

HUMAN NUTRITION

Title: Ounce-Equivalents” in the Protein Foods Group: Benefits of Quality Protein, **NPB #17-001**

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Industry Summary:

Over the past 35 years the United States Department of Agriculture (USDA) Dietary Guidelines for Americans (DGAs) has sought to translate recommendations on nutrient requirements into practical nutritional advice for the American public. The most recent (2015) included specific advice to “vary your protein” and to rely less on meat as a source of protein. To help the consumer meet protein needs while achieving the goal of varied protein food sources, the DGAs Committee published “ounce equivalents” in the protein foods group. It is stated, among other equivalents cited, that 1 ounce (oz) of meat is equivalent to 1 tablespoon (Tbsp) of peanut butter or 1/2 cup (0.5 oz) of cooked kidney beans. This study investigated the metabolic response to the ounce-equivalents of these protein sources. Three groups of 8 subjects, balanced for gender, were randomly given 2 ounce-equivalents of various protein sources, including: 2 oz of pork loin, 4 oz of tofu, or 1 oz of mixed nuts. The response of muscle and whole-body protein was studied, with particular emphasis on determining body protein accretion. The gain of protein, metabolically called net balance, was greatest with pork. The primary mechanism responsible for a greater gain in body protein with pork intake was a significant decrease in the loss, or breakdown, of body protein. These results indicate that the ounce-equivalents of protein sources are not equivalent in terms of the amount or quality of protein, the caloric content, and most importantly, the resultant metabolic response of body protein. These findings strongly suggest that the recommendation of dietary protein intake based upon the “ounce equivalents” in the DGA guidelines requires revision.

Keywords:

Dietary Guidelines for Americans (DGA)
Protein Metabolism
Essential Amino Acids (EAA)
Ounce-Equivalents
Net Protein Balance
MyPlate

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Scientific Abstract:

Background: The USDA Dietary Guidelines for Americans (DGAs) Committee published “ounce equivalents” guidelines for the protein foods group, stating that 1 ounce of pork (meat) is equivalent to 2 oz of tofu or 1 oz of mixed nuts. The inequities of the “ounce equivalents” are glaring when considering both the caloric intake and the protein quality differences between animal and plant protein sources. The DGAs do not take this into account, nor the importance of the amount and profile of EAAs in individual proteins. The purpose of this study was to test the impact of differing amounts of protein in the ounce equivalents on the accumulation of body protein.

Objective: The objective of this project was to compare the gain in body protein, the principal benefit of protein nutrition, following the ingestion of ounce equivalents of pork loin, tofu, and mixed nuts.

Design: We measured the status of muscle and body protein before and after ingestion of the ounce equivalents of pork loin, tofu, and mixed nuts. We also measured the effects of these foods on blood EAA levels, since this is the primary basis for eating protein.

Results: Ingestion of pork resulted in a significant increase in peripheral EAA, and in turn, a greater net protein balance. Greater net protein balance with pork was the result of both increased protein synthesis and decreased protein breakdown. Tofu also resulted in a significant improvement in net balance that was greater than mixed nuts, but not as substantial as pork. Like pork, the improvement in net balance with tofu was due to an increase in protein synthesis, but a lesser decrease in protein breakdown. Plasma EAA concentrations were greatest with pork, followed by tofu and mixed nuts. There were no differences in muscle protein synthesis, most likely due to the small amount of protein intake.

Conclusion: The “ounce equivalents” proteins in the DGA guidelines were not equivalent in terms of metabolic responses or plasma EAAs concentrations.

Introduction

Over the past 35 years the United States Department of Agriculture (USDA) Dietary Guidelines for Americans (DGAs) has sought to translate recommendations on nutrient requirements (i.e., Recommended Dietary Allowances, RDAs) from the Food and Nutrition Board of the Institute of Medicine (IOM) into practical nutritional advice for the American public. In addition to the RDAs, the DGAs are intended to incorporate additional scientific evidence as it arises into the recommendations for a healthy diet. The recommendations of the DGA have impacted the dietary patterns in the United States, resulting in a shift from eggs, meat, and whole milk consumption towards greater consumption of grains, vegetable oils, vegetables and fruits [1]. These changes in dietary patterns are in accord with the recommendations of the DGAs [2]. The most recent (2015) DGAs include specific advice to “vary your protein” and to rely less on red meat as a source of protein. Whereas the final recommendations of the DGAs Committee still allow room for consumption of a small amount of meat as part of a varied diet, the sentiments of the Committee regarding the nutritional role of meat was clear in the accompanying scientific report. The argument against meat consumption was expanded from citing concerns not only related to nutritional factors, but also to factors peripheral to nutritional considerations, such as the environmental impact of the livestock industry.

The lack of appropriate focus on protein nutrition is a major shortcoming of the DGAs. Not only is the amount of protein not a major focus, absolutely no mention is made of protein quality. Protein quality refers to the amount, profile, and true ileal digestibility of the essential amino acids (EAAs) in the protein. The concept of protein quality is not new, as the Protein Digestible Corrected Amino Acid Score was published by the Food and Agriculture Organization of the World Health Organization in 1993. This scoring system was supplanted by the same organization in 2013 by the Digestible Indispensable Amino Acid Score (DIAAS) [3]. In general, animal proteins have much higher DIAASs than plant proteins, often by as much as two-fold. Account has not been taken of DIAAS scores, nor the general concept of the importance of the amount and profile of EAAs in individual proteins, in the formulation of MyPlate [4] or the scientific report of the DGAs Committee [2]. This is despite the fact that in the IOM report stating the RDA for protein refers to “high quality protein, a classification that does not apply to most plant proteins [5].

To help the consumer meet protein needs while achieving the goal of varied protein food sources, the DGAs Committee published “ounce equivalents” in the protein foods group. It is stated among other equivalents cited, that 1 ounce of pork is equivalent to 2 oz of tofu and 1 oz of mixed nuts [4]. But are they really equivalent? For example, one oz of pork loin contains approximately 14 g of protein and 59 kcal, 2 oz of tofu contains approximately 3g of protein and 40 kcal, and 1/2 oz mixed nuts contains 2.5 g of protein and 84 kcal [6]. The inequities of the “ounce equivalents” are even more glaring when protein quality as quantified by

the respective DIAAS scores of animal and plant protein sources are considered. For the examples provided, the DIAAS of pork is around 110, soy 92, and mixed nuts 40-50 [6].

Dietary protein intake serves many physiological roles, but the most prominent is the maintenance or gain of body protein. This is accomplished by stimulation of protein synthesis, the inhibition of protein breakdown, or a combination thereof. A net gain in protein balance (i.e., synthesis minus breakdown) defines an anabolic response, as opposed to a catabolic response caused by the rate of protein breakdown exceeding the rate of protein synthesis. An anabolic response usually refers to gain of muscle protein, but actually involves the entire body. Thus, the functional response to consumption of a given amount of a protein food source is best assessed by quantifying the rates of protein synthesis and breakdown at the whole-body level, as well as at the muscle level in order to ascertain the overall the anabolic response. In this study, we have made these measurements in response to the consumption of “equivalent” (according to MyPlate) amounts of pork loin, tofu, and mixed nuts.

We have tested the impact of differing amounts of protein in the ounce equivalents on the anabolic response. Our specific hypothesis was that ingestion of pork loin would induce a greater anabolic response, i.e., increase in the difference between protein synthesis and breakdown, than consumption of tofu or mixed nuts.

Objective(s): The objective of this project was to compare the gain in body protein following the ingestion of so-called ounce equivalents of pork, tofu, and mixed nuts. We hypothesized that the complete protein source, with higher EAA content, would result in a greater accretion of body protein.

Materials and Methods: We studied a total of 24 healthy male and females between 21 and 40 years of age (Table 1).

	Pork Loin	Tofu	Mixed Nuts
Subject number (Male/Female)	8 (4/4)	8 (4/4)	8 (4/4)
Age, yr	22.1 ± 1.0	25.9 ± 2.2	24.3 ± 2.1
Body weight (kg)	74.3 ± 3.5	75.9 ± 5.2	74.3 ± 5.3
Body mass index (kg/m ²)	24.5 ± 0.9	25.9 ± 1.0	24.9 ± 1.2
Lean body mass	51.8 ± 4.6	49.7 ± 4.2	49.3 ± 3.9
Fat mass, %	29.9 ± 3.9	33.0 ± 3.0	32.3 ± 2.2

Subjects were not participating in any organized exercise program, and did not perform any strenuous exercise 72 hours prior to the metabolic experiment. We used a treatment-randomized, two-period study: 4-hour basal fasted period followed by a 4-hour post-meal period (total 8-h time period). The principal end-point was the total anabolic response (whole body protein synthesis minus breakdown) over the 4 hours following a meal containing either 2 oz of pork loin, 4 oz of tofu, or 1 oz of mixed nuts. These amounts were chosen because we know from our previous work that 2 oz of meat would be sufficient to induce an anabolic response.

The inclusion and exclusion criteria are listed below

Inclusion Criteria:

- Men and women, ages 21 – 40 years in good health.

Exclusion Criteria:

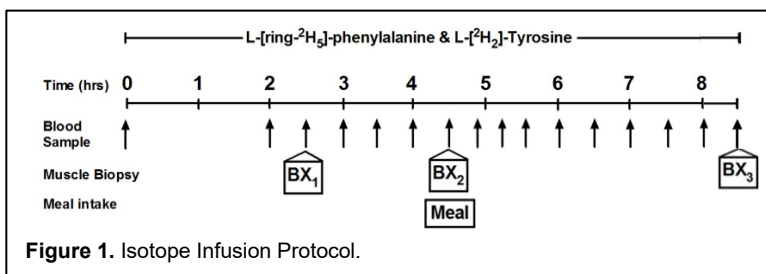
- History of diabetes
- History of malignancy in the 6 months prior to enrollment
- History of gastrointestinal bypass surgery (Lapband, etc.)
- History of a chronic inflammatory condition or disease (Lupus, HIV/AIDS, etc)
- Pregnant females
- Subjects who do not or will not eat animal proteins
- Subjects who cannot refrain from consuming protein or amino acid supplements during their participation in this study
- Subjects who report regular resistance exercise (more than once per week)
- Hemoglobin less than 9.5mg/dL at the screening visit
- Platelets less than 250,000 at the screening visit
- Concomitant use of corticosteroids (ingestion, injection or transdermal)

- Any other disease or condition that would place the subject at increased risk of harm if they were to participate, at the discretion of the study physician

Experimental Design. During the screening visit, dual-energy X-ray absorptiometry (DEXA) was performed to determine body composition. This enabled us to normalize whole-body protein kinetics in terms of lean body mass. Subjects were then randomly assigned by a study coordinator to one of 3 meal intervention groups: Pork Loin (Kroger, center cut pork loin), Tofu (Kroger, Simple Truth Organic), or Mixed Nuts (Planters unsalted mixed nuts). Subjects were instructed to abstain from strenuous physical activity for >72 h before the initiation of the metabolic study. Meals were provided in the 3-day run-in period before the metabolic study on day 4 [7]. All food was prepared by a study dietician in the Metabolic Kitchen at the RIOA. The 3-day run-in dietary control served as a dietary normalization period. Subjects obtained the 3-d meal allotments from the study coordinator and were also given a dietary record and point-and shoot digital camera. Subjects were asked to record time of meal consumption, percentage of meal consumption, and to photograph the meal prior to, and after, consumption. Subjects returned all unused or empty meal/supplement packaging and camera on the morning of the fourth day when reporting for the metabolic study. These data enabled the Research Dietician to ascertain caloric/protein intake as well as study compliance. Subjects who would achieve a minimum compliance of 80% consumption of meals progressed to the metabolic study (all subjects exceeded compliance cutoff). Caloric intake for the diet stabilization phase was based on the Harris Benedict equation for each subject, and protein intake was 1.2 g protein/kg/day.

Stable Isotope Tracer Infusion Protocol. The 8.5-h tracer infusion protocol is presented in **Figure 1**. On the fourth day, subjects reported to the overnight (from 2200 hrs) fast.

a venous catheter was inserted into one arm for tracer infusion, and in a vein of the contralateral arm for sampling utilizing the heated hand After obtaining a blood sample to background enrichments, infusion phenylalanine and L-[ring-²H₂]



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started and maintained throughout the study period. Priming doses of L-[ring-²H₅] phenylalanine and L-[ring-²H₂] tyrosine, as well as L-[ring-²H₄] tyrosine was given intravenously. Blood samples were drawn at specific intervals between 2.5 – 4.5 hours elapsed time to obtain values for determination of the basal rates of whole body protein synthesis and breakdown. After basal samples were obtained, subjects ate a meal containing one of the two food sources as described. Blood samples were taken at 0, 120, 150, 180, 210, 240, 270 min before consumption of a test food (the fasted blood samples) and at 290, 310, 330, 360, 390, 420, 450, 480, and 510 min (for fed blood samples) to measure tracer enrichment and plasma responses of essential amino acids. A total of 15 blood samples were taken during the study (approximately 100 ml).

Calculations of Protein Kinetics at Whole Body and Muscle Levels. Whole body protein kinetics were calculated based upon the determinations of the rate of appearance (R_a) into the plasma of phenylalanine and tyrosine, and the fractional R_a of endogenous tyrosine converted from phenylalanine, as we have previously described [7,8]. Briefly, the area under the curve (AUC) of plasma enrichments of phenylalanine and tyrosine tracers were calculated using Graphpad Prism 6 (Graphpad Software, La Jolla, CA). Whole body protein kinetics were calculated by dividing kinetic values of phenylalanine by its fractional contribution to protein (4%) [9]. For the calculations for whole body protein breakdown rate, the contribution of phenylalanine from the exogenous meal and the infused tracer were subtracted from total R_a . The following equations were used for the calculations of whole body protein kinetics:

- Total rate of appearance of tracer into plasma (R_a) = F / E
- Fractional R_a of Tyrosine from Phenylalanine (Fractional R_a of Tyr from Phe) = $E_{Tyr M+4} / E_{Phe M+5}$
- Rate of phenylalanine hydroxylation to tyrosine (Phe hydroxylation rate) = Fractional R_a of Tyr from Phe x $R_{a Tyr}$
- Protein synthesis rate = $[(R_{a Phe} - \text{Phe hydroxylation rate}) \times 25]$
- Protein breakdown rate = $[(R_{a Phe} - F_{Phe}) \times 25 - \text{PRO}]$
- Net protein balance (NB) = Protein synthesis rate (PS) - Protein breakdown rate (PB)

- Muscle protein fractional synthesis rate (FSR, %/h) = $[(E_{BP2} - E_{BP1}) / (E_m \times t)] \times 60 \times 100$

Enrichment (E) is expressed as tracer-to-tracee ratio (TTR) or mole percent excess (MPE), calculated as $TTR / (TTR + 1)$. TTR was used for calculations of rates of protein breakdown whereas MPE was used for calculation of rates of protein synthesis. E is enrichment of respective tracers. *F* is the tracer infusion rate into a venous site: F_{Phe} for phenylalanine tracer. $E_{Tyr\ M+4}$ and $E_{Phe\ M+5}$ are plasma enrichments of tyrosine tracer at M+4 and of phenylalanine tracer at M+5 relative to M+0, respectively. The correction factor of 25 is for conversion of phenylalanine kinetics to protein based on the assumption that contribution of phenylalanine to protein is 4% ($100/4 = 25$) [9]. PRO is the amount of exogenous amino acids appearing in the circulation as a result of the exogenous protein digestion, accounting for splanchnic extraction (29%) of amino acids in young adults [10]. Phe hydroxylation rate is the rate of appearance of tyrosine derived from phenylalanine through process of hydroxylation.

The calculation of the rate of protein breakdown is central to quantifying endogenous protein kinetics. This calculation requires knowledge of the contribution of the Phe from ingested protein to the observed plasma kinetics. Different approaches have been used for this calculation, with differing results for protein breakdown being obtained. For that reason, we undertook a careful analysis of all approaches to this problem, particularly including an assessment of the use of intrinsically-labeled protein as the preferred method to quantify exogenous Ra. This analysis resulted in an extensive manuscript that has been submitted for publication. A copy of this manuscript will be submitted to ENC. It was on the basis of this analysis that we elected to use the “bioavailability” approach to quantify exogenous Ra.

Analytic Methods. Plasma samples were processed as previously described for determination of enrichment by gas chromatography-mass spectrometry (GCMS: Models 7890A/5975; Agilent Technologies, Santa Clara CA) [7,8]. Briefly, 125 μ l of 10% sulfosalicylic acid was added to plasma samples to precipitate protein. Plasma free amino acids were then extracted from 300 μ l supernatant fluid by cation exchange chromatography (Strata-X-C; Phenomenex, Torrance, CA) and dried under Speed Vac (Savant Instruments, Farmingdale, NY). Enrichments of phenylalanine and tyrosine were measured on the *tert*-butyldimethylsilyl derivative with the use of GCMS. Ions of mass to charge ratios of 234, 235, and 239 for phenylalanine and of 466, 467, 468, and 470 for tyrosine will be monitored with electron impact ionization and selected ion monitoring. Plasma AA concentrations were determined by using liquid chromatography-mass spectrometry (QTrap 5500 MS; AB Sciex) using internal standard method [7,11].

Statistics. One-way repeated-measures of ANOVA were used to compare differences in protein kinetics (NB, PS, and PB), FSR, and area under the curve (AUC) of plasma EAAs concentrations. The variables including concentrations and sampling time in plasma EAAs were determined by using two-way repeated-measures of ANOVA. If there were significant main effects or interactions, a two-tailed student's t-test was performed for specific comparisons. $P < 0.05$ was considered to be statistically significant.

Results:

Whole body protein synthesis, breakdown, and net balance (**Figure 2**) are presented as g protein throughout the measured 4-hour period following intake. Pork ingestion resulted in a greater NB than tofu or nuts. There was a significant increase in PS with both pork and tofu; however, the greater NB with pork ingestion was due primarily to the greater decrease in PB. Tofu (soy) having the highest DIAAS score among plant proteins

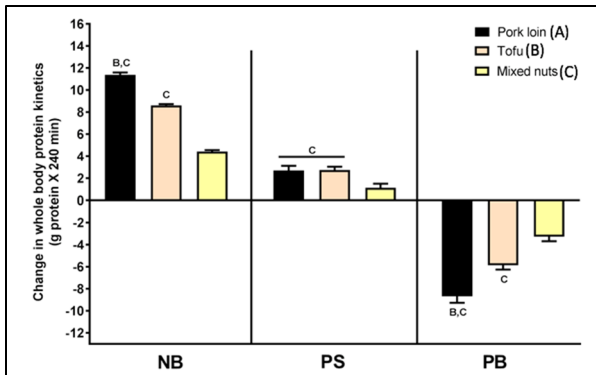


Figure 2. Whole body net balance (NB), protein synthesis (PS), and breakdown (PB) before and after the ounce equivalents meal intake with pork, tofu, or mixed nuts. Values are expressed mean \pm SEM. Letters represent group differences.

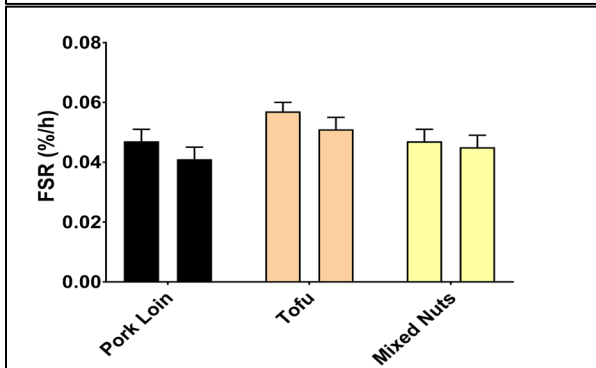


Figure 3. Muscle protein fractional synthetic rate (FSR) before and after the ounce equivalents meal intake with pork, tofu, or mixed nuts. Values are expressed mean \pm SEM. There were no significant treatment effects.

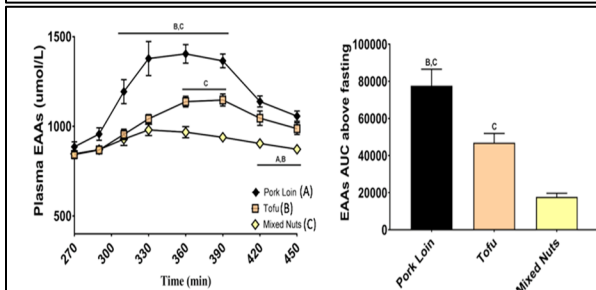


Figure 4. Plasma essential amino acids (EAAs) peripheral response and area under the curve (AUC) calculation following the ounce equivalents meal intake with pork, tofu, or mixed nuts. Values are expressed mean \pm SEM. Letters represent group differences.

resulted in improved EAA concentrations that were greater than nuts, but less than pork (Figure 4). The modest increases in whole-body PS with pork and tofu appears to be attributable to proteins other than muscle, as muscle protein synthesis was not significantly elevated with either treatment (Figure 3). The absence of muscle effect is most likely due to the small serving size of protein, as we have shown effects with twice the protein intake [7]. Pork intake exhibited higher peak plasma EAAs concentrations than tofu or nuts, as well as a greater integrated area under the curve (AUC; $P < 0.05$, Figure 4). Plasma EAA response was greater with pork intake versus tofu for 2.5 hrs following the meal intake ($P < 0.05$; Figure 4). The magnitude of the peripheral EAA increase is consistent with the absence of response of muscle protein synthesis, as our experience indicates that peripheral EAA concentrations must increase more than 50% above basal levels to stimulate an increase in muscle protein synthesis. In summary, the inclusion of these 3 foods provided for the investigation of a wide range of DIAAS scores and the resultant effects on protein accretion. These findings are consistent with the prevailing wisdom that higher DIAAS proteins result in greater protein accrual.

We are in the process of reviewing these findings in a combined manuscript (with all commodity groups) that will be submitted to NPB in the future.

Discussion: The results of this study indicate that the “ounce equivalents” as expressed in the DGA guidelines are in fact, not equivalent in their ability to affect body protein balance. They are not equivalent in terms of the amount of protein provided, the quality of the proteins in terms of DIAAS, nor the caloric content. From a functional standpoint, the anabolic response to the ounce equivalents also differed: the anabolic response (NB) to pork was significantly greater than the anabolic response to tofu and peanut butter (Figure 2). The greater net protein balance is the result of a significant increase in plasma EAAs concentrations, a main stimulus and regulator of anabolic response (Figure 4). The concept of ounce equivalents was presumably for the purpose of facilitating the consumption of varied protein sources in the context of a complete diet plan. However, our findings indicate that the recommendation of dietary protein based on the ounce equivalents in the DGA guidelines needs to be

reconsidered.

The recommendations of the DGA have impacted the dietary patterns in the United States, as reflected by changes in food consumption. The consumption of the following nutrients has increased between 1970 and 2005: grains (41%); vegetable oils (91%); fish and shell fish (37%); vegetables (23%); and fruits (13%). Over the same time interval the consumption of eggs and red meat has declined 17% and whole milk consumption has decreased by 73% [1]. This change in dietary patterns is in accord with the recommendations of the DGAs [2]. The most recent (2015) DGAs have continued along the same path of recommendations, including specific advice to “vary your protein” and to rely less on animal proteins. Whereas the final recommendations of the DGAs Committee still leave room for consumption of a small amount of animal protein as part of a varied diet, the sentiments of the Committee regarding the nutritional role of animal protein was clear. The argument against animal protein consumption was expanded to factors peripheral to nutritional considerations such as

the environmental impact of producing animal proteins. Clearly there is a direct assault from various interests on the consumption of animal proteins.

Over the same time interval in which the Dietary Guidelines have existed the occurrence of nutrition-related health problems in the United States has escalated dramatically. Most prominently, obesity has become an epidemic. In the decade before the DGAs were issued, obesity (body mass index greater than 30) increased 1.7% [12]. Between 1980, when the first DGAs were issued, and 2010, obesity increased more than 7% per decade, to a total of 35.8% [12]. Clearly the increased incidence of obesity and related health issues over the past 40 years cannot be attributed solely to the DGAs, but the changes in dietary patterns cited above that are consistent with the recommendations of the Dietary Guidelines have not prevented the development of an epidemic of obesity and nutrition-related health issues.

The problem faced by advocates for higher rates of protein consumption, including animal sources, as an important component of a healthy diet is that there are limited data upon which to base a convincing argument. Prospective studies are needed in which the responses to different levels of animal protein intake as part of an overall increase in protein intake are documented. To be convincing, such studies would have to be tightly controlled with other variables maintained constant. Further, the duration of dietary control and subject numbers would have to be sufficiently powered to assess health outcomes. The results of the current study provide a concrete basis on which to design an outcome study to assess the impact of protein quality on functional parameters.

In summary, the “ounce equivalents” proteins in the DGA guidelines were not equivalent in terms of functional responses and plasma EAAs concentration. The animal-based protein, pork, exhibited a greater whole-body net protein balance, a greater reduction in protein breakdown, and a greater increase in plasma EAA concentrations than tofu or mixed nuts. The comparison of protein quality is complicated by the potential role of the non-protein components (i.e., carbohydrate) of the protein food sources. These findings strongly suggest that the recommendation of dietary protein intake based upon the “ounce equivalents” in the DGA guidelines requires revision.

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