

THE EFFECT OF MEAL COMPOSITION TO MODULATE THE ANABOLIC  
RESPONSE DURING RECOVERY FROM RESISTANCE EXERCISE

BY

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THESIS

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## **ABSTRACT**

Two of the most powerful anabolic stimuli to skeletal muscle tissue are resistance exercise (RE) and protein ingestion. RE and protein ingestion synergistically enhance the muscle protein synthetic response. Evidence suggests that the digestion and absorption kinetics, particularly protein derived amino acid availability in circulation, of the ingested protein source is an important regulator of postprandial muscle protein synthesis rates. Moreover, recent work has shown that dietary protein digestion and absorption kinetics is altered when other macronutrients are co-ingested with isolated protein powders. Indeed, current research has focused primarily on the ingestion of isolated protein powders to support the repair and remodeling process of skeletal muscle tissue. However, this does not accurately reflect the human diet as protein is generally consumed as part of energy-dense whole foods. In this thesis, we aimed to investigate to what extent dietary protein derived amino acid availability modulated the anabolic response to protein-dense whole foods during recovery from resistance exercise in healthy young adults using sophisticated stable isotope amino acid tracer methods. The study in this thesis demonstrates that whole eggs and egg whites are similar in the amount of dietary derived amino acids that become available to skeletal muscle after ingestion.

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## CHAPTER 1: GENERAL INTRODUCTION

### Introduction

Skeletal muscle plays a central role in human physiology. Beyond its fundamental importance in locomotion, muscle is an essential regulator of 24 h glucose metabolism, lipid oxidation, basal metabolic rate (BMR) and the largest reservoir of amino acids (AAs) for other tissues in times of stress [1] [2] [3]. Understanding the important role of skeletal muscle in human health has fostered decades of scientific investigation to identify modulatory variables that can influence the maintenance of skeletal muscle mass across a lifespan.

Two of the most powerful stimuli of this adaptive response are resistance exercise (RE) and protein ingestion [4] [5]. RE is the intentional performance of any exercise that causes the muscle to contract against an externally imposed load. It has been well established in previous work that one of the primary anabolic characteristics of RE is the ability to enhance skeletal muscle sensitivity to feeding during the post exercise recovery period [6]. Specifically, an acute bout of RE has been shown to increase the anabolic sensitivity of skeletal muscle to protein feeding for up to 24-h [6] and probably longer.

Recommendations have historically stressed the importance of consuming isolated protein powders that results in a rapid digestion and subsequent rise in circulating amino acids to optimize muscle anabolism. However, methodological constraints and perhaps a lack of interest may have limited the amount of research that used protein dense whole foods to assess the muscle protein synthetic (MPS) in response to resistance exercise. Consequently, a cohesive understanding as to the comparable

anabolic efficacy of consuming protein in its *whole food* or *isolated* form has yet to be adequately summarized.

Previous work has proposed that the quantity and composition of AAs that become available in circulation after protein ingestion dictate the capacity of a food source to augment the MPS response [7]. The consumption of isolated proteins that are rapidly digested in an effort to enhance the appearance rate of dietary protein derived AA availability in circulation to skeletal muscle has been proposed as a strategy to optimize this response [8] [9]. These isolated sources, including bovine milk's constituents, such as whey [10] [8] and casein [11], have been the predominant sources to assess acute skeletal muscle remodeling after a bout of resistance exercise. However, isolated protein sources represent a small fraction of available protein options, as most individuals consume whole meals that include protein within a highly complex and diverse macronutrient profile. Ingesting protein with fat or carbohydrates either present in its inherent food matrix or provided separately within the bolus has been shown to modulate protein digestion and amino acid absorption kinetics [12] [13] [14]. Despite the observation that protein digestion and absorption kinetics are modulated by macronutrient co-ingestion, the overall MPS response was not significantly different between sources of comparable quality (AA profile) [11] [15]. As such it is still unclear as to whether high quality whole food protein offers similar anabolic potential to the classically studied isolated forms.

Recommendations for whole food consumption is often supported by the concept that whole food offers an array of micronutrients and bioactive compounds not obtained from standard isolated alternatives. Although the influence of these factors on overall

skeletal muscle anabolism is ambiguous, it has been a provocative topic of recent investigation. Despite efforts to develop isolated protein powders of an equivalent micronutrient profile, other factors naturally incorporated in the nutrient matrix of intact food, such as miRNAs, could modulate a source's physiologic effect [16]. The purpose of this thesis, however, is to summarize the comparative efficacy of whole food in augmenting skeletal muscle adaptations to resistance exercise. Although potential relationships may exist between micronutrients and anabolism, no causal data will be presented, rather nutrient abundance corresponding to the studied sources will be provided as a contextual framework for establishing dietary recommendations.

### **Measuring the anabolic potency of a protein source**

The diverse methodology between studies assessing the postprandial aspects of anabolism creates some ambiguity in efforts to synthesize and summarize the available data. It is first important to understand the primary measures used to evaluate the anabolic potential of a protein source to better understand how to appropriately interpret the available evidence on assessing the anabolic potential of food. Nitrogen balance has conventionally established protein requirements based on the estimated quantity of protein sufficient to offset a nitrogen deficiency [17]. By definition this technique identifies the minimum amount of protein required to achieve and maintain balance, but it does not elucidate an optimal quantity to maximally stimulate the repair and remodeling of skeletal muscle. Advancement in stable isotope amino acid tracer methodology have made it possible to directly assess muscle protein synthesis within specific protein fraction pools, such as myofibrillar (contractile) proteins. Direct insight in the effects of exercise and

protein ingestion on myofibrillar protein synthesis allows for better determination of the optimal quantity and quality of protein to maximize the muscle contractile anabolic response.

### **Muscle protein synthesis (MPS)**

Previous work has established both dietary and training variables that modulate skeletal muscle anabolic sensitivity in magnitude and duration during the post exercise recovery period. This sensitization of skeletal muscle to the anabolic properties of amino acids persists for at least 24 hours post-exercise, primarily driven by two inter-related factors: (1) contraction volume (sets x reps x load); and (2) degree of muscle fiber recruitment with the recruitment of type II muscle fibers being exceedingly important [4]. Although resistance exercise alone is inherently anabolic [18], protein and its constituent amino acids are required to adequately facilitate the repair and remodeling of muscle. Muscle plasticity, or the muscles ability to adapt to specific stimuli manifests through a combination of acute (protein signaling, MPS, etc.) and chronic (protein accretion and hypertrophy) in muscle. Acutely, the skeletal muscle adaptive response is often characterized by the balance between anabolic (Muscle Protein Synthesis, MPS) and catabolic (Muscle Protein Breakdown, MPB) processes in muscle. When the aggregated effect of MPS exceeds MPB this would result in a positive Net Protein Balance (NPB); creating a requisite environment for protein accretion. Comparatively, MPS is the primary focus because of its highly robust reaction to protein ingestion and resistance exercise relative to MPB. This sensitivity to protein and exercise suggests MPS is the primary modulator of NPB. Studies often focus on the 5 hours after protein ingestion (acute post-



prandial phase) to assess the muscle protein synthetic response to feeding and exercise. This has been used as a reasonable proxy to assess long-term phenotypic adaptations [19]. This concept implies when the muscle experiences repeated and sustained acute rises in anabolism over a period of time, it will often result in the manifestation of physical and metabolic adaptations in skeletal muscle. Although this assumption seems reasonable, it is not without limitation, as the available evidence has demonstrated inconsistency in these extrapolated outcomes [19]. However, the main outcome variable that has been used to translate the acute muscle protein synthetic response to chronic adaptations has been gauged by the amount of muscle hypertrophy that occurs after prolonged interventions. The acute stimulation of muscle protein synthesis is more representative of the remodeling process which can also encompass non hypertrophic remodeling such as improved metabolic quality of muscle. Thus, it is no surprise that acute rates of MPS after 'exercise' and feeding do not provide a quantitative prediction for chronic adaptations, if only viewed through a lens of muscle hypertrophy.

### **Intrinsically labeled proteins**

Despite the fields expanding capability to measure the acute anabolic response using intravenous amino acid tracers, a number of factors that regulate the postprandial muscle protein synthetic response are not accurately comprised in such methods. Protein digestion and absorption, splanchnic amino acid extraction, and postprandial protein derived amino acid availability in circulation, are just some of the primary regulatory factors that can significantly influence the rate and magnitude of post prandial MPS [20] [21] [5]. The development of intrinsically labeled protein has created a novel

technique that better illustrates a protein sources capacity to influence specific adaptations in muscle [22]. Recent findings have perpetuated this uniquely effective tool to better characterize the anabolic potential of intact whole food proteins [14]. In contrast to either intravenous infusion or co-ingestion of an AA tracer within a bolus, the intrinsically labeled amino acid has been dynamically incorporated into the actual protein matrix of its host food. This unique feature is what allows researchers to appropriately describe the metabolic fate of amino acids from an intact whole food source. Using this technique, researchers can differentially measure exogenous rates of amino acid appearance in circulation and the incorporation of these labeled tracers into the contractile units of muscle [7]. Previously protein quality was largely thought to be dictated by the inherent amino acid profile of the protein source preceding ingestion. However, this assumes no influence of dietary protein digestion and absorption kinetics on the subsequent muscle anabolic response. However, with intrinsically labeled proteins researchers can assess the rate and quantity of which amino acids that become available in circulation after protein digestion and absorption, which are important and independent modulators of the muscle anabolic response [21]. Thus evaluating the influence of dietary protein digestion and absorption kinetics after the consumption of protein dense food is important for a greater understanding of factors that modulate the muscle anabolic response.

## Protein digestion and amino acid absorption

MPS is regulated by extracellular and/or intracellular amino acid availability [24]. The rate of protein digestion and absorption has been suggested to be a primary modulator of the anabolic potential of an individual protein source [7]. If true, differences in protein digestion and absorption kinetics of various protein sources would dictate the respective capacity to augment the adaptive response. The macronutrient profile of the protein source or the meal in which it is consumed has been shown to influence the metabolic fate of the dietary derived amino acids [13]. Consequently, whey protein, has been recommended at least partially because of its comparatively fast rate of amino acid appearance in peripheral circulation relative to alternatively slower sources (i.e. whole foods) [11] [25, 26]. However, recent work studying the influence of co-ingestion of other macronutrients (i.e. fat and carbohydrates) to modulate protein digestion and absorption patterning of a protein source have illustrated, despite having different rates of AA appearance in circulation, the total anabolic response was otherwise similar when comparing two protein sources of equivalent quality [13]. Many researchers now believe, it is less the *rate* of AA appearance but rather the *quantity* and *composition* of AAs ultimately becoming available in peripheral circulation (i.e. muscle) that comprises the strongest driver of the acute anabolic response. It is important to note, although a mechanistic understanding of differences between dietary protein sources in the acute postprandial period is imperative, physical adaptations to muscle are the end result research is trying to predict. Considering the observed metabolic variability between protein sources, it is clear future work needs to directly compare the long term efficacy of intact whole foods most prevalent in the human diet. The combination of acute and

chronic measures of anabolism could characterize optimal dietary recommendations for clinical and healthy populations.

### **Protein source**

Common sources of protein are varied among different cultures/geographical location, but typically comprise a range of animal and plant based proteins. Previous studies have focused on isolated protein sources implicated in acute skeletal muscle remodeling after a bout of resistance exercise, including bovine milk and its constituents, whey [7] and casein [27]; and plant-based protein, particularly soy [28]. However, these sources reflect a small fraction of available protein options, as most individuals consume whole meals that include protein among a highly complex and diverse macronutrient profile. It is currently understood that “high-quality” protein-dense food sources robust in essential amino acids [29] – and particularly the amino acid leucine – elicit a stronger muscle protein synthetic response than isonitrogenous consumption of lower-quality proteins [30] [31]. However, as the word denotes, isolated proteins do not include the diverse macronutrient and micronutrient components that comprise standard mixed meals, and thus are unrepresentative of typical dietary patterning of humans. Recent work has studied intact protein dense whole foods: milk [14], beef [14], egg [29] and pork [32] to better reflect the diets of modern humans. Few studies directly compare isolated vs. intact whole food proteins, but those that do demonstrate similarity in anabolism [15]. Studies comparing intact protein dense foods show significant differences in the exogenous rate of AA appearance and time to peak of MPS. However, when accounting for the 5 hour post prandial period, high quality sources particularly sufficient in the AA

leucine seem to have similar overall impacts on the acute MPS response [15]. More equivalence would likely be seen the longer you extend the observation period post consumption. A unique exception seems to be that of milk, which demonstrates a unique capacity to effect several aspects of body composition [33], and a superior muscle protein synthetic response to beef in the early postprandial period [34]. Attempts to reveal potential explanations for the observed efficacy of milk have led to recent investigations into physiologic factors beyond the macronutrient or AA profile of the protein source.

### **Other nutritional factors: miRNAs & micronutrients**

Presumably, protein dense whole foods offer an array of micronutrients not available from standard isolated alternatives. When comparing whey against standard dietary proteins, whey fails to hold up in a conventional panel of nutrients. Although whey offers an attractive combination of high quality protein at minimal cost (total energy), using these sources to *replace* traditional whole foods could significantly reduce the nutrient quality of a diet. Appropriately, these supplements can be viewed as just that, a supplementary tool to support a diverse intake of whole foods. Additionally, there are other nutritional factors of recent focus, such as miRNAs, that could influence a proteins physiologic effect. For instance, bovine milk has demonstrated the capacity to transport biologically meaningful amounts of microRNA's (miRNA) into the host consuming it. These short non-coding RNAs, intrinsic in milk and other foods, propose a meaningful dietary modulator of human health by inhibiting aberrant gene regulation, stimulating bone mineralization, and prevention of cancer risk in humans [16]. Clearly more work needs

to be done on these ancillary nutritional factors to better illustrate their potential role in muscle health and human longevity.

### **Nutrient density**

Establishing population specific dietary programs is a multi-faceted process. Traditionally recommendations emphasized avoiding foods containing perceptively problematic ingredients (i.e. sugar or saturated fat) and general guidelines for reducing overall energy intake. However, the American diet is increasingly energy rich (total kcal) but often nutrient poor; perpetuating a paradox of overconsumption in nutritionally deficient individuals. It should be no surprise then, rising rates of obesity and type 2 diabetes are growing trends in American society, partially linked to the habitual consumption of high energy foods combined with a sedentary lifestyle [35]. Recent efforts have developed new standards of evaluating food quality by establishing 'nutrient density' scores. According to the FDA, these values establish the amount of beneficial nutrients relative to the food's energy content per reference amount customarily consumed [36]. Despite having higher overall total energy, milk, beef and whole eggs demonstrate a uniquely nutrient rich option (per kcal) compared to protein supplements or nutrient manipulated foods (i.e. egg whites).

Some research shows a higher MPS response with nutrient dense food sources and/or other macronutrients. In these studies, the co-ingestion of omega-3 fatty acids [37] or whole milk [38] resulted in a stronger anabolic response in muscle relative to corn oil or fat free milk, respectively. Similarly previous work that specifically examined the co-ingestion of fat or carbohydrate with isolated dairy protein (i.e. casein) demonstrated that the co-ingestion of other macronutrients did not negatively impact the muscle protein synthetic response [13, 39]. These findings provide further support for consuming intact protein rich whole foods to potentially augment the adaptive response of skeletal muscle and overall nutrient quality of the diet, two critical factors in maintaining metabolic and functional capacity as we age.

### **Specific Objectives and Hypothesis Tested**

The primary goal of the study within this thesis was to compare differences in digestion and absorption kinetics, which is an important modulator of MPS, between whole eggs and egg whites when consumed in their natural whole food form. The chapter that follows gives specific details on the experimental design and methods used to test the hypothesis and the main findings. In Chapter 3, we offer an interpretation of these findings within the context of the available literature (discussion), limitations of the current study, and recommendations for future directions of research.

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## CHAPTER 2: WHOLE EGG vs EGG WHITE

Protein Digestion and Absorption Kinetics of Whole Eggs vs Egg Whites during recovery from resistance exercise in healthy young men.

### Introduction

Dietary patterns that include the regular ingestion of nutrient and high quality protein dense foods are important for skeletal muscle mass maintenance and/or growth throughout adult life. Protein quality in human nutrition can be partly determined by the availability of food protein derived amino acids in circulation to support metabolic need, such as protein synthesis, after ingesting a meal. Research has shown that protein type [40, 41] and amount [42] are modifying factors that can determine the amount of protein-derived amino acids available in circulation and the stimulation of postprandial muscle protein synthesis. However, most work has focused on the effects of consuming isolated protein fractions (e.g., dairy-based whey and casein) in liquid beverages [40, 41, 43-54] with far less known about how the ingestion of nutrient and protein dense whole foods impacts postprandial protein metabolism.

To better define whole food protein quality in human nutrition, we developed intrinsically L-[5,5,5-<sup>2</sup>H<sub>3</sub>]leucine eggs to allow for the detailed assessment of postprandial protein metabolism *in vivo* in humans [55]. Eggs are a nutritionally complete food source and commonly consumed at breakfast by US adults [60]. Interestingly, the removal of the yolk is often promoted for improved health when multiple eggs are consumed; an unsubstantiated belief related to the cholesterol and fat content of an egg yolk [56]. The yolk, however, is nutrient dense and contains nearly half of an egg's total protein content

(Table 1). Since the protein density of a meal is essential to maximally stimulate postprandial muscle protein synthesis rates [57, 58] it is important to define how a food matrix in which the protein is naturally consumed modulates postprandial protein derived amino acid availability in circulation and the subsequent stimulation of the postprandial muscle protein synthetic response.

Therefore, the purpose of this study was to compare protein digestion and absorption kinetics generalizable and nutritionally complete protein sources, such as whole egg and egg whites, during recovery from resistance exercise. We hypothesized that the consumption of 3 whole eggs (18 g protein and 17 g fat) will result in a delayed appearance of postprandial protein derived amino acids in circulation when compared to the ingestion of an equivalent amount of protein from egg whites (18 g protein and 0 g fat). However, we expect that total 5h dietary protein derived amino acid availability in circulation to be similar between the two conditions.

## **Methods**

### **Participants and ethical approval**

Ten healthy young men (mean  $\pm$  SEM: age: 21 $\pm$ 1 y; weight: 88 $\pm$ 3 kg; body fat: 16 $\pm$ 1%) volunteered to participate in this study. Participants were regularly engaged in structured resistance exercise training (mean  $\pm$  SEM: 5  $\pm$  1 y). Further detailed participant characteristics are presented in **Table 1**. All participants were deemed healthy based on responses to a routine medical screening questionnaire and had no prior history of participating in stable isotope amino acid tracer experiments. All participants were informed about the experimental procedures to be used, the purpose of the study, and all

potential risks before giving written consent. The experiment conformed to all standards for the use of human subjects in research as outlined in the Helsinki Declaration and were approved by the local Institutional Review Board at the University of Illinois at Urbana-Champaign.

Subjects' characteristics

<b>(n=10)</b>		
Age (y)	21	± 1
Weight (kg)	88	± 3
Fat (%)	16	± 1
Lean Body Mass (kg)	72	± 2
Systolic BP (mmHg)	121	± 4
Diastolic BP (mmHg)	70	± 2
Fasting Glucose (mg/dL <sup>-1</sup> )	76	± 1
Training Status (y)	5	± 1
10-RM Leg Press (kg)	242	± 32
10-RM Leg Extension (kg)	112	± 10

Table 1. Mean  $\pm$  SE of health characteristics for all study participants.

## Experimental design

A within-subject crossover design was used for this study. At least one week before the first infusion trial, participant reported to the laboratory for familiarization with the exercise equipment and for maximum strength testing as determined by their ten-repetition maximum (10-RM) for leg press and leg extension. In addition, body weight and height were measured as well as body composition by dual-energy X-ray absorptiometry (Hologic QDR 4500A, Bedford, MA, USA). On a separate occasion, at least 3 days after initial maximum strength testing and at least 3 days before the first infusion trial, subjects re-visited the lab to confirm their 10-RM for leg press and leg extension. The highest 10-RM for leg press and leg extension were  $233 \pm 32$  kg and  $112 \pm 10$  kg, respectively. Participants were instructed to refrain from vigorous physical activity for 3 d prior to each trial and to record their dietary intake in a food diary for 2 d before each trial. Upon completion of the first infusion trial, a copy of the food diary was returned to the participants. Participants were subsequently instructed to follow their food diary as closely as possible during the 2 d leading into the second infusion trial and record their dietary intake in another food diary. Average 2 d macronutrient intake was similar between the whole egg and egg white trials ( $P=XX$ ). Participants were counterbalanced in random fashion to consume either whole eggs or egg whites for their first trial. The time between crossover trials was 7–14 d (mean  $\pm$  SEM:  $8 \pm 1$  d)

## Infusion protocol

On both infusion trials, participants reported to the laboratory at 0700 h after an overnight fast. Upon arrival to the lab, a baseline breath sample was collected for determination of  $^{13}\text{CO}_2$  enrichment by isotope ratio mass spectrometry (BreathMat Plus; Finnigan MAT GmbH, Bremen, Germany). A Teflon catheter was inserted into an antecubital vein for baseline blood sample collection ( $t=-210$  min) and then participants received priming doses of L-[1- $^{13}\text{C}$ ]leucine ( $7.6 \mu\text{mol}\cdot\text{kg}^{-1}$ ) and  $\text{NaH}^{13}\text{CO}_2$  ( $2.35 \mu\text{mol}\cdot\text{kg}^{-1}$ ). Subsequently, a continuous L-[1- $^{13}\text{C}$ ]leucine ( $0.10 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) intravenous infusion was initiated ( $t=-210$  min) and maintained over the experimental infusion trial. A second Teflon catheter was inserted into a heated dorsal hand vein for repeated arterialized blood sampling and remained patent by a 0.9% saline drip. At -30 min, the participants performed resistance exercise that consisted of 4 sets of 10 repetitions at a pre-determined load for both leg press and leg extension exercise. The external work (repetitions  $\times$  load) was matched between exercise trials (mean  $\pm$  SEM: whole egg trial:  $9896 \pm 524$  kg; egg whites=trial:  $9893 \pm 522$  kg;  $P=0.34$ ). After completion of the exercise bout, participants consumed 3 whole eggs or an equivalent amount of protein from egg whites ( $t=0$  min). Participants were also provided 300 mL of water to consume with the meal. Completion of the meal marked the start of the postprandial phase ( $t=0$  min). Arterialized blood samples and breath samples were collected every 30 or 60 min during the postabsorptive- and postprandial states. The blood samples were immediately analyzed for whole blood glucose concentrations (2300 Stat Plus, YSI Life Sciences, Springs, OH) and subsequently centrifuged at  $3000 \times g$  for 10 min at  $4^\circ\text{C}$ .

The plasma samples were subsequently stored at -20°C for future analysis. The collected breath samples were stored at room temperature for future analysis.

### **Meal composition**

Intrinsically L-[5,5,5-<sup>2</sup>H<sub>3</sub>]leucine labeled eggs were produced by supplementing the diet of laying hens (Lohmann LSL Whites) with 0.3% L-[5,5,5-<sup>2</sup>H<sub>3</sub>]leucine as described previously [55]. For this study, a portion of the eggs had the yolks removed prior to storage at -20°C in aliquots of 18 g protein. The other portion of eggs had the yolks and whites completely mixed prior to storage in aliquots of isonitrogenous amounts. The L-[5,5,5-<sup>2</sup>H<sub>3</sub>]leucine enrichment of the whole egg and white aliquots were determined by gas chromatography mass spectrometry (GC-MS) and averaged 27.3 and 26.8 mole percent excess (MPE), respectively. Proximate analyses for protein and lipid concentrations were determined by using the combustion method (method 990.03; AOAC International, 2000; TruMac; LECO Corp., USA) [55]. Before the infusions, the whole egg or whites were thawed overnight in a refrigerator at 4°C. On the morning of the experiment, the whole egg or whites were scrambled in a skillet until solid with no visible liquid remaining. The macronutrient composition and energy content 18 g protein, 17 g fat, and 225 kcals and 18 g protein, 0 g fat, and 72 kcals for the whole egg and egg white treatments, respectively.

### **Blood analyses**

Glucose concentrations were analyzed in whole blood using an automated glucose analyzer (YSI 2300 Stat Plus, Yellow Springs Instruments, USA). Plasma insulin



concentrations were determined using a commercially available enzyme-linked immunosorbent assays (Alpco Diagnostics; Salem, NH). Plasma amino acid concentrations and enrichments were determined by GC-MS (Agilent 7890A GC/5975C; MSD, Little Falls, DE) as described in our previous work [55]. Briefly, plasma samples were prepared for amino acid analysis using a mixture of isopropanol:acetonitrile:water (3:3:2, v/v) and centrifuged for 10 min at 4°C. Subsequently, the supernatant was dried and the amino acids converted into tert-butyldimethylsilyl (*t*-BDMS) derivatives prior to GC-MS analysis. The plasma leucine and phenylalanine <sup>13</sup>C and <sup>2</sup>H enrichments were determined using selective ion monitoring at *m/z* 302, 303 and 305 for unlabeled and labeled (L-[1-<sup>13</sup>C] and L-[5,5,5-<sup>2</sup>H<sub>3</sub>]) leucine, respectively; and *m/z* 336 and 341 for unlabeled and L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine, respectively. Plasma amino acid concentrations were determined by integrating amino acid peak areas in comparison to an internal standard (DL-*p*-chlorophenylalanine) with the use of the AMDIS software package (v. 2.71; NIST) [59].

## Results

### Plasma metabolites

Plasma glucose concentrations decreased during the early recovery phase (time effect:  $P=0.05$ ) with no differences between groups ( $P=0.27$ ) (**Figure 2**). Lowest concentrations of plasma glucose were observed at 60 min after protein ingestion and with values of  $74.6 \pm 1.2 \text{ mg}\cdot\text{dL}^{-1}$  and  $74.4 \pm 1.2 \text{ mg}\cdot\text{dL}^{-1}$  for the egg white and whole egg condition, respectively. Plasma insulin concentrations increased during the early recovery phase (time effect:  $P=0.003$ ) with no differences between groups ( $P=0.158$ ) (**Figure 3**).

Peak concentrations of plasma insulin were observed at 30 min of the postprandial period with values of  $7.1 \pm 1.4$  and  $9.3 \pm 2.0 \mu\text{U} \cdot \text{mL}^{-1}$  for the egg white and whole egg condition, respectively. Plasma phenylalanine concentrations increased in both treatment groups (time effect:  $P < 0.001$ ) with higher peak concentrations observed after ingestion of egg whites when compared to whole eggs ( $P < 0.001$ ) (**Figure 4**). Peak concentrations of plasma phenylalanine were observed at 75 min ( $92 \pm 8 \mu\text{mol} \cdot \text{L}^{-1}$ ) and 120 min ( $86 \pm 4 \mu\text{mol} \cdot \text{L}^{-1}$ ) after ingestion of egg whites and whole eggs, respectively. Plasma leucine concentrations increased after protein ingestion (time effect:  $P < 0.001$ ) with no differences between groups ( $P = 0.603$ ) (**Figure 5**). Peak concentrations of plasma leucine were observed at 75 min ( $172 \pm 16 \mu\text{mol} \cdot \text{L}^{-1}$ ) and 120 min ( $174 \pm 11 \mu\text{mol} \cdot \text{L}^{-1}$ ) after ingestion of egg whites and whole eggs, respectively.

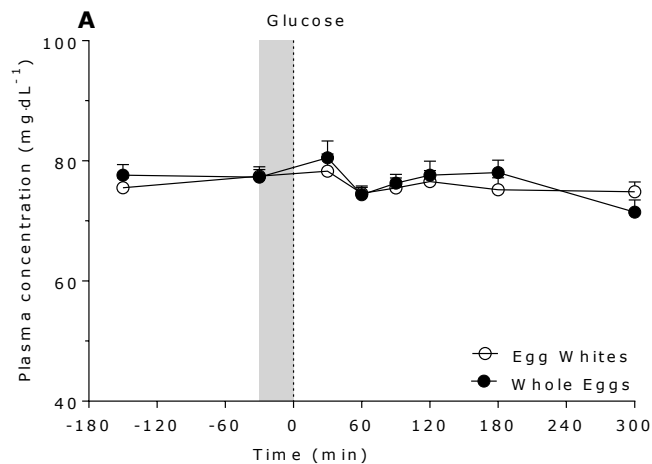


Figure 1. Plasma glucose concentrations throughout study duration.

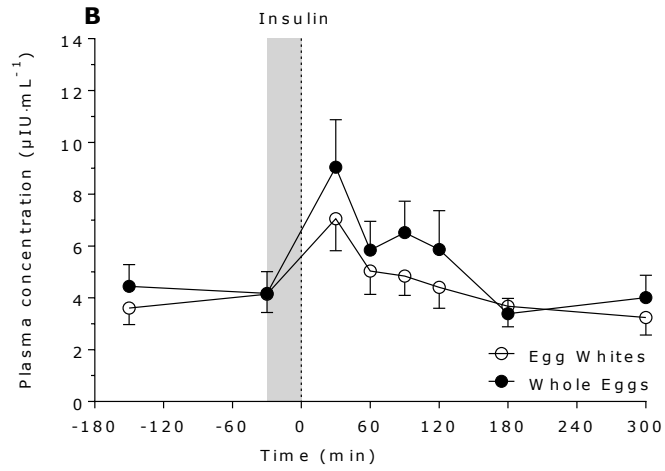


Figure 2. Plasma insulin concentrations throughout study duration.

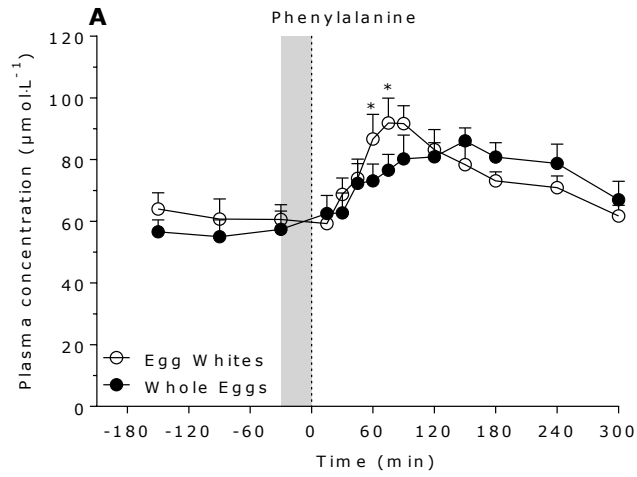


Figure 3. Plasma phenylalanine concentrations throughout study duration.

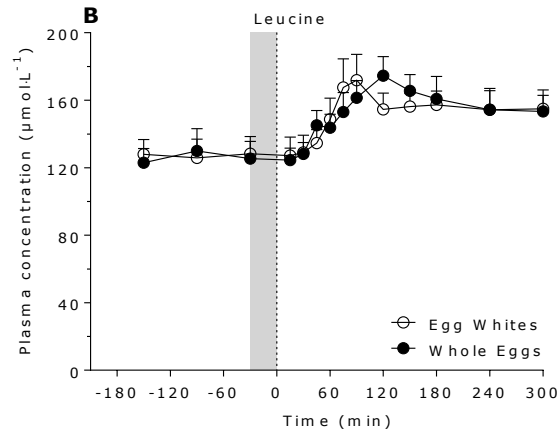


Figure 4. Plasma leucine concentrations throughout study duration.

### Plasma amino acid enrichments

Plasma L-[5,5,5-<sup>2</sup>H<sub>3</sub>]leucine (oral tracer) enrichment (**Figure 6**) increased in both treatment conditions (time effect:  $P < 0.001$ ) with a trend for higher enrichments observed after ingestion of whole eggs when compared to egg whites ( $P = 0.12$ ). Peak plasma L-[5,5,5-<sup>2</sup>H<sub>3</sub>]leucine enrichment were observed at 75 min ( $6.3 \pm 0.6$  MPE) and 120 min ( $7.0 \pm 0.5$  MPE) after ingestion of egg whites and whole eggs, respectively. Basal plasma L-[1-<sup>13</sup>C]leucine (intravenous tracer) enrichments (**Figure 7**) did not differ between groups ( $P = 0.725$ ). Plasma L-[1-<sup>13</sup>C]leucine (intravenous tracer) enrichments (**Figure 7**) decreased after protein ingestion (time effect:  $P = 0.006$ ) with no differences between groups ( $P = 0.754$ ). Basal plasma L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine (intravenous tracer) enrichments (**Figure 7**) did not differ between groups ( $P = 0.946$ ). Plasma L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine (intravenous tracer) enrichments (**Figure 8**) decreased after protein ingestion (time effect:  $P < 0.001$ ) with no differences between groups ( $P = 0.693$ ).

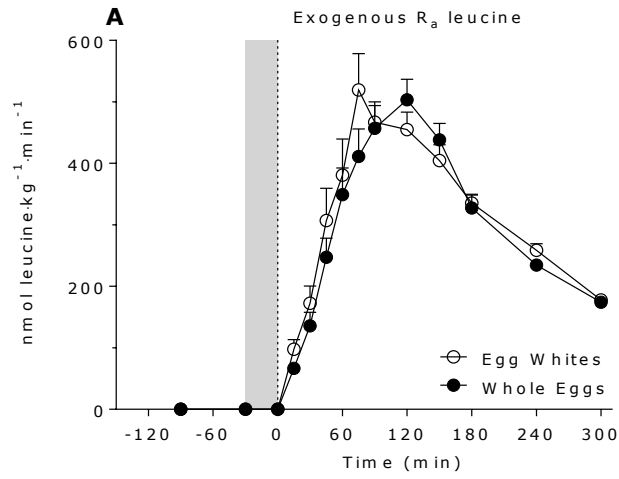


Figure 5. Plasma L-[5,5,5-<sup>2</sup>H<sub>3</sub>]leucine enrichment throughout study duration.

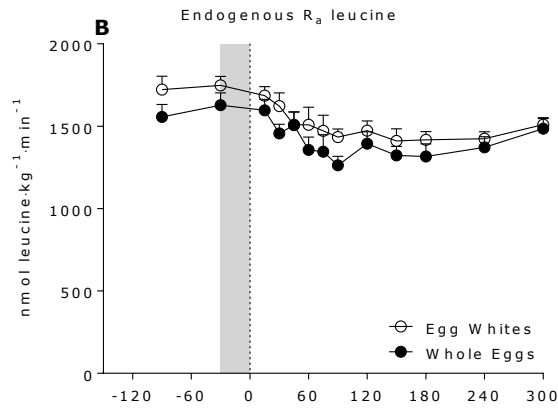


Figure 6. Basal plasma L-[1-<sup>13</sup>C]leucine (intravenous tracer) enrichments throughout study duration.

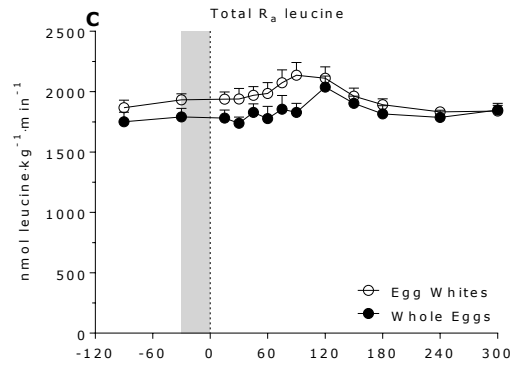


Figure 7. Plasma L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine (intravenous tracer) enrichments throughout study duration.

### Plasma amino acid kinetics

Exogenous leucine rates of appearance (representing the appearance of dietary protein-derived leucine into circulation) increased after the ingestion of whole eggs and egg whites (time effect:  $P < 0.001$ ) with no differences between groups ( $P = 0.17$ ). The total amount of protein derived leucine that became available in circulation over the 300 min postprandial period was similar ( $P = 0.53$ ) between whole egg ( $75 \pm 2.3\%$ ) and egg white ingestion ( $77 \pm 1.7\%$ ). Endogenous leucine rates of appearance (representing the appearance of leucine derived from whole-body protein breakdown into circulation) decreased after the ingestion of whole eggs and egg whites (time effect:  $P < 0.001$ ) with no differences between groups ( $P = 0.440$ ). These data indicate that both egg white and whole egg ingestion decreased whole body protein breakdown.

## **Conclusion**

The evidence from this research supports that egg ingestion, regardless of whole or egg white form, increases protein derived amino acid (i.e. leucine) appearance in circulation in healthy young males. The novelty of this research is the unique comparison of intact whole eggs vs. egg whites. The latter is a highly popular dietary manipulation among the weight lifting community when multiple eggs are consumed in order to limit energy intake. In order to make this dynamic contrast, we intrinsically labeled the eggs to examine the metabolic fate of dietary derived amino acids in two notably different macronutrient profiles (i.e. different fat content of meal). The findings from this research suggest that there is no differences in total amount of dietary derived amino acids that became available in circulation after consuming egg whites and whole eggs, respectively. These data indicate that consuming protein as part of nutrient dense whole food sources is a good strategy to drive adaptations in skeletal muscle mass.

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## CHAPTER 3: GENERAL DISCUSSION

### **Effect of meal composition on modulating the anabolic response to food ingestion**

The current thesis outlined a study that assessed the effect of consuming protein within its natural nutrient dense food matrix on modulating dietary protein digestion and amino acid absorption kinetics using an indirect model. In this thesis, we gained new information by using a novel approach of labeling egg protein with D3Leucine. By combining intrinsically labeled eggs with primed intravenous constant infusions of 1-<sup>13</sup>Cleucine, we were able to assess differences in dietary derived amino acid availability in peripheral circulation after the consumption of an isonitrogenous bolus consisting of whole eggs and egg whites. The present chapter will discuss any potential limitations of the study described in chapter 2. In addition, future directions for protein metabolism research in the area of sports nutrition will also be presented.

The current thesis outlined a study that assessed digestion and amino acid absorption of dietary derived amino acids, an important modulator of the muscle protein synthetic response, to the ingestion of isonitrogenous whole eggs and egg whites after resistance training. Although amino acid digestion and absorption kinetics appear to play an influential role in the anabolic response, exclusively focusing on this measure only serves as a proxy for downstream, arguably more relevant effects on muscle. Future investigation should aim to identify how differences in amino acid availability between competing sources impact muscle growth. Such research would require long-term

training studies ( $\geq 8$  weeks), with controlled dietary interventions using intrinsically labeled protein sources.

The evidence and the consensus of this thesis, as well as the introductory topical review suggests, the quality and quantity of protein, based largely on the amino acid profile ingested appears to be the most relevant factor required to initiate and sustain the adaptive response of skeletal muscle to resistance exercise, independent of its intact or isolated form. Multiple studies have demonstrated that despite diverging kinetics in the initial 2 h postprandial phase, when assessing the total impact over the 0 to 5-hour period, protein sources comparable in amino acid profile, have similar accumulated anabolic effects on skeletal muscle [12] [13, 14]. This indicates a wide range of whole food options to meet the protein demands essential to support the repair and remodeling process in response to resistance exercise.

### **Study limitations and future directions**

The relevance of these findings are particularly significant for the majority of people whom whole food predominates the way in which they consume dietary protein. Despite the exponential growth in supplement companies marketing forms of isolated protein powders [37], it is still unclear to whether these options meet essential micronutrient profiles that are inherent within the diverse consumption of whole food. The physiological significance of essential micronutrients demands the focus of new research in human health and sports nutrition. Several acute studies have indicated a superior anabolic response to the consumption of protein dense whole foods when compared to their isolated forms (i.e. whole milk compared to skim milk) [37] [60]. The observed differences

warrant a closer look into the mechanisms modulating this enhanced effect on skeletal muscle. The limitations of looking at acute markers of digestion and absorption or even muscle protein synthesis, is not necessarily predictive of long-term adaptations in muscle size or function. Long-term training studies are needed, using intrinsically labeled proteins in whole food in order to adequately assess to what extent, if any, competing protein sources differentially impact long-term phenotypic changes in skeletal muscle.

Some studies have demonstrated unique effects beyond protein accretion between otherwise equal protein sources (similar amino acid profile). With the growing obesity crisis and associated metabolic diseases, decreases in fat mass [33] and modulatory factors of gene regulation [16] have been central themes of recent investigation. Collectively, evaluating protein sources comparing a wide range of physiologic outcomes could provide a viable proxy for assessing nutrient quality. This insight could provide a comprehensive basis for dietary recommendations to the general public and athletes beyond the sole perspective of muscle growth.

In conclusion, although small differences were observed in the rate of amino acid availability between egg whites and whole eggs, removing the yolk does not demonstrate a superior anabolic response in muscle. In fact, some evidence exist that the co-ingestion of other macronutrients (in particular dietary fat) with protein may further enhance whole body or muscle anabolism, although the underlying mechanism remain unclear at present. Despite potential divergence in protein digestion and amino acid absorption kinetics, intact whole food comprises a potent stimulus in augmenting the adaptive response to resistance exercise, at least when a modest dose of protein is consumed in healthy young males. The consumption of a diverse set of protein dense whole foods to

adequately facilitate health and longevity should be considered when developing global dietary recommendations.

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