trying to achieve an optimal strength to weight ratio to remove the tissue that generates strength.

The old model of 24h energy balance has many shortcomings, as illustrated by this comparison of studies with similar subjects and variables, but different methods of analysis. One such study examines 24h energy balance and body composition in juvenile elite gymnasts (Filaire & Lac, 2002). Body composition, dietary intake, and energy expenditure were examined in 12 elite female gymnasts with 15+ hours/wk physical activity and in 9 control subjects age-matched with less than 4 hours/wk physical activity. Results indicate that the gymnasts were significantly shorter and had lower body weight than controls. The primary finding of this investigation is that in both groups, the mean daily energy intake met the energy requirement. Thus, the gymnasts did not restrict total energy and were presumably in energy balance. Using 24-hour energy balance does not reveal when and for how long energy deficits occurred during the day. It must rationally be assumed that something must be causing this significant height difference between gymnasts and controls. The most likely culprit is relative energy deficiency for much of the day due to increased expenditure in locomotion which will inhibit the other four major metabolic activities, namely growth.

A more complete understanding of energy balance is achieved by a study utilizing real-time energy balance analysis (Deutz et al. 2000). This study examined intake and expenditure using Computerized Time-Line Energy Analysis (NutriTiming) to determine number and frequency of within-day energy deficits, surpluses, and relative balance. Results indicate that those with the most hours spent in energy deficit had the highest percentage body fat. Furthermore, the magnitude of the deficits was also positively associated with body fat percentage. In this study, 24hr energy balance showed no relationship with body fat percentage. Further refuting the 24hr energy balance theory is evidence of energy surpluses being inversely associated with body fat percentage. Although it must be noted that the energy surpluses observed were typically of a lower magnitude and frequency than the deviations of the relatively larger energy deficits. Mechanistically, this manifests as a potential lower insulin response and concomitant increase in fat storage as a result of the relatively small energy surpluses. Therefore, it cannot be stated that large magnitudes or frequencies of energy surplus is advised (due to basic thermodynamics of weight stability), but rather small energy surpluses appear to have a favorable effect on body composition.

Comparing these two studies which examine similar populations and variables illustrate the problem with the often employed 24hr energy balance analysis. Some athletes with high body fat percentage in the Deutz et al. (2000) study had pronounced and/or prolonged energy deficits, but ended the day in perfect energy balance. The issue is manifest using real-time energy balance analysis, but these same athletes would erroneously be considered in perfect energy balance using 24hr energy balance methods.

CHAPTER III

METHODS

INCLUSION CRITERIA

Individuals were eligible for inclusion only if they were on the Georgia State University Women's Basketball Team. For this reason, there are no minors or men in the study. The team consisted of 17 potential subjects, aged 19-26 years old. Procedures were approved by the Georgia State University Institutional Review Board.

SUBJECTS

Of the 17 potential subjects, 13 completed the full study protocol. Recruitment occurred in the Georgia State University Sports Annex. Recruitment proceeded as follows:

1. The student PI and PI were invited to talk about sports nutrition to the team and coaches. The study was introduced at this time.
2. Players interested in volunteering for the study were provided the email address of the student PI, and could contact the student PI directly.
3. A mutually acceptable time for the student PI and volunteer subject was established for them to meet in Room 455, Petit Science Center. At that time, the student PI covered the content of the informed consent form with the player.
4. Subjects interested in volunteering for the student then signed the informed consent form, which was further co-signed by the student PI.

5. Following data acquisition, participants were given the option to review the results of their data after analysis had been performed.

DATA ACQUISITION PROTOCOLS

All evaluations took place in the Laboratory for Elite Athlete Performance (455 Petit Science Center) on the campus of Georgia State University. Data collection occurred in a 2-month period from October to December, 2014. On arrival in the laboratory, subjects were asked to describe the previous day's schedule to the interviewer (student PI). NutriTiming® (NutriTiming® LLC, Atlanta, GA), a computerized timeline energy analysis (CTLEA) program, was used to assess real-time energy balance. This method of analysis has been validated previously (Benardot, 1996), although not with collegiate basketball athletes.

A full description of CTLEA methodology has been previously described (Benardot, 1996. Deutz et al. 2000). Briefly, CTLEA simultaneously assesses food intake and energy expenditure. The energy content of consumed foods in NutriTiming was based upon the USDA nutrient database for standard reference (Version 26). Foods reported by the athlete that were not included in the nutrient database were manually added from information on the food label or data provided by the food producer. Programmed into CTLEA is the methodology for determination of energy expenditure data as presented by the Physical Activity Guidelines Advisory Committee (U.S. Department of Health and Human Services, 2008) and the National Research Council (NRC, Food and Nutrition Board, 1989). As opposed to a daily activity factor, real time energy expenditure was assessed by asking participants to assign an activity factor to all daily activities using a 13 point scale (in 0.5 increments) of 1 (sedentary) to 7 (exhaustive). Duration of each activity was emphasized. Energy intake data were obtained using a method similar to 24-hr recall, except that activity and food intake information are obtained simultaneously (Deutz et al. 2000).

Subjects entering 455 Petit Science Center had their height, weight, body composition, and previous day dietary record and energy expenditure recorded.

1. Measurement of Height: Height was measured on a standard physician stadiometer in inches (then converted to cm). Subjects were asked to stand straight with no socks or shoes for the measurement. There is no risk or discomfort associated with this measurement.
2. Measurement of Weight and Body Composition (fat mass and fat-free mass in kg): A multi-current BIA body composition analyzer (Tanita BC-418) was used to assess body weight and composition. Subjects stood on the scale without their shoes and socks and held additional handles in each hand. There is no discomfort associated with this test, which took approximately 2 minutes to set up and run. There is no harm or risk associated with this assessment.
3. Measurement of Diet/Fluid Intake and Energy Expenditure: Subjects completed a questionnaire and interview. Hourly energy intake and expenditure were recorded, with an emphasis placed on timing of intake and expenditure, using the NutriTiming Data Entry Form (Appendix II). There are no risks associated with this task.

All assessments were conducted by the Student PI.

DATA ANALYSIS

Statistical analysis of data was analyzed using SPSS for Windows 7 (version 22.0, SPSS, Inc., Chicago, IL). Abnormal distribution of the data were assumed due to the small sample size (n = 13). Thus, analyses were performed utilizing non-parametric statistical methods. Numerous indices of protein intake in relation to energy balance were examined. Variables examined for determination of energy balance included Net Energy Balance (24hr Starting EB – Ending EB), Hours anabolic (EB > 0 kcal), Hours catabolic (EB < 0 kcal), Hours in EB Surplus (EB > +400 kcal), Hours in EB Deficit (EB < -400 kcal), and Hours Within Optimal EB (EB within ± 400 kcal). Variables examined for determination of protein intake and distribution included, Protein (grams ingested), Optimal Protein (ingested within ± 400 kcal EB), Protein in EB (ingested in > 0 kcal EB), Protein Eating Occurrences (PEO) (number of meals containing > 10 g PRO), and Optimal PEO (number of meals containing >10g PRO within ± 400 kcal).

Descriptive statistics, frequencies, independent samples t-tests (normal variances not assumed), and Spearman correlations were utilized to evaluate potential relationships between energy balance, protein intake and distribution, and body composition. In all cases, statistical significance was set at $P < 0.05$.

DATA SECURITY

Special care was taken to assure that no coercion was involved with participation in this study. The subject pool consists of the Georgia State University Women's Basketball Team, and the coach has agreed that participation is totally voluntary. The coach and other administrators associated with the team were not aware of who on the team has volunteered for the study. Non-participation in the study in no way affected their membership on the team or their status as a student at Georgia State University.

Volunteer subjects were assessed individually, so no other person on the team was aware of their participation. Only subjects who voluntarily agree to participate with the study were included in the study. Subjects used a ‘code’ and no individually identifiable information was included on any study documents or on information that summarizes study results. This code sheet was kept in a locked drawer in a separate room (413 Petit, the PI’s office). All Data sheets were kept in a locked file cabinet in 455 Petit Science Center, and electronic databases associated with this study were kept on a secure, code-requiring, computer in 455 Petit Science Center. The electronic data had no personally identifiable information.

CHAPTER IV

RESULTS

ANTHROPOMETRIC DATA

Of the 16 eligible participants on the basketball team, 13 completed the study. Mean weight, height, and BMI were determined to be 75.70 kg (± 12.92), 175.638 cm (± 7.47), and 24.4 kg/m² (±3.13), respectively. Mean percentage body fat was 23.3% (± 5.2%), with a high of 31.3% and a low of 16.0%. Mean body fat mass and lean body mass were 18.10 kg (± 6.95 kg) and 57.60 kg (± 6.91 kg), respectively.

Table 1: Descriptive Statistics of Collegiate Female Basketball Players (N = 13)

	Minimum	Maximum	Mean	Std. Deviation
Age (yrs)	**19.0**	**26.0**	**20.5**	**1.8**
Height (cm)	**165.1**	**190.5**	**175.6**	**7.5**
Weight (kg)	**59.8**	**105.9**	**75.7**	**12.9**
Lean Body Mass (kg)	**47.0**	**72.7**	**57.6**	**6.9**
Body Fat Mass (kg)	**10.5**	**33.1**	**18.1**	**7.0**
Body Mass Index	**19.3**	**29.1**	**24.4**	**3.1**
Percent Body Fat (%)	**16.0**	**31.3**	**23.3**	**5.2**

ENERGY BALANCE

The average energy intake for all subjects was 2,259 kcal (± 411), and the average energy expenditure was 2,463 kcal (± 472). Thus, the average 24-hr net energy balance was

-204 (± 629). The average largest within-day surplus was 454 kcal (± 423), and the average greatest within-day deficit was -862 kcal (± 783). The greatest energy deficit observed was -2,255 kcal, and the largest energy surplus was 1,460 kcal. Athletes spent more time in energy deficit (17.46, ± 4.66) than in energy surplus (6.54, ± 4.66). Additionally, the average number of hours spent in energy deficit greater than 400 kcal 6.46 (± 6.09). In comparison, the average number of hours spent in energy surplus greater than 400 kcal was 2.46 (± 3.66). Athletes spent 15.08 hours (± 6.33) in relative energy balance (± 400 kcal EB). No participant was in a state of optimal energy balance (± 400 kcal) for the full 24 hrs (2 subjects were in EB ± 400 kcal for 23 hrs, however). A thorough report of intake and energy balance data can be found in Table 2.

Table 2: Energy Balance Descriptive Statistics of Collegiate Female Basketball Players (N = 13)

	Minimum	Maximum	Mean	Std. Deviation
Kcal (in)	**1638**	**3088**	**2259.8**	**410.7**
Kcal (out)	**1968**	**3676**	**2463.2**	**472.0**
Energy Balance (24-hr Net)	**-1374**	**953**	**-204.2**	**629.0**
Highest Energy Surplus (kcal)	**10.00**	**1460.0**	**454.2**	**423.0**
Lowest Energy Deficit (kcal)	**-2255.0**	**-374.0**	**-956.6**	**654.3**
Energy Balance (hrs > +400 kcal)	**0**	**10**	**2.5**	**3.7**
Energy Balance (hrs < -400 kcal)	**0**	**18**	**6.5**	**6.1**
Energy Balance (hrs >0 kcal)	**0**	**14**	**6.5**	**4.7**
Energy Balance (hrs < 0 kcal)	**10.0**	**24.00**	**17.5**	**4.7**
Energy Balance (hrs within +/- 400 kcal)	**5**	**23**	**15.1**	**6.3**

PROTEIN INTAKE AND DISTRIBUTION

Mean total protein intake and protein intake/kg were 79.21g (± 22.01) and 1.06 (± 0.27), respectively. Protein intake was examined in relation to various energy states and thresholds (30g). Protein intake in >0 kcal EB averaged 37.34g (± 28.77), and protein intake while in relative energy balance (± 400 kcal) averaged 49.13g (±29.17). Lastly, number of eating occurrences containing >10g protein (PEO) were examined, as well as energy status modifiers. Mean PEO, PEO in ± 400 kcal EB, and PEO in > 0 kcal EB were 2.54 (± 1.19), 2.00 (± 1.29), and 1.46 (± 1.19), respectively. Table 3 summarizes protein intake and distribution data.

Table 3: Protein Intake and Distribution Descriptive Statistics of Collegiate Female Basketball Players (N = 13)

	Minimum	Maximum	Mean	Std. Deviation
Protein (g_)	**49.0**	**114.0**	**79.2**	**22.0**
Protein (g/kg)	**0.69**	**1.69**	**1.06**	**.27**
Protein (>0 kcal EB)	**0.0**	**100.2**	**37.3**	**28.8**
Protein (within +/- 400 kcal EB)	**0.0**	**100.2**	**49.1**	**29.2**
PEO (>10g)*	**1**	**4**	**2.5**	**1.2**
PEO (>10g, within +/- 400 kcal EB)**	**0**	**4**	**2.0**	**1.3**
PEO (>10g, > 0 kcal EB)***	**0**	**4**	**1.5**	**1.2**

*Number of meals containing >10g protein
**Number of meals containing >10g protein while within ± 400 kcal EB
*** Number of meals containing >10g protein while > 0 kcal EB

RELATIONSHIP BETWEEN ENERGY BALANCE, PROTEIN INTAKE AND DISTRIBUTION, AND BODY COMPOSITION

Net 24-hr energy balance was not associated with percentage body fat ($R = .137$; $p = .655$). There was no statistically significant relationship between total protein intake and percentage body fat ($R = .533$; $p = .061$), although a notable trend was detected (Figure 1). Furthermore, there was no statistically significant relationship between protein ingested in relative energy balance (± 400 kcal) and percentage body fat ($R = -.187$; $p = .541$). Additionally, there was no statistically significant relationship between PEO

(>10g), PEO (>10g, ± 400 kcal EB), and PEO (>10g, >0 kcal EB) and percentage body fat (R = .117; p = .704, R = -.313; p = .298, R = -.051; p = .868).

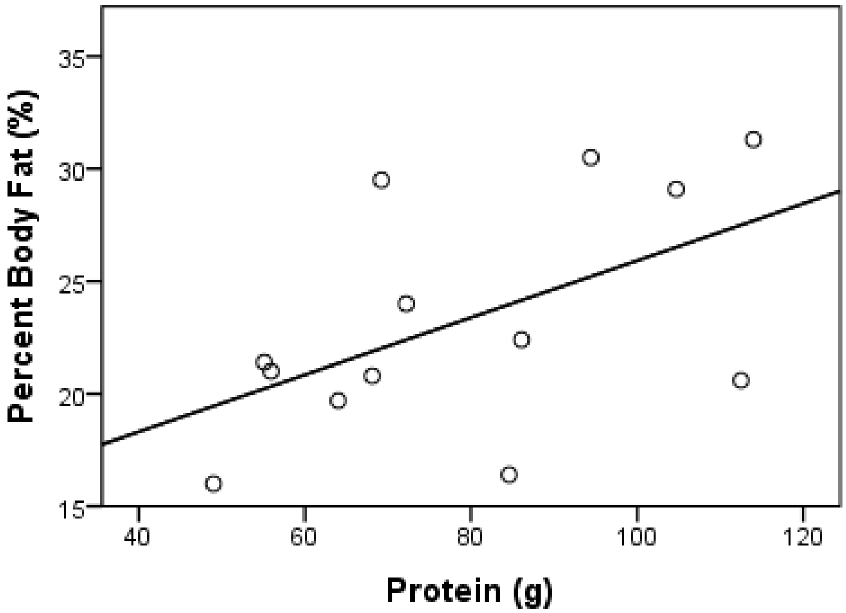

Figure 1: Scatterplot of Total Protein Intake and Percentage Body fat

A statistically significant relationship was observed between total protein intake and body fat mass (r = .597*; P = .031). PEO (>10g, ± 400 kcal EB) was inversely correlated with BMI (r = -.650; P = .016). No correlation between total energy intake and protein intake was detected (R = .549; p = .052).

Table 4: Correlations Between Total Protein Intake, Body Fat Mass, and Total Kcal Intake of Collegiate Female Basketball Players (N = 13)

			Kcal (in)	Protein (g)	Body Fat Mass (kg)
Spearman's rho	Kcal (intake)	Correlation Coefficient	**1.000**	**.549**	**.223**
		Sig. (2-tailed)	**.**	**.052**	**.464**
	Protein (g)	Correlation Coefficient	**.549**	**1.000**	**.597**
		Sig. (2-tailed)	**.052**	**.**	**.031**

*. Correlation is significant at the 0.05 level (2-tailed).

The highest energy surplus and net 24-hr energy balance were both inversely associated with lean body mass ($R = -.577$; $p = .012$, $R = -.670$; $p = -.670$). Additionally, there was a significant inverse association between the lowest energy deficit and lean body mass ($R = -.736$; $p = .004$).

A Kruskal-Wallis H test was conducted to determine if there were differences in body fat percentage, lean body mass, fat mass, and BMI between tertiles of protein intake in relative EB (± 400 kcal) (lowest: $n = 4$, moderate: $n = 4$, highest: $n = 5$). Distribution of body fat mass ($p = .694$), lean body mass ($p = .173$), BMI ($p = .221$), and percentage body fat ($p = .985$) were not statistically significantly different between tertiles of protein intake in relative EB (± 400 kcal).

CHAPTER V

DISCUSSION AND CONCLUSIONS

This study sought to investigate new indices to assess dietary adequacy in promoting optimal body composition from dietary recall techniques. Typical recall analysis examines total energy and specific macronutrients in 24-hr time blocks. CTLEA has been utilized in an attempt to better understand dynamic requirements previously (Deutz et al. 2000), and the hypotheses were developed and tested to primarily assess differential energy availability on body composition. Accordingly, the present study was designed to build upon these findings by expanding the focus into analysis of specific macronutrient content (protein) in relation to real-time energy balance. This study, like previous CTLEA analysis (Deutz et al. 2000), observed no association between 24hr net energy balance and percentage body fat. Our findings suggest that within-day protein distribution relative to energy balance is associated with BMI. In addition, the greatest energy balance deficit during the day was inversely associated with lean body mass, indicative of potentially deleterious effects of energy restriction. However, the present study failed to detect differences in percentage body fat across within-day energy balance variables.

There is general agreement that almost all dietary assessment methodologies are subject to reporting bias; typically in the form of underreporting as opposed to over-reporting (McCrory et al. 2011). However, under-reporting of energy intake is not random, and varies by key health determinants (Garriguet, 2008). The author concluded that "under-reporting was greater among people who were overweight or obese, those who were physically active, adults compared with teenagers, and women compared with men." (Garriguet, 2008) The present study population meets three out of four characteristics of inaccurate reporting. Furthermore, the only significant difference in under-reporting of six classified age groups occurred between men and women in the 19-30 yr old group, with women significantly under-reporting more than men (Garriguet, 2008). Under-reporting is a manifestation of social desirability, and women have been shown to score higher in the social desirability trait and be influenced more strongly by social desirability in patterning responses (Herbert et al. 1997). Social desirability is defined as 'the tendency to respond in such a way as to avoid criticism' (Herbert et al. 1997). Essentially, it is telling the investigator what they believe the investigator wants to hear.

As part of the recruitment process, the student PI and PI were invited to speak with the basketball team and give a presentation. The presentation provided information on the importance of relative energy availability in sport, and may have given potential subjects a model to pattern optimal responses on. Since the presentation described optimal eating for sport as distributing energy and macronutrient intake to meet dynamic energy needs, the paradigm may have shifted the desirability trait-derived response from underreporting (the classical manifestation) to frequent/balanced meals. As previously cited, this inaccurate reporting would not be random, and overweight/obese respondents would likely modify reporting to a greater extent (Garriguet et al. 2008). Thus, a potential explanation for lack of significant body composition findings could be differential social-desirability reporting based upon the model of optimal meal patterning demonstrated to participants before the study.

Subject data is consistent with past CTLEA analysis. Athletes spent a far greater proportion of the day in an energy deficit (~17 hours) than in a surplus. Additionally, the subjects averaged spending ~6.5 hours in a sizable energy deficit (<-400 kcal EB), which has been associated with higher percentage body fat (Deutz et al., 2000). The inverse association between lowest energy deficit and lean body mass is not surprising, as there is no inactive storage for amino acids (Volpi et al. 2003) and periods of fasting are associated with lean tissue losses (Nourouzy et al. 2013). Further, a potential gender difference exists, where one study found high eating frequency to be associated with leanness in men, but no link existing between eating frequency and body weight status in women (Drummond et al., 1998).

The substantial gap between mean total protein intake (79.2g) and protein estimated to be usable for MPS (49.1g) is cause for concern in this population. The mean protein intake displayed as g/kg intake was 1.1 g/kg/d; below the ACSM recommendation of 1.2-1.7 for athletes. When protein over the maximal threshold and protein ingested in significant energy deficits (< - 400 kcal EB) are controlled for, the mean intake is 0.69 g/kg/d; approximately half of the low end of the ACSM recommendation.

The present study does not reveal an overt connection between protein intake relative to current energy balance on percentage body fat. In particular, we hypothesized that those with a higher consumption of protein in a positive energy balance (excluding protein intake above the posited threshold) would display a lower percentage body fat.

Statistical analysis failed to detect any difference in body composition amongst protein intake variables. Likely, nonparametric cross-sectional analysis of single-day dietary recall was not powerful enough to detect differences very specific protein variables on body composition in the small study population (N=13). Further limitations include potential response bias and lack of random selection. An interesting finding is that total daily protein intake was associated with higher body fat mass. Moreover, a near-significant trend was detected between total protein intake and percentage body fat ($R = .533$; $p = .061$). No association between total protein intake and total energy intake (the most logical explanation) was found, however a near-significant trend was observed ($R = .549$; $p = .052$).

CONCLUSIONS

This study is exploratory in nature and was designed to test the validity of new indices to assess dietary adequacy. However, as old indices (net energy balance, meal frequency, total protein intake, etc.) did not exhibit associations with body fat percentage to be considered superior measurements, the question remains unresolved.

The rationale for dynamic analysis of energy balance is sound (Benardot, 1996; Deutz et al. 2000; Benardot, 2013), and literature elucidating the importance of timing and distribution of protein intake (Mamerow et al. 2014; Symons et al. 2009; Moore et al. 2009) make the two a natural marriage. The finding that plasma amino acid concentrations do not linearly correlate with MPS across various time points and that energy status of the muscle is often the limiting factor (Wilson et al. 2011) further strengthens the argument to simultaneously investigate energy availability and protein distribution. Future investigations should increase subject pool size and take protein quality into consideration. Experimental control over balanced and skewed protein intake in various states of energy balance (skewing or balancing energy intake within-day) would provide clarity to the issue. Complex relationships exist between specific nutrients and available energy which cannot be explained by simplistic net value analyses. Just as a human body does not make a single calculation at the end of a 24hr period, we should not do the same to assess dietary adequacy and expect an accurate representation. Methods of determining dietary adequacy should no mirror a bomb calorimeters net ‘calories in, calories out’ model, but instead should reflect the complexity inherent to having an endocrine system and metabolic adaptions by analyzing much smaller units of time.

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APPENDICES

APPENDIX A: Informed Consent form

Georgia State University
Byridine F. Lewis School of Nursing and Health Professions
Department of Nutrition
Informed Consent

Title: The Relationship Between Within-Day Protein Distribution and Energy Balance in Female Collegiate Basketball Players

Principal Investigator: Dan Benardot, PhD, RD, LD, FACSM

Student Investigator: Robert Bergia

I. Purpose
You are invited to participate in a research study. The purpose of the study is to investigate the relationship of what/when you eat and how much of your body weight is bone, muscle, or fat. There is little information on diet and protein intake in female basketball players. You are invited because you are a basketball player and member of the Georgia State Female Basketball team. A total of 17 players will be recruited for this study. 30 minutes of your time will be needed on a day that works for you and the research staff. Should you wish to see the outcomes of your own data, another meeting of up to 30 minutes can be arranged. This meeting will require you to bring the code card to identify your data sheet.

II. Procedures
If you decide to participate, you will be assessed in several ways that include:
1. Height
2. Weight
3. Diet, activity questions (completed by yourself followed by a talk with student investigator)
4. Body Composition (fat mass and fat-free mass) using a machine called a Multi-current BIA

All of the tests will be done in such a way that protects your identity. You will be given a card with a code number. Only that code number and not your name will be used on all of the forms to make sure that your results cannot be traced to you. The test procedures are explained more fully here.

1. **Measurement of Height:** You will have your height measured. You will be asked to stand straight with no socks or shoes for the measurement, which takes approximately 15 seconds. There is no risk or discomfort associated with this measurement. Robert Bergia will take your height.
2. **Measurement of Weight and Body Composition (Fat mass and Fat-free mass):** A multi-current BIA body composition analyzer will be used to assess body composition and weight. You will stand on the machine with your shoes and socks removed and hold additional handles in your hands. The machine works by running

GSU APPROVED IRB NUMBER: H15152
IRB APPROVAL DATE: 10/17/2014
IRB EXPIRATION DATE: 10/16/2015

an electrical current through body tissues to estimate fat-free mass (and fat-mass by subtraction from total weight). It is important that you are not dehydrated when this test is performed as it will throw off the accuracy. There is no discomfort associated with this test which will take approximately 2 minutes to set up and run. There is no harm associated with the assessment. Robert Bergia will be performing the body composition assessment in 455 Petit Science Center.

3. **Measurement of Diet/Fluid Intake and Energy Expenditure:** You will complete a questionnaire and interview. These will help us understand what you eat, drink, and your activities that burn energy during a normal day. If you complete the questionnaire in advance, we may ask you some questions to make sure the questionnaire is correct and complete. For instance, if you indicated that you ate a hamburger, you may be asked if you had ketchup or lettuce. This will ensure the questionnaire is complete. The total time required to complete the questionnaire with interview will take 15 to 30 minutes. There are no risks associated with this task.

III. Risks
In this study you will not have any more risks than you would in a normal day of life.

IV.Benefits
Participation in this study may or may not benefit you personally. It may give you a better understanding of nutrition and body composition of basketball players. You will have access to all of the information you have provided at the end of the study. The information obtained may provide information that helps us better understand the relationships between energy intake, protein intake, and body composition. This information may help to improve our understanding of why many active people find it difficult to lower body fat levels.

V. Voluntary Participation and Withdrawal
Participation in research is voluntary. You do not have to be in this study. If you decide to be in the study and change your mind, you have the right to drop out at any time. You may skip questions or stop participating at any time. Whatever you decide, you will not lose any benefits to which you are otherwise entitled. Your decision will not have any impact on your status as a member of the Georgia State Women's Basketball team.

VI. Confidentiality
We will keep your records private to the extent allowed by law. Only study personnel will have access to the information you provide. Information may also be shared with those who make sure the study is done correctly (GSU Institutional Review Board, the Office for Human Research Protection (OHRP). We will use a code number rather than your name on study records, which will be stored in a locked file cabinet. When your information is entered into a computer, it will be entered into a computer that is password protected. Your name and other facts that might point to you will not appear when we present this study or publish its results. The findings will be summarized and reported in group form. You will not be identified personally.

VII. Contact Persons

Contact Robert Bergia at 412-849-9759 or rbergia1@student.gsu.edu if you have questions, concerns, or complaints about this study. You can also call if you have been harmed by the study. Call Susan Vogtner in Georgia State University Office of Research Integrity at 404-413-3513 or svogtner1@gsu.edu if you want to talk to someone who is not part of the study team. You can talk about questions, concerns, offer input, obtain information, or suggestions about the study. You can also call Susan Vogtner if you have questions or concerns about your rights in this study.

VIII. Copt of Consent Form to Subject:

We will give you a copy of this consent form to keep.

If you are willing to volunteer for this research, please sign below.

____ ____________

Participant Date

____ ____________

Principal Investigator or Researcher Obtaining Consent Date

GSU APPROVED IRB NUMBER: H15152
IRB APPROVAL DATE: 10/17/2014
IRB EXPIRATION DATE: 10/16/2015

Appendix B: NutriTiming Data Entry Form (ex.)

Dietary and Activity Analysis Data Entry Form

Subject Code:________________________

Time of Last Meal Before Day of Analysis:_________________

Instructions: Completing this form will help us understand whether the amount of energy (calories) you consume comes close to matching the energy (calories) you expend. This form provides a way of entering your energy expended by using an 'Activity Factor', and your energy consumed by using a description of the foods and drinks you ate. The information is entered by hourly units, so you don't have to remember precisely the time you had an activity or ate some food. Rather, you are asked to enter when you had an activity, its intensity by using the activity factor scale, and how long you did it (example: I had a slow jog between 10 and 11 in the morning that lasted for 30 minutes). Use the Activity Factor Scale Descriptions to help you figure out the best factor to enter when describing an activity. When entering food, describe the food and the way it was prepared fully (example: chicken breast with no skin that was baked; or fried, battered chicken breast, etc), and the amount you consumed (example: 1 apple; 1 ½ cups; 15 red grapes; 1 large banana, etc.). A factor of 1.5 is considered normal daytime activity, and we will assume a factor of 1.5 unless you indicate otherwise. A factor of 1 is equal to sleep, and a factor greater than 1.5 suggests you are doing something more vigorous than normal daytime activity. Please enter a full 24 hours of all your activities and all the foods/drinks you consume. Use the example below to help you understand how to enter the information.

Activity Factor Scale

Factor	Description
1	**Resting, Reclining:** Sleeping, reclining, relaxing
1.5	**Rest +:** Normal, average sitting, standing daytime activity
2.0	**Very Light:** More movement, mainly with upper body. Equivalent to tying shoes, typing, brushing teeth
2.5	**Very Light +:** Working harder than 2.0
3.0	**Light:** Movement with upper and lower body. Equivalent to household chores
3.5	**Light +:** Working harder than 3.0; Heart rate faster, but can do this all day without difficulty
4.0	**Moderate:** Walking briskly, etc. Heart rate faster, sweating lightly, etc but comfortable
4.5	**Moderate +:** Working harder than 4.0. Heart rate noticeably faster, breathing faster
5.0	**Vigorous:** Breathing clearly faster and deeper, heart rate faster, must take occasional deep breath during sentence to carry on conversation
5.5	**Vigorous +:** Working harder than 5.0. Breathing noticeably faster and deeper, and must breath deeply more often to carry on conversation
6.0	**Heavy:** You can still talk, but breathing is so hard and deep you would prefer not to. Sweating profusely. Heart rate very high
6.5	**Heavy +:** Working harder than 6.0. You can barely talk but would prefer not to. This is about as hard as you can go, but not for long
7.0	**Exhaustive:** Can't continue this intensity long, as you are on the verge of collapse and are gasping for air. Heart rate is pounding

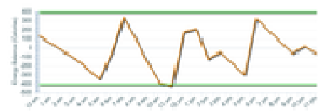
Begin Hour	End Hour	Activity Factor	Activity Description	Food/Drink Description	Food/Drink Amount
****Begin Example****					
12am	7am	1.0	Sleep		
7am	8am	1.5	Nothing Special	Whole Wheat Waffles (Frozen-Kellogg)	3
				Maple Syrup	2 Tablespoons
				1 % Milk	1 Cup
				Orange Juice (from concentrate)	1.5 Cups
				Coffee	2 Cups
				1 % Milk for Coffee	2 Tablespoons
10am	11am	5.0	Jog 30 minutes	Gatorade	16 Ounces
12noon	1pm	1.5	Nothing Special	Medium size beef sandwich with white	1 Sandwich
				bread, mayonnaise, lettuce, and tomato.	
				Coffee	2 Cups
				Artificial Coffee Creamer	2 Packets
				Apple Pie	1 Slice (small)
5pm	6pm	4.0	Walk 1 hour	Water	16 ounces
7pm	8pm	1.5	Nothing Special	Lasagna with ground beef and cheese	Large Plate
				Lettuce Salad with Tomatoes and Cucumbers	Medium Size Salad
				Blue Cheese Salad Dressing	1 Tablespoon
				Red Wine	1 Medium Glass
10pm	11pm	1.5	Nothing Special	Popcorn (air popped; no butter)	100 Calorie Pack
End Example					

Page _____

APPENDIX C: NutriTiming Data Output (ex.)

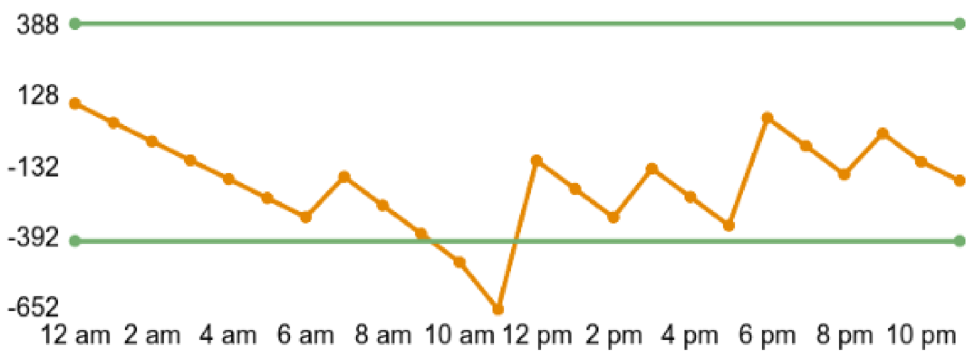
NutriTiming
Energy Balance Experts

Professional: Dr Dan Benardot
Bball Study, 323 - Energy Balance - 2015-01-22



Account: 323 Bball Study Age: 27 yrs. Height: 6 feet, 1 in.
Weight: 184.5 pounds Gender: Female BMI: 24.4
Analysis of Wed, Dec 10 2014

Energy Balance



Calories Out

Factor	Hours	Calories
1.0	8	558
1.5	15	1568
3.5	1	174
	Total	2300

24 Hour Calorie Balance

Starting Energy Balance	176
Calories In	1946
Calories Out	2300
24 Hour Energy Net	-354
Ending Energy Balance	-178
KCal(in)/Kg - total	23.2
KCal(in)/Kg - active	0.0

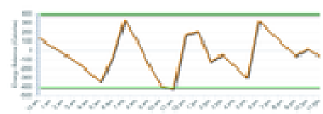
Highs and Lows

Hours optimum (+- 400 KCal)	22
Hours surplus (> 400 KCal)	0
Hours deficit (< -400 KCal)	2
Ratio surplus to deficit	0.00
Hours anabolic (> 0 KCal)	3
Hours catabolic (< 0 KCal)	21
Ratio anabolic to catabolic	0.14
Highest energy	176
Lowest energy	-653

Summary Notes:

NutriTiming
Energy Balance Experts

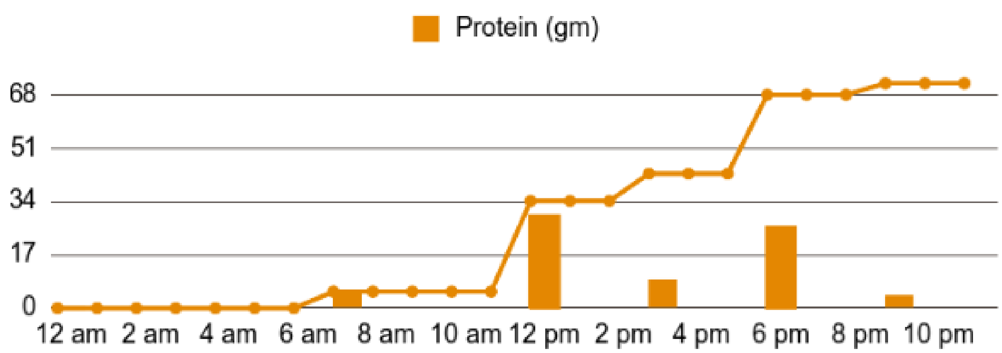
Professional: Dr Dan Benardot
Bball Study, 323 - Protein (gm) - 2015-01-22



Account: 323 Bball Study   Age: 27 yrs.   Height: 6 feet, 1 in.
Weight: 184.5 pounds   Gender: Female   BMI: 24.4

Analysis of Wed, Dec 10 2014

Nutrient Intake



Summary Notes: