

## Higher Net Protein Balance following the Ingestion of Free Range Reindeer compared to Commercial Beef

Melynda S. Coker<sup>1</sup>, Kaylee R Ladd<sup>2</sup>, Scott E Schutzler<sup>4</sup>, Sanghee Park<sup>4</sup>, Rick H. Williams<sup>4</sup>, Nicolaas EP Deutz<sup>5</sup>, Robert R. Wolfe<sup>4</sup>, Robert H. Coker<sup>2,3</sup>

Department of Natural Resources and Environment<sup>1</sup>, Department of Biology and Wildlife<sup>2</sup>, Institute of Arctic Biology<sup>3</sup>, University of Alaska Fairbanks, Fairbanks, AK; Reynolds Institute on Aging<sup>4</sup>, University of Arkansas for Medical Sciences, Little Rock, AR; Department of Health and Kinesiology<sup>5</sup>, Texas A and M University, College Station, TX

Running Title: Red meat and protein metabolism

Funding: Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health (NIH) under award numbers UL1GM118991, TL4GM118992, or RL5GM118990 and an Institutional Development Award under grant number P20GM130443. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. University of Alaska Fairbanks, University of Arkansas for Medical Sciences, and Texas A and M University are affirmative action / equal employment opportunity employers and educational institutions.

Acknowledgements: We wish to thank graduate committee members, Drs. Todd Brinkman, Josh Greenberg, and Barbara Taylor for their guidance and direction on this project. We also recognize Tiffany Aguilar for processing of blood samples and Gwendolyn Quigley for her organization of references. Lastly, we would like to express our sincere appreciation to our participants from the Fairbanks, Alaska area that volunteered their time and effort for this study.

Word count: 2940

Address for Correspondence:

Robert H. Coker, PhD, FACSM, FTOS  
Institute of Arctic Biology  
University of Alaska Fairbanks  
2140 Koyukuk Drive  
PO Box 757000  
Fairbanks, AK 99775-7000  
(907) 474-6701  
[rcoker@alaska.edu](mailto:rcoker@alaska.edu)

## Abstract

Wild game consumption has been associated with health benefits, but the influence on protein metabolism remains unknown. We compared the feeding-induced response to free-range reindeer (FR) versus commercial beef (CB) using stable isotope methodology. Seven male and female participants (age:  $38 \pm 12$  years; body mass index:  $24 \pm 3$  kg/m<sup>2</sup>) completed two studies using a randomized, crossover design in which they ingested 2 oz of FR or CB. L-[ring <sup>2</sup>H<sub>5</sub>]phenylalanine & L-[ring <sup>2</sup>H<sub>2</sub>]tyrosine were delivered via primed, continuous intravenous infusion. Blood samples were collected during the basal period and following consumption of FR or CB. Feeding-induced changes in whole body protein synthesis (PS), protein breakdown (PB), and net protein balance (NB) were determined via analysis of plasma samples for phenylalanine and tyrosine enrichment by gas chromatography mass spectrometry; plasma essential amino acid concentrations were determined by liquid chromatography-electrospray ionization-mass spectrometry. Plasma post-prandial essential amino acid (EAA) concentrations were higher with the ingestion of FR compared to CB ( $P=0.02$ ). The acute feeding-induced response in PS was not different in either trial, but PB was reduced with the ingestion of FR compared to CB ( $P<0.0001$ ). The difference in PB contributed to a superior level of NB ( $P<0.0001$ ). When protein kinetics were normalized relative to the amino acids ingested, PB/EAA and total amino acids ingested were reduced ( $P<0.01$  and  $0.001$ , respectively) in FR compared to CB; contributing to greater NB/total amino acid ingested ( $P<0.0001$ ) between FR and CB. We conclude that the nutrient profiles of FR may have a more favorable benefit on protein metabolism

compared to CB. These data support the potential health benefits of wild game in the preservation of whole-body protein.

## Introduction

Alaska's aging indigenous people are growing in number, and their functional disabilities are disproportionately greater than those experienced by other populations<sup>1,2,3</sup>. In Alaska, the factors responsible for increased functional disabilities are complex due to limited economic competition, higher profit margins, expensive medical infrastructure, and unique environmental and socio-demographic elements<sup>4</sup>. Such inherent difficulties already contribute to the most extreme healthcare costs in the United States, 2.5 times the national average<sup>5</sup>. Instead of addressing these challenges after individuals reach the older stages of life, healthy aging strategies throughout the lifespan are necessary to counteract the risks of sarcopenia, disability, and rising healthcare costs<sup>6</sup>.

Sarcopenia is defined as an age-related decline in muscle mass (reflected by loss of lean tissue), strength, and function<sup>7</sup>. In order to maintain a healthy state of dynamic equilibrium with regard to lean tissue, rates of whole body protein synthesis (PS) and protein breakdown (PB) are modulated by dietary intake and/or physical activity. For PS to occur in response to nutrient ingestion, essential amino acids (EAA's) must be available in sufficient amounts<sup>8</sup>. Consumption of proteins providing EAA's is therefore necessary for PS to exceed PB, and primarily indicates the net balance of muscle protein<sup>9</sup>.

The quality of a dietary protein is reflected by the digestible indispensable amino acid score (DIAAS), and is a function of the amount and profile of EAAs as well as

digestibility of that protein<sup>11</sup>. Much research has been done concerning protein quality and quantity<sup>10</sup>, but practical questions remain concerning the potential for greater benefits relative to serving in wild game. Free range cervids contain a more desirable balance of  $\omega$ -6: $\omega$ -3 fatty acid ratios<sup>12</sup>. Relevant to protein metabolism, amino acid-enriched medical formulas fortified with a balanced provision of omega-6 ( $\omega$ -6) to omega-3 ( $\omega$ -3) fatty acid ratios have demonstrated a favorable influence on protein metabolism<sup>13</sup>.

To our knowledge, the acute influence of the same amount of wild game meat compared commercial meat ingestion on protein kinetics in humans has not been evaluated. We have investigated the acute response of human protein metabolism to consumption of 2 ounces of FR and commercial beef (CB).. FR is similar to the caribou found in Alaska Native traditional diets<sup>14</sup>, and is USDA or state-approved for consumption. We chose 70% lean/30% fat CB for comparison, as it is the most-purchased meat in the US by millions of pounds<sup>15</sup>. We hypothesized that NB would be higher in FR compared to CB; due to existing differences in the total amount of protein in FR. If our data support the superior efficacy of FR consumption per ounce on NB, it is our assertion that traditional amino and fatty acid sources such as wild red meats may augment the retention of whole body protein largely through existing differences in total protein intake/serving size.

## **Materials and Methods**

Eight male and female participants were recruited from the Fairbanks, Alaska area through a combination of local newspaper advertisements and flyers posted

around the University of Alaska Fairbanks (UAF) campus. All testing occurred at the Clinical Research and Imaging Facility (CRIF) except baseline blood sampling for medical screening performed at Labcorp, Inc (1626 30th Ave, Fairbanks, AK 99701). All participants were properly consented, which included a comprehensive verbal and written review of all procedures, and copies of the consent forms were provided. Participants completed clinical assessments to determine eligibility during the initial consent and screening visit. Exclusion criteria included a creatinine level of  $>1.4$  and/or a serum glutamate pyruvate transaminase  $>2$  times normal; a resting blood pressure above 160/90 mmHg; previously diagnosed diabetes (fasting blood glucose  $\geq 126$  mg/dl; history of kidney or liver disease; heart disease as indicated by interventional procedures; recent history of alcoholism; and/or active cancer. Volunteers with a chronic inflammatory condition or taking corticosteroids were not eligible. Volunteers were excluded from the study if the study physician recognized a medical condition or medication that in his opinion represented an unacceptable risk. Participant confidentiality was ensured for all participants. Protected records were kept in a locked file cabinet behind a locked door in a facility with restricted swipe card access. The study was reviewed and approved by the UAF Institutional Review Board (IRB) under protocol 986801-17.

One participant dropped out due to circumstances unrelated to the study. Eligible participants visited the CRIF at UAF on three separate occasions: a) consent and determination of eligibility status, b) feeding/ tracer study #1, and c) feeding/tracer study #2. The feeding/tracer studies were performed in a randomized, double blind fashion. Using stable isotope methodology on participant visits #1 and #2, the response

to the ingestion of equivalent 2 ounce pre-cooked servings of randomly assigned FR or 70% lean/30% fat CB was evaluated. These amounts are consistent with the variable portion sizes listed in the Dietary Guidelines 2015-2020<sup>16</sup>. The nutritional profiles for reindeer and beef are listed in Table 1. A dual-energy X-ray absorptiometry (GE iDXA) scan for the determination of body composition was also performed.

<b>Table 1. Nutritional Composition</b>	<b>Free-Range Reindeer</b>	<b>Commercial Beef</b>	<b>Difference (Reindeer – Beef)</b>
<i>Essential Amino Acids (mg)</i>			
Leucine	1376	998	+378
Isoleucine	754	576	+178
Valine	784	632	+152
Threonine	712	476	+236
Methionine	372	314	+58
Phenylalanine	742	514	+228
Histidine	660	394	+266
Tryptophan	256	44	+212
Lysine	1510	1048	+462
Sub Total	7166	4996	+2170
<i>Non-Essential Amino Acids (mg)</i>			
Cysteine	120	124	-4
Tyrosine	546	376	+170
Arginine	992	880	+112
Alanine	892	394	+498
Aspartic Acid	1474	856	+618
Glutamic Acid	2616	1148	+1468
Glycine	700	1882	-1182
Proline	518	1058	-540
Serine	586	766	-180
Sub Total	7858	6718	+1140
<i>Polyunsaturated Fatty Acids (mg)</i>			
Linoleic Acid	174	110	+64
Arachidonic Acid	101	22	+79
Gamma Linoleic Acid	0	6	-6
Docosahexaenoic Acid	34	0	+34
Eicosapentaenoic Acid	17	0	+17
Sub Total	325	138	+187
<i>Monounsaturated Fatty Acids (mg)</i>			
Gadoleic Acid	0	35	-35
Oleic Acid	684	3866	-2882
Myristoleic Acid	0	78	-78
Palmitoleic Acid	62	342	-280
Sub Total	746	4021	-3275
<i>Saturated Fat</i>			

Arachidic Acid	0	6	-6
Myristic Acid	67	274	-207
Palmitic Acid	498	1984	-1486
Stearic Acid	330	568	-238
Margaric Acid	0	104	-104
Pentadecanoic Acid	0	43	-43
Lauric Acid	0	7	-7
Subtotal	895	2987	-2092
Percent PUFA/Total Fat	10.7	1.2	+9.5
Percent MONO/Total Fat	24.6	36.1	-11.5
Percent SAT/Total Fat	29.5	26.8	+2.7
Total Fat	3036	11150	-8114

### Experimental protocol

The isotope infusion/feeding studies were completed on two occasions at least three days apart and after an overnight fast beginning at 2200 hr<sup>13</sup>. To achieve simultaneous infusion/sampling, polyethylene catheters were placed into each forearm; one for the infusion of stable isotope tracers and the other for obtaining “arterialized” blood sampling via the heated hand technique<sup>17</sup>. Prior to the initiation of the tracer infusion, a baseline blood sample was collected to determine background isotopic enrichments. Primed continuous infusions of I-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine (prime, 5.04 μmol/kg body weight; rate, 5.04 μmol·kg<sup>-1</sup>·h<sup>-1</sup>) and I-[ring-<sup>2</sup>H<sub>2</sub>]tyrosine (prime, 2.16 μmol/kg; rate, 1.995 μmol·kg<sup>-1</sup>·h<sup>-1</sup>) were performed to determine rates of PS, PB, and net protein balance (NB) at the whole body level<sup>13</sup>. All isotope tracers were purchased from Cambridge Isotope Laboratories (Andover, MA). To measure tracer enrichment and plasma responses of AAs, blood samples were taken at 0, 60, and 180 min to provide the fasted blood samples. Consumption of FR and CB occurred at 180 minutes, and additional blood samples followed at t=210, 240, 300, 330, 360, and 420 min. A total of 12 blood samples were drawn during the study.

The rate of appearance ( $R_a$ ) of phenylalanine and tyrosine into the plasma and the rate of phenylalanine hydroxylation to tyrosine were determined from the tracer enrichments in plasma. The area under the curve (AUC) for plasma enrichments of phenylalanine and tyrosine tracers following ingestion of meat was calculated using Graphpad Prism 5 for Mac (Graphpad Software, La Jolla, CA). The AUC was used in the calculation of protein kinetics in the fed state to minimize the impact of non-steady state tracer enrichments. Whole body protein kinetics were calculated as described previously<sup>18</sup>. Account was taken of the contribution of phenylalanine from the exogenous meal to the total  $R_a$  of phenylalanine. The amount of phenylalanine in the ingested meat was multiplied by the digestibility, and that quantity was then multiplied by (1- fraction hydroxylation of phenylalanine in the liver). To calculate endogenous  $R_a$  of phenylalanine, the exogenous appearance of phenylalanine into the peripheral circulation was subtracted from the total measured  $R_a$  of phenylalanine. The following equations were used for the calculations of whole body protein kinetics:

1. Total rate of appearance into plasma ( $R_a$ ) =  $F/E$
2. Fractional  $R_a$  of Tyr from Phe =  $E_{Tyr M+4}/E_{Phe M+5}$
3. Phe hydroxylation rate = fractional  $R_a$  of Tyr from Phe x  $R_a$  Tyr
4. Protein synthesis rate (PS) = [(Total  $R_a$  Phe – Phe hydroxylation rate) x 25]
5. Protein breakdown rate (PB) = [Endogenous ( $R_a$  Phe –  $F_{Phe}$ ) x 25 – PRO]
6. Net protein balance = PS – PB

Enrichment (E) was expressed as the tracer-to-tracee ratio (TTR), or mole percent excess (MPE), and calculated as  $TTR/(TTR + 1)$ . TTR was used for calculations of NB, whereas MPE was used for calculations of PS. E is the enrichment of respective



tracers.  $F$  is the tracer infusion rate into an intravenous infusion site:  $F_{\text{Phe}}$  for phenylalanine tracer.  $E_{\text{Tyr M+4}}$  and  $E_{\text{Phe M+5}}$  were the plasma enrichments of tyrosine tracers at  $M + 4$  and  $M + 5$  relative to  $M + 0$ , where  $M$  represents mass, respectively. Phe hydroxylation rate was the rate of appearance of tyrosine derived from phenylalanine through the process of hydroxylation<sup>18</sup>. The correction of 25 was utilized for the conversion value for phenylalanine to protein based on the assumption that the contribution of phenylalanine to protein was 4%<sup>19</sup>.

**Analytical methods.** Plasma samples were deproteinized using dry sulfosalicylic acid, and frozen at  $-80^{\circ}\text{C}$  until analysis. Enrichment analysis was performed by gas chromatography-mass spectrometry (Agilent 5975)<sup>9</sup>. Ions of mass to charge ratios of 234, 235, and 239 for phenylalanine and 466, 467, 468, and 470 for tyrosine were utilized. Plasma amino acid analysis was performed by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) (QTrap 5500 MS; AB Sciex) with ExpressHT Ultra LC (Eksigent Div.; AB Sciex) after derivatization with 9-fluoren-9-methoxycarbonyl, and concentrations were measured using the internal standard method<sup>20</sup>.

**Statistical Methods.** A one-way ANOVA with repeated measures was used to examine overall differences in plasma essential amino acid concentrations between FR and CB; a Bonferroni post-test was used to detect potential differences in the plasma concentrations of essential amino acids at individual time points. A two-way ANOVA was utilized to evaluate differences in PS, PB, and NB in response to the ingestion of FR or CB. All data were analyzed using the Graphpad Prism 6 for Mac (Graphpad Software, Inc. La Jolla CA), and presented as mean  $\pm$  SD.

## Results

Eight participants ( $40.4 \pm 14.0$  years of age) with a body mass index (BMI) of  $24.0 \pm 2.9$  kg/m<sup>2</sup> were recruited and enrolled in the study (Table 2). All participants provided their own transportation to the CRIF at UAF. One participant dropped out due to a personal matter completely unrelated to the study. Therefore, seven participants completed all aspects of the study; reflected in Table 2.

<b>Table 2. Clinical Characteristics</b>	
M/F	2/5
Age (years)	$40.4 \pm 14.0$
Weight (kg)	$67.5 \pm 15.8$
Height (m)	$1.7 \pm 0.1$
BMI (kg/m <sup>2</sup> )	$24.0 \pm 2.9$
Fat Mass (kg)	$19.0 \pm 4.4$
Fat Free Mass (kg)	$47.5 \pm 11.2$

Plasma concentrations of essential amino acids were higher with FR compared to CB at  $t=240$  and  $270$  min (Figure 1). The EAA and branched chain amino acids area under the curve (AUC) during the fed state were also greater following FR consumption ( $P=0.009$  and  $P=0.04$ , respectively), but there were no differences in leucine AUC (data not shown). Basal post-absorptive rates of PS, PB, and NB were not different in FR and CB. As previously described in feeding studies using identical stable isotope methodologies<sup>9,13</sup>, we have calculated and expressed PS, PB, and NB relative to their changes from the fasted to the fed state. While there was a similar increase in PS after FR and CB ingestion, the heightened suppression of PB ( $P<0.0001$ ) contributed to a substantially greater enhancement of NB after ingestion of FR ( $P<0.0001$ ) compared to CB (Figure 2). We also calculated the rates of PS, PB, and NB relative to the ingested amounts of EAAs and total amino acids. There was a greater reduction in PB/EAA<sub>s</sub> ingested in FR compared to CB ( $P<0.01$ ), and there were no significant differences

between PS and NB when expressed relative to the amount of EAAs ingested (Figure 2). PB, with respect to the total amino acids, was suppressed significantly greater after consumption of FR compared to CB ( $P<0.001$ ); contributing to higher NB/total amino acids ingested with FR as well ( $P<0.0001$ ) (Figure 2).

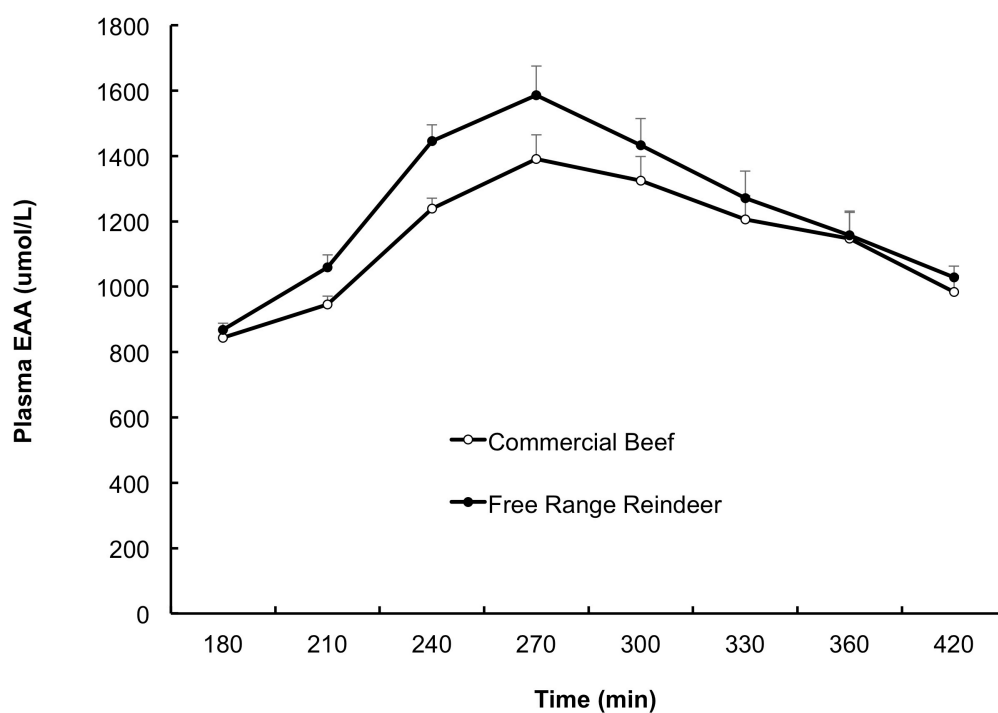


Figure 1. Plasma essential amino acid (EAA) concentrations during the fasted ( $t=180$  min) and fed states ( $t=210-420$  min). There was a significant difference ( $P=0.082$ ) in the overall concentration based on the type of meat ingested but no significant differences between the individual time points.

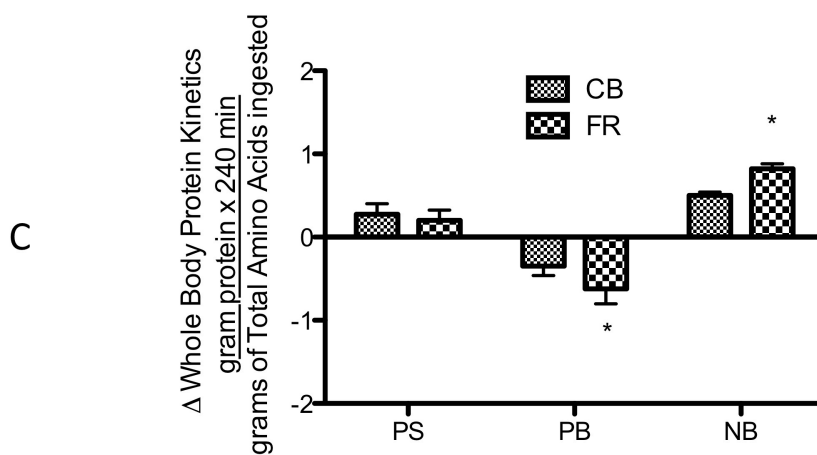
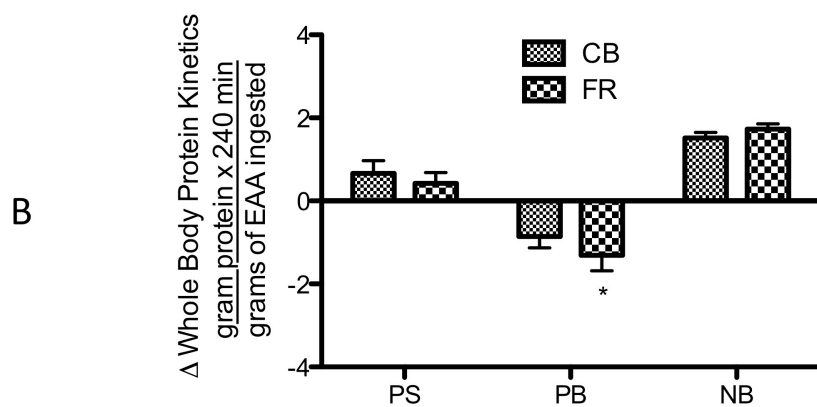
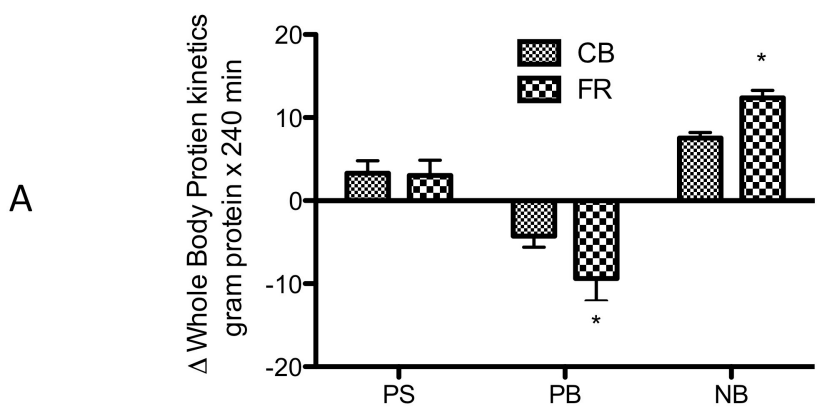


Figure 2. Whole body protein kinetics: A) changes in rates of whole body protein synthesis (PS), breakdown (PB), and net balance (NB) from the fasted state; B) changes in rates of whole body protein synthesis (PS), breakdown (PB), and net balance (NB)/EAA from the fasted state; and C) changes in rates of whole body protein synthesis (PS), breakdown (PB), and net balance (NB)/Total Amino Acids from the fasted state. \*Denotes significant difference between FR and CB ( $P<0.05$ ).

## Discussion

In this study, we examined the responses of whole body protein kinetics to the dietary ingestion of 2 ounces of FR and CB on whole body protein kinetics in humans. We demonstrated significantly greater increases in plasma EAAs after consumption of FR compared to CB. Despite these differences in plasma concentrations of EAAs, the PS response to FR was not greater than that to CB. Increased suppression of PB contributed to superior differences in NB after ingestion of FR compared to CB. The profile of amino acid composition was apparently linked to greater whole body protein retention, as the NB after FR consumption was greater than CB even when normalized for the amount of total amino acids ingested. To our knowledge, these data represent the first acute feeding studies to illustrate the superior benefits of free-range red meat on direct changes in protein metabolism.

Modulation of PS occurs in conjunction with alterations in PB during fasting and fed conditions and/or physical activity<sup>21</sup>. For the most part, changes in PS and PB reflect feeding or activity-based changes in muscle protein synthesis and breakdown<sup>21</sup>. Consumption of nutrients providing EAA's is necessary in order to maintain optimal

NB<sup>22</sup>. Differences in the total amount of amino acids ingested were linked to higher plasma EAA concentrations following ingestion of FR, and may have contributed to the suppression of PB<sup>9,13</sup>. It is particularly interesting that in another recent study, the suppression of PB was even greater in response to 4 ounces and 11 ounces of red meat ingested, respectively<sup>23</sup>. Therefore, the influence of the total amount of amino acids ingested seems to remain consistent across the dose response; supporting the rationale behind the importance of the amount of dietary protein in the modulation of NB<sup>9</sup>.

It is well accepted that EAAs are largely responsible for the stimulation of muscle protein synthesis<sup>24</sup>. Non-essential amino acids are not primary factors in the stimulation of muscle protein synthesis<sup>8</sup>. That being said, significant correlations have been reported between overall protein intake and NB ( $R^2=0.765$ ;  $P<0.0001$ )<sup>25</sup>. This correlation indicates the potential importance of non-essential amino acids and overall amino acid intake on the suppression of PB. This assertion is further supported by the consensus that the delivery of 0.24 g of amino acids/kg/meal in 2 oz of FR equates to the maximal response of PS relative to the overall influence on NB<sup>26</sup>. While isolating the individual contributions of each non-essential amino acid is beyond the scope of the current investigation, the ingestion of arginine, glutamine, glutamate, proline, and glycine have all been implicated in the preservation of lean tissue<sup>27</sup>. Recommendations for amino acid take (essential and non-essential) should consider variations in growth rates, reproductive status, and aging,<sup>28</sup> and that the “indispensable” characterization of non-essential amino acids may represent an oversimplification<sup>29</sup>.

In addition to the importance of amino acids in the modulation of protein kinetics, the beneficial influence of  $\omega$ -3 fatty acids on protein metabolism has also gained some attention<sup>30,31</sup>. Many of the benefits of  $\omega$ -3 fatty acids are derived from potential improvements in the sensitivity of mTOR and downstream signaling targets that positively affect muscle protein synthesis<sup>32</sup>. In our study, there were no differences in PS between FR and CB. Even though the combined  $\omega$ -3 fatty acids eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) was just 51 mg from FR, CB provided neither one. The relatively small amount of  $\omega$ -3 fatty acids may not have produced a sufficient stimulus to augment PS. Nonetheless, substantial evidence suggests that EPA may mitigate protein degradation<sup>33</sup>, potentially through its inhibitory influence on the NF- $\kappa$ B pathway<sup>34</sup>. DHA has also been demonstrated to reduce protein degradation through the PPAR $\gamma$ /NF- $\kappa$ B pathway<sup>34</sup>. Data from these mechanistically-oriented studies corresponds with favorable findings from clinical studies that support the role of  $\omega$ -3 fatty acids on maintenance of lean tissue mass and physical function<sup>35,36</sup>. Therefore, differences in the amount of DHA and EPA may have contributed to the differences in NB between FR and CB.

The interactive combination of amino acids and  $\omega$ -3 fatty acids is particularly interesting given that the consumption of  $\omega$ -6 to  $\omega$ -3 fatty acids has progressively increased from the 1:1 ratio of our hunter/gatherer ancestors to 20:1 or more in our modern, Westernized diets<sup>37-38</sup>. Alterations in  $\omega$ -6 to  $\omega$ -3 fatty acid ratios have increased in conjunction with the widespread availability of highly processed foods, and comingled with a reduced consumption of wild and natural proteins with higher overall protein quality. Transformations in these nutritive proportions coincide with gradual and

deleterious declines in metabolic health, as progressive elevations in  $\omega$ -6 to  $\omega$ -3 fatty acid ratios have been linked to increases in obesity and other chronic illnesses<sup>39</sup>. The overall level of DHA and EPA in FR is still relatively in the context of the current study but may become relevant over time.

While many studies have compared the effect of various isolated amino acids profiles on muscle protein synthesis, we are not aware of any studies comparing intact proteins found in wild game, to beef generated from feed-lots. We have demonstrated that the acute consumption of FR elicited greater increases in the plasma concentrations of EAA's compared to CB. We have also demonstrated the superior benefits of ingestion of FR compared to CB on NB, largely derived from the improved suppression of PB. Results of this study extend the overall influence of amino acid intake on anabolism derived from a combination of PS and PB, which affects NB. Future studies are warranted to evaluate the influence of FR on the molecular regulation of muscle protein kinetics in humans who have been more affected by the industrialization of meat protein over a relatively short period in human evolutionary history.



## References

1. Burge, R., Dawson-Hughes, B., Solomon D.H., Wong, J.B., King, A., Tosteson, A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res.* **2007** 22, 465–475. doi: 10.1359/jbmr.061113
2. Goins, R.T., Moss, M., Buchwald, D. Guralnik, J.M. Disability among older American Indians and Alaska Natives: an analysis of the 2000 Census Public Use Microdata Sample. *Gerontologist.* **2007** 47, 690–696). doi: 10.1093/geront/47.5.690
3. Schure, M.B., Goins, R.T. An Examination of the Disablement Process among Older American Indians: The Native Elder Care Study. *Gerontologist* **2016** 56, 948–955. doi:10.1093/geront/gnv001
4. Foutz, J.D., Cohen, S.A., Cook, S.K. Challenges and barriers to health care and overall health in older residents of Alaska: Evidence from a national survey. *Int J Circumpolar Health* **2016** 75, 30348. doi:10.3402/ijch.v75.30348
5. Grazko, J. Alaska medical costs are more than double the national average | Premera Voices. *Premera Voices* **2017**.
6. Lewis, J. The future of successful aging in Alaska. *Int J Circumpolar Health* **2013** 72,10.3402. doi:10.3402/ijch.v72i0.21186
7. Beaudart, C., Zaaria, M., Pasleau, F., Reginster, J.Y., Bruyère, O. Health outcomes of sarcopenia: A systematic review and meta-analysis. *PLoS One* **2017** 12(1) e0169548. doi: 10.1371/journal.pone.0169548
8. Børsheim, E., Tipton, K.D., Wolf, S.E., Wolfe, R.R. Essential amino acids and

- muscle protein recovery from resistance exercise. *Am J Physiol - Endocrinol Metab* **2002**. 283(4), E648-E657. doi:10.1152/ajpendo.00466.2001
9. Kim, I.-Y., Schutzler, S., Schrader, A., Spencer, H., Kortebein, P., Deutz, NEP., Wolfe, R.R., Ferrando, A.A. Quantity of dietary protein intake, but not pattern of intake, affects net protein balance primarily through differences in protein synthesis in older adults. *Am J Physiol Metab* **2015** 308(1), E21-E28. doi:10.1152/ajpendo.00382.2014
  10. Lonnie, M., Hooker, E., Brunstrom, J.M., Corfe, B.M., Green, M.A., Watson, A.W., Williams, E.A., Stevenson, E.J., Penson, S., Johnstone, A.M. Protein for life: Review of optimal protein intake, sustainable dietary sources and the effect on appetite in ageing adults. *Nutrients* **2018** 10(3). doi:10.3390/nu10030360
  11. Data, S. N. SELF Nutrition Data | Food Facts, Information & Calorie Calculator. *NutritionData* (2014).
  12. Pham, T.H., Vidal N.P., Manful, C.F., Fillier T.A. Pumphrey, R.P., Doody, K.M., Thomas, R.H. Moose and caribou as novel sources of functional lipids: Fatty acid esters of hydroxy fatty acids, diglycerides and monoacetyldiglycerides. *Molecules* **2019** 24(2). doi:10.3390/molecules24020232
  13. Coker, R. H. *et al.* Essential amino acid-enriched meal replacement promotes superior net protein balance in older, overweight adults. *Clin Nutr* **2019** 38(6), 2821-2826. doi:10.1016/j.clnu.2018.12.013
  14. Richardson J, UAF news and information archives. Diet of traditional Native foods revealed in hair samples, July 24, 2019.
  15. Cattlemen's Beef Board & National Cattlemen's Beef Association.

16. Agriculture, U. S. D. of H. and H. S. and U. S. D. of. 2015 – 2020 Dietary Guidelines for Americans. *2015 – 2020 Diet. Guidel. Am. 8th Ed.* **2015**.  
doi:10.1097/NT.0b013e31826c50af
17. Abumrad, N.N., Rabin, D., Diamond, M.P. & Lacy, W.W. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* **1981** 30(9), 936-940. doi:10.1016/0026-0495(81)90074-3
18. Wolfe, R.R., Park, S., Kim, I.-Y., Moughan, P.J., Ferrando, A.A. Advances in stable isotope tracer methodology part 2: New thoughts about an "old" method-measurement of whole body protein synthesis and breakdown in the fed state. *J Investig Med* **2020** **68** (1). doi:10.1136/jim-2019-001108
19. Chinkes, David L.; Wolfe, R. R. *Isotope Tracers in Metabolic Research: Principles and Practice of Kinetic Analysis*. (Wiley, 2005).
20. De Betue, C.T.I., Joosten, K.F.M., Deutz, N.E.P., Vreugdenhil, A.C.E., Van Waardenburg, D.A. Arginine appearance and nitric oxide synthesis in critically ill infants can be increased with a protein-energy-enriched enteral formula. *Am J Clin Nutr* **2013** 98(4), 907-916. doi:10.3945/ajcn.112.042523
21. Holwerda, A.M., Paulussen, K.J.M., Overkamp, M., Goessens, J.P.B., Kramer, I.F., Wodzig, W.K.W.H., Verdijk, L.B., van Loon, L.J.C. Dose-dependent increases in whole-body net protein balance and dietary protein-derived amino acid incorporation into myofibrillar protein during recovery from resistance exercise in older men. *J Nutr* **2019** 149(2), 221-230. doi:10.1093/jn/nxy263
22. Wolfe, R.R. The underappreciated role of muscle in health and disease. *Am J Clin*

- Nutr* (2006). doi:10.1093/ajcn/84.3.475
23. Kim, I.-Y., Schutzler, S., Schrader, A., Spencer, H.J., Azhar, G., Ferrando, A.A., Wolfe, R.R. The anabolic response to a meal containing different amounts of protein is not limited by the maximal stimulation of protein synthesis in healthy young adults. *Am J Physiol - Endocrinol Metab* **2015** 310(1), E73-E80.  
doi:10.1152/ajpendo.00365.2015
  24. Volpi, E., Kobayashi, H., Sheffield-Moore, M., Mittendorfer, B. & Wolfe, R. R. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* **2003** 78(2), 250-258. doi:10.1093/ajcn/78.2.250
  25. Kim, I.-Y., Deutz, N.E.P., Wolfe, R.R. Update on maximal anabolic response to dietary protein. *Clin Nutr* **2018** 37(2), 411-418. doi:10.1016/j.clnu.2017.05.029
  26. Moore, D.R., Churchward-Venne, T.A., Witard, O., Breen, L., Burd, N.A., Tipton, K.D., Philips, S.M. Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *J Gerontol - Ser A Biol Sci Med Sci* **2015** 70(1), 57-62. doi:10.1093/gerona/glu103
  27. Hou, Y., Yin, Y. & Wu, G. Dietary essentiality of “nutritionally non-essential amino acids” for animals and humans. *Exp Biol Med* **2015** 240(8): 997-1007.  
doi:10.1177/1535370215587913
  28. Phang, J. M., Liu, W., Hancock, C. Bridging epigenetics and metabolism: Role of non-essential amino acids. *Epigenetics* **2013** 8(3), 231-236.  
doi:10.4161/epi.24042
  29. Wu, G. Dietary requirements of synthesizable amino acids by animals: A

- paradigm shift in protein nutrition. *J Anim Sci Biotech* **2014** 5:34.  
doi:10.1186/2049-1891-5-34
30. Smith, G.I., Atherton, P., Reeds D.N., Mohammed B.S., Rankin, D., Rennie, M.J., Mittendorfer B. Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. *Clin Sci* **2011** 121(6), 267-278.  
doi:10.1042/CS20100597
31. Di Girolamo, F.G., Situlin, R., Mazzucco, S., Valentini, R., Toigo, G., Biolo, G. Omega-3 fatty acids and protein metabolism: Enhancement of anabolic interventions for sarcopenia. *Curr Opin Clin Nutr Metab Care* **2014** 17(2), 145-150. doi:10.1097/MCO.0000000000000032
32. Drummond, M.J., Dreyer, H.C., Fry, C.S., Glynn, E.L. Rasmussen, B.B. Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. *J Appl Physiol* **2009** 106(4), 1374-1384.  
doi:10.1152/jappphysiol.91397.2008
33. Magee, P., Pearson, S., Whittingham-Dowd, J., Allen, J. PPAR $\gamma$  as a molecular target of EPA anti-inflammatory activity during TNF- $\alpha$ -impaired skeletal muscle cell differentiation. *J Nutr Biochem* **2012** 23(11), 1440-1448.  
doi:10.1016/j.jnutbio.2011.09.005
34. Kamolrat, T., Gray, S.R. The effect of eicosapentaenoic and docosahexaenoic acid on protein synthesis and breakdown in murine C2C12 myotubes. *Biochem Biophys Res Commun* **2013** 432(4), 593-598. doi:10.1016/j.bbrc.2013.02.041
35. Yoshino, J., Smith, G.I., Kelly, S.C., Julliand, S., Reeds, D.N., Mittendorfer, B.

- Effect of dietary n-3 PUFA supplementation on the muscle transcriptome in older adults. *Physiol Rep* **2016** 4(11). doi:10.14814/phy2.12785
36. Smith, G.I., Julliand, S., Reeds, D.N., Sinacore, D.R., Klein, S., Mittendorfer, B. Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults. *Am J Clin Nutr* **2015** 102(1), 115-122. doi:10.3945/ajcn.114.105833
37. Simopoulos, A.P. An increase in the Omega-6/Omega-3 fatty acid ratio increases the risk for obesity. *Nutrients* **2016** 8(3), 128. doi:10.3390/nu8030128
38. Manson, J.E., Cook, N.R., Lee, I.-M., Christen, W., Bassuk, S.S., Mora, S., Gibson, H., Albert, C.M. Gordon, D., Copeland, T., D'Agostino, D., Friedenberg, G., Ridge, C., Bubes, V., Giovannucci, E.L., Willet, W.C., Buring, J.E., VITAL Research Group. Marine n-3 Fatty Acids and Prevention of Cardiovascular Disease and Cancer. *N Engl J Med* **2019** 380, 23–32. doi: 10.1056/NEJMoa1811403
39. Simopoulos, A.P. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med* **2008** 233(6), 674-688. doi: 10.3181/0711-MR-311
40. Burton, L.A., Sumukadas, D. Optimal management of sarcopenia. *Clin Interv Aging* **2010** 5, 217-228.
41. Liljas, A.E.M., Walters, K., Jovicic, A., Lliffee, S., Manthorpe, J., Goodman, C., Kharicha, K., Strategies to improve engagement of 'hard to reach' older people in research on health promotion: a systematic review. *BMC Public Health* **2017** 17(1), 349. doi: 10.1186/s12889-017-4241-8
42. Knechel, N.A. The challenges of enrolling older adults into intervention studies.

- Yale J Biol Med* **2013** 86, 41–47.
43. Gabriel, A. S., Ninomiya, K. & Uneyama, H. The role of the Japanese traditional diet in healthy and sustainable dietary patterns around the world. *Nutrients* **2018** 10(2), 173. doi: 10.3390/nu10020173
  44. Murphy, N.J., Schraer, C.D., Thiele, M.C., Boyko, E.J., Bulkow, L.R., Doty, B.J., Lanier, A.P. Dietary Change and Obesity Associated with Glucose Intolerance in Alaska Natives. *J Am Diet Assoc* **1995** 95(6), 676-682. doi:10.1016/S0002-8223(95)00184-0
  45. Mohatt, G.V., Plaetke, R., Klejka, J., Luick, B., Lardon, C., Bersamin, A., Hopkins, S., Dondanville, M., Herron, J., Boyer, B., Center for Alaska Native Health Research. The Center for Alaska Native Health Research Study: a community-based participatory research study of obesity and chronic disease-related protective and risk factors. *Int J Circumpolar Health* **2007** 66(1), 8-18. doi:10.3402/ijch.v66i1.18219
  46. Makhoul, Z., Kristal, A.R., Gulati, R., Luick, B., Bersamin, A., Boyer, B., Mohatt, G.V. Associations of very high intakes of eicosapentaenoic and docosahexaenoic acids with biomarkers of chronic disease risk among Yup'ik Eskimos. *Am J Clin Nutr* **2010** 91(3): 777-785. doi:10.3945/ajcn.2009.28820