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Research Article

A Pharmacokinetic Evaluation of Isolated Chicken Protein as Compared to Beef Protein in Healthy Active Adults

Douglas Kalman[1,3], Susan Hewlings[2,3,*], Robin Lee[1], Jacob Bentley[1], Richard Foster[1], Kayce Morton[1] and Darryn S Willoughby[4]

[1]Department of BD, Nutrition and Clinical Research, QPS-BKCA, Springfield, USA

[2]Department of Nutrition, Central Michigan University Mount Pleasant, USA

[3]Substantiation Sciences, Weston, USA

[4]Department of Health, Human Performance and Recreation, Baylor University, Waco, USA

Abstract

High-quality proteins of various sources stimulate Muscle Protein Synthesis (MPS); however, less is known about the comparative bioavailability and Pharmacokinetics (PK) of typical dietary proteins. This was a prospective, randomized, pharmacokinetic, exploratory clinical trial to evaluate the amino acid bioavailability of chicken protein isolate [Chik│Pro™ (CKP)] compared to Beef Protein Isolate (BFP). Twenty-two participants were randomized to receive both proteins in a cross-over design. Participants fasted overnight for at least eight hours and in a single blind fashion consumed 25 grams protein of CKP or BFP on day 1 and the alternative treatment on day

*Corresponding author: Susan Hewlings, Department of Nutrition, Central Michigan University, USA; Substantiation Sciences, Weston, USA, Tel: +321 3774522; E-mail: sue.hewlings@gmail.com

4. Venous blood samples were collected for Total Amino Acid (TAA), Essential Amino Acid (EAA), Sulfur-containing Amino Acid (SAA), leucine, and arginine analysis one hour prior to ingestion (pre-ingestion) and post-ingestion at 30, 60, 90, 120, and 180 minutes. Statistical analyses were performed with paired t-tests, ANOVA, and the Wilcoxon's signed-rank test (p<0.05). Pharmacokinetic analysis was determined by mixed-model effects ANOVA. CKP produced significant increases in TAA, EAA, SAA, arginine and leucine concentrations at 30 through 180 minutes (C30 min-C180 min) following ingestion, while BFP was only capable of this for arginine (p<0.05). Furthermore, these increases were shown to be significantly greater for CKP (p<0.05). The Maximum Concentration (Cmax), Area Under the Curve (AUC0-t), and Time of Delivery (Tmax) for EAA and leucine were significantly greater for CKP compared to BFP (p<0.05). For SAA, the CKP Cmax and AUC0-t for arginine was significantly greater than BFP (p<0.05). CKP was found to be superior to BFP in relative bioavailability for EAA, SAA, and leucine suggesting it may stimulate MPS and enhance recovery more effectively than BFP.

Keywords: Amino acid; Bioavailability; Protein synthesis; Skeletal muscle

Introduction

Dietary protein sources, particularly complete sources, can have different rates of bioavailability based on many factors. These factors affect relative bioavailability including the fat and carbohydrate content, amino acid composition, while also noting that peptide size within the protein can slow stomach digestion, gastric motility, and subsequent absorption into circulation [1]. Additionally, food processing can also impact bioavailability. For instance, the D-amino acids and lysinoalanine (LAL, an unnatural amino acid) formed during the alkaline/heat treatment of proteins such as casein are only 40% digestible, and their presence can reduce the digestibility of protein by up to 28% [2]. Once a protein meal is ingested, approximately 50% of the amino acids are taken up by the splanchnic tissues and the remainder absorbed into the plasma circulation for use by extra-splanchnic tissues [3]. It has been shown that from 20 grams of casein protein (a slower absorbing protein than whey), only 2 grams (11%) of the amino acids were used for incorporation into Muscle Protein Synthesis (MPS), despite 55% availability in the peripheral circulation following splanchnic extraction [3]. Nevertheless, independent of these factors, the amino acid composition of dietary proteins can have differential effects on MPS, perhaps as a result of their rates on bioavailability.

Whey protein has a high bioavailability compared to other protein sources, such as casein [4-6] and beef [1]. However, it has been shown that meat protein serves as an important protein source for augmenting muscle growth and increasing strength gains [7]. Recently, beef and chicken protein isolates have become popular in the exercise/sport nutrition arena as well within the medical nutrition therapy community based on their amino acid composition and propensity to be able to augment the rate of MPS associated with intense exercise training. In humans, comparing the bioavailability of whey, chicken, and beef protein isolates, it was shown that whey and

chicken protein isolate contained a higher content of Essential Amino Acids (EAAs) with high bioavailability, being absorbed into plasma at peak concentrations at 30 minutes following ingestion. Conversely, beef protein isolate contained a greater proportion of conditionally EAAs that progressively increased over a three-hour period [1].

Within the exercise and sport nutrition industry, efforts are being made to determine alternate sources of protein isolate that may be superior to whey isolate. The purpose of this approach is for more rapid bioavailability and subsequent augmentation in MPS since protein source is an important factor in up-regulating MPS following protein consumption [5,6,8].

The purpose of this study was to determine the bioavailability (rate of appearance in blood) and Pharmacokinetic (PK) evaluation of amino acids due to ingestion of Chicken Protein Isolate (CKP) in comparison to Beef Protein Isolate (BFP) in healthy, physically-active, adult males. The specific aims were to determine the kinetic effects of CKP and BFP on Total Amino Acids (TAA), Essential Amino Acids (EAA), Sulfur-containing Amino Acids (SAA), leucine, and arginine. TAA were assessed to determine overall amino acid absorption, EAA were assessed because they are required for optimal protein synthesis, SAA were assessed because the profile amino acids in meats are different than non-meat sources, leucine was assessed because it is the amino acids responsible for stimulating protein synthesis, arginine was assessed because it is typically higher in meat protein than non-meat.

Methodology

Experimental approach

This was a prospective, randomized, pharmacokinetic, exploratory, blinded, clinical trial pilot study performed in a cross-over fashion involving 22 physically-active adult males to evaluate the bioavailability and PK for CKP in comparison to BFP.

Participants

Twenty-two apparently healthy, physically-active males between the ages of 18 and 45 with a Body Mass Index (BMI) of 19.0-34.9 kg/m^2 completed the study. In order to be included in the study subjects were required to meet the following criteria: non-smokers; maintained a stable weight, had consistent exercise and dietary habits, and were in good health, able to exercise, willing and able to comply with the protocol requirements; met requirements of pre-study physical examination and clinical laboratory tests. All participants understood the study protocol and consistent with the guidelines of the American College of Sports Medicine [(ACSM) ACSM's Guidelines for Exercise Testing and Prescription, 10th edition] and provided written informed consent. The study (#64516) was approved on 8/28/2017 by the Bio-Kinetic Applications Institutional Review Board (Springfield, MO). All experimental procedures involved in the study conformed to the ethical consideration of the Declaration of Helsinki.

Screening visit

Each prospective participant underwent a pre-study screening to determine eligibility for the study. The screening was performed and/or assessed by the principal physician investigator and study personnel within 28 days prior to visit 1. Each participant underwent morphometric measurements, including weight (kg), height (cm), BMI calculation and vital signs (blood pressure and heart rate). A medical history was reviewed for past and current medical conditions, surgical history, allergy information, and concomitant or recently taken (in the past 30 days) medications including over-the-counter, non-prescription products, nutritional supplements, herbals, and investigational products. A physical examination was performed by a licensed physician and included examination of the head, ears, eyes, nose, throat, neck, chest, skin, heart and lungs and the gastrointestinal, musculoskeletal and neurological systems. In addition, venous blood was obtained and used to assess clinical screening and safety variables [i.e., glucose, hemoglobin, hematocrit, Leukoyctes (WBC), Erythrocytes (RBC), Blood Urea Nitrogen (BUN), creatinine, total bilirubin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST)]. All participants were questioned and monitored during each return visit about any changes in their health status since the previous visit.

Procedures

In a cross-over fashion, on visit 1 (day 1), at the study site, each participant randomly received one of the dietary proteins (CKP or BFP) and the other protein at visit 2 (day 4). The protein products were in powder form and were prepared by the study staff for consumption in a single-blind fashion. At each visit at day 1 and day 4, participants reported to the study center the morning of each visit following an overnight fast of at least eight hours. Compliance with protocol was monitored by dichotomous questionnaire. These visits were separated by 3 days for wash-out purposes.

Supplementation protocol

Subjects consumed a single dose (delivering 25 grams of protein) of either Beef Protein Isolate [(BFP) IsoPrime, Maximum Human Performance, West Caldwell, NJ, USA] or Chicken Protein Isolate [(CKP) Chik│Pro™, International Dehydrated Foods, Inc., Springfield, MO, USA] on day 1 (visit 1) at the study site, and the alternative treatment was provided on day 4 (visit 2). See table 1 for a complete list of ingredients for both protein powders. The contents were mixed with water for approximately 30 seconds until all the powder was dissolved. A small amount of water was added for any residual powder mix and consumed immediately after mixing the contents. Participants were instructed to consume the entire dose within 2 minutes and were supervised in order to make sure the entire product was ingested.

Venous blood sampling

Blood samples of 6 ml each (total 36 ml) were collected through repeated venipuncture for pre-study medical screening. Also, at day 1 and day 4, blood was drawn at six time points: pre-ingestion (within 1 hour of dose) and 30, 60, 90, 120, and 180 minutes post-ingestion.

Amino acid analysis

Venous blood samples were collected in sodium heparin vacutainer tubes. After the blood sample was obtained, the tube was gently inverted 8 to 10 times to allow the sample to mix with the anticoagulant. Samples were then sent to Mercy Hospital Laboratory (Springfield, MO, USA) for processing and subsequent shipment to Mayo Medical Laboratories (Rochester, MN, USA) for the determination of plasma amino acids utilizing quantitative Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) using standard procedures for amino acid analysis [1-11].

Study Product 1: Test Powder Mix
Chicken protein (Chik │ Pro™) 25 grams
Chocolate ChikPro 100% chicken protein isolate
Ingredients: Chocolate ChikPro 100% chicken proteins isolate, Dutched cocoa powder, natural flavors, maltodextrin, sucralose, chocolate flavor natural
International Dehydrated Foods, Inc.
Study Product 2: Reference Product Mix
Beef protein isolate 25 grams (MHP IsoPrime 100% Beef (pure beef protein isolate))
25 g of protein/28.1 g serving size [89.0% protein]
Ingredients: IsoPrime 100% beef protein isolate, TasteTech flavoring system (natural and artificial flavors, gum blend (cellulose gum, xanthan gum and carrageenan), acesulfame potassium and sucralose)
Maximum Human Performance (MHP)

Table 1: Ingredients for CKP and BFP Products.

Note: The serving size consisted of a once daily dose of 25 grams of the assigned study product as bolus. For this study, a single dose of study product (s) was taken on visit 1 (day 1) and visit 2 (day 4) at study site.

For TAA analysis, the sum of all measured amino acids (taurine, threonine, serine, asparagine, glutamic acid, glutamine, proline, alanine, citrulline, alpha-amino-n-butyric acid, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, beta-alanine, ornithine, lysine, histidine, argininosuccinic acid, allo-isoleucine, arginine, phosphoserine, phosphoethanolamine, hydroxyproline, glycine, aspartic acid, ethanolamine, sarcosine, 1-methylhistidine, 3-methylhistidine, carnosine, anserine, homocitruline, alpha-aminoadipic acid, gamma-amino-n-butyric acid, beta-aminoisobutyric acid, hydroxylysine, cystathionine, and tryptophan) was determined and comprehensive values reported. For EAA, the sum of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine was determined and reported. Also, the sum of the SAA as methionine and cystathionine was determined and reported. Leucine and arginine values were also reported on their own.

Pharmacokinetic parameters

The PK parameters for TAA, EAA, SAA, leucine, and arginine were calculated by non-compartmental methods. Actual elapsed PK sampling times were used for the estimation of PK variables. The PK parameters were the Maximum Observed Concentration (Cmax), Time to Maximum Observed Concentration (Tmax), and Area Under the Concentration-Time Curve (AUC0-t). Specifically, AUC0-t was calculated using the linear up/log down trapezoidal method from time zero to time t, where t is the time of the last quantifiable concentration (Clast).

Statistical analysis

No formal statistical calculations of sample size were conducted for this study. Sample size was selected based upon prior published pharmacokinetic protein and amino acid studies [1,5,9]. For baseline correction, each participant's baseline value was defined as the pre-ingestion sample (within 1 hour of dose) on day 1. The baseline value was subtracted from each measured concentration, including the pre-ingestion concentration. Negative values obtained because of adjusting the data were set to zero. Time-wise comparisons, where appropriate, were made for tests at the visit 2, namely comparisons for changes from visit 1 pre-ingestion to all five post-ingestion blood draws (30, 60, 90, 120 and 180 minutes) at visit 2. Within-and

between-treatment effects for TAA, EAA, SAA, leucine, and arginine at C30 min-C180 min for each protein were analyzed with paired t-tests. Baseline-corrected PK variables (Cmax and AUC0-t) were analyzed using the general linear model procedure for the log-transformed values. The terms used in the ANOVA model were sequence, period and treatment as fixed effects and subject within sequence as random effect. Back-transformed statistics and inferential results are reported and the 90% Confidence Intervals (CIs) were generated for the Geometric Mean Ratios (GMR) of Cmax and AUC0-t between CKP and BFP. The Tmax was analyzed using non-parametric Wilcoxon's signed-rank test. A p-value was of significance was set at ≤0.05 throughout. Phoenix WinNonlin version 6.3 (Certara USA, Inc., Princeton, NJ, USA) was used for PK analyses. All statistical procedures were performed using SAS® v9.3 (SAS Institute Inc., Cary, NC, USA).

Results

A total of 51 individuals were recruited and screened; however, 29 did not meet the eligibility criteria. Therefore, 22 who were recruited, screened, and eligible were enrolled in the study. Of the 22 participants, the mean±SD age, height, total body mass, and BMI was 31.50±6.99, 178.27±6.80 cm, 87.14±16.60 kg, and 27.24±3.86 (kg, m^2), respectively. There were no adverse events observed or reported. None of the participants discontinued the study.

Amino acid analyses

Both CKP and BFP resulted in significant elevations in plasma TAA at C30 min, C60 min, C90 min, and C120 min and C180 min (p<0.05), there was no significance between-group difference at C180 min following ingestion (p>0.05) (Table 2). The Cmax, AUC0-t, and Tmax after the ingestion of CKP were comparable to BFP, but not significantly different (p>0.05) (Table 3).

For EAA, both CKP and BFP resulted in significant elevations at C30 min, C60 min, and C90 min (p<0.05); however, CKP also significantly increased EAA at C120 min and C180 min. The CKP isolate significantly enhanced EAA absorption to a greater degree at all five time points compared to the BFP (p<0.05) (Table 4). The Cmax, AUC0-t, and Tmax were significantly greater for CKP than BFP (p<0.05) (Table 3).

For SAA, both CKP and BFP resulted in significant elevations at C30 min, C60 min, C90 min, and C120 min, but CKP was also significantly increased at C180 min (p<0.05). Significantly greater increases in SAA absorption for CKF compared to BFP occurred at all time points (p<0.05) (Table 5). The Cmax and AUC0-t after the ingestion of CKP were significantly higher than BFP (P<0.05). However, the Tmax was comparable between CKP and BFP, but not significantly different (p>0.05) (Table 3).

Similar to EAA, both CKP and BFP resulted in significant elevations in plasma leucine at C30 min, C60 min, and C90 min (p<0.05); however, CKP also significantly increased leucine at C120 min and C180 min. Significantly greater increases in leucine absorption for CKF compared to BFP occurred at all time points (p<0.05) (Table 6). The Cmax, AUC0-t, and Tmax for CKP were significantly greater than BFP (p<0.05) (Table 3).

Variable/Time Point	Chik Pro (N=22)	Beef Protein (N=22)	p-value* (A vs B)
Pre-ingestion	3428.09±455.68 3444.50 (2690.00-4958.00)	3450.09±377.67 3455.50 (2695.00-4307.00)	0.6338^w
30 minutes post-ingestion	4203.95±793.97 3876.00 (3544.00-6795.00)	4084.59±486.09 3964.00 (3375.00-5408.00)	1.0000^w
30 minutes post-ingestion - pre-ingestion	775.86±715.67 586.00 (-63.00-3254.00) <0.0001w	634.50±532.26 585.50 (-367.00-2012.00) <0.0001t	0.8330^w
60 minutes post-ingestion	4321.86±528.31 4276.50 (3723.00-5946.00)	4120.41±496.44 4065.50 (3339.00-5635.00)	0.1995^t
60 minutes post-ingestion - pre-ingestion	893.77±561.91 831.50 (-645.00-1999.00) <0.0001^w	670.32±586.76 669.50 (-712.00-2239.00) <0.0001^t	0.2041^t
90 minutes post-ingestion	4560.82±878.95 4218.00 (3618.00-6848.00)	4259.50±609.80 4250.00 (3535.00-6559.00)	0.4814^w
90 minutes post-ingestion-pre-ingestion	1132.73±701.96 943.50 (406.00-3307.00) <0.0001w	809.41±728.13 822.00 (-545.00-3163.00) <0.0001^w	0.2009^w
120 minutes post-ingestion	4301.91±745.13 4118.00 (3053.00-6476.00)	4174.86±702.74 4033.50 (3048.00-5942.00)	0.3776^w
120 minutes post-ingestion-pre-ingestion	873.82±743.03 672.00 (-136.00-3243.00) <0.0001^w	724.77±747.09 564.00 (-312.00-2595.00) <0.0001^w	0.3902^w
180 minutes post-ingestion	3823.23±506.07 3714.50 (2974.00-4913.00)	3811.59±387.12 3760.50 (2735.00-4602.00)	0.9321^t
180 minutes post-ingestion-pre-ingestion	395.14±352.92 326.00 (-139.00-1238.00) <0.0001^w	361.50±444.37 368.50 (-790.00-1321.00) 0.0010^t	0.7824^t

Table 2: Plasma TAA concentrations before and after administration of CKP and BFP.

Note: *Tested by the independent student t test (t) or by the non-parametric Wilcoxon rank sum test (w) if non-normally distributed. **Tested by the paired t test (t) or by the non-parametric Wilcoxon signed-ranks test (w) if non-normally distributed.

Parameters	Chicken Protein		Beef Protein	
	N	Mean (SD)	N	Mean (SD)
Leucine				
Cmax, nmol/mL	22	113 (43.4)	20[b]	43.4 (21.5)
Tmax, h[a]	22	1.50 (0.50, 3.00)	20[b]	1.00 (0.50, 2.00)
AUC0-t, h•nmol/mL	22	190 (68.9)	20[b]	50.6 (33.4)
Arginine				
Cmax, nmol/mL	22	80.5 (40.3)	22	72.8 (23.4)
Tmax, h[a]	22	1.25 (0.50, 2.00)	22	0.50 (0.50, 1.50)
AUC0-t, h•nmol/mL	22	128 (55.4)	22	101 (41.2)
Total Amino Acids (TAA)				
Cmax, nmol/mL	22	1410 (813)	20[b]	1200 (691)
Tmax, h[a]	22	1.50 (0.50, 2.00)	20[b]	1.50 (0.50, 3.00)
AUC0-t, h•nmol/mL	22	2190 (1140)	20[b]	2000 (1190)
Essential Amino Acids (EAA)				
Cmax, nmol/mL	22	663 (308)	20[b]	268 (181)
Tmax, h[a]	22	1.50 (0.50, 2.00)	20[b]	0.75 (0.50, 2.00)
AUC0-t, h•nmol/mL	22	1120 (457)	20[b]	329 (297)
Sulfur Containing Amino Acids				
Cmax, nmol/mL	22	32.5 (11.7)	21[c]	9.33 (3.89)
Tmax, h[a]	22	1.50 (0.50, 2.00)	21[c]	1.00 (0.50, 2.00)
AUC0-t, h•nmol/mL	22	54.5 (17.9)	21[c]	11.9 (8.82)

Table 3: Pharmacokinetic parameters for amino acids after administration of CKP and BFP.

Note: Median (Min, Max);

Cmax, Tmax and AUC0-t of leucine, TAA, and EAA for two (2) subjects, subjects 012 and 016, were not be calculated because the baseline-corrected concentrations were all zero for each time point after administration of beef protein product;

Cmax, Tmax and AUC0-t of sulfur containing amino acids for one (1) subject, subject 016, were not be calculated because the baseline-corrected concentrations were all zero for each time point after administration of beef protein product.

Variable/Time Point	Chik Pro (N=22)	Beef Protein (N=22)	p-value* (A vs B)
Pre-ingestion	1099.64±164.61 1078.00 (779.00-1622.00)	1130.27±192.93 1112.00 (798.00-1706.00)	0.4736^w
30 minutes post-ingestion	1459.32±293.08 1381.00 (1125.00-2406.00)	1293.50±185.91 1270.50 (1039.00-1848.00)	0.0251^w
30 minutes post-ingestion - pre-ingestion	359.68±294.40 291.00 (17.00-1279.00) <0.0001^w	163.23±168.61 134.00 (-210.00-595.00) 0.0002^w	0.0052^w
60 minutes post-ingestion	1570.82±158.18 1555.50 (1331.00-2027.00)	1227.82±199.62 1188.00 (868.00-1874.00)	<0.0001^w
60 minutes post-ingestion - pre-ingestion	471.18±213.39 474.50 (-84.00-874.00) <0.0001^w	97.55±187.59 115.00 (-287.00-621.00) 0.0246^w	<0.0001^t
90 minutes post-ingestion	1656.77 ± 315.86 1538.50 (1236.00-2517.00)	1265.50 ± 235.44 1248.00 (926.00-2111.00)	<0.0001^w
90 minutes post-ingestion - pre-ingestion	557.14±283.03 476.00 (211.00-1390.00) <0.0001^w	135.23±227.36 136.50 (-229.00-858.00) 0.0051^w	<0.0001^w
120 minutes post-ingestion	1553.23±267.53 1532.00 (1004.00-2296.00)	1194.05±201.19 1179.00 (795.00-1802.00)	<0.0001^t
120 minutes post-ingestion - pre-ingestion	453.59±255.44 380.00 (95.00-1233.00) <0.0001^w	63.77±205.68 42.50 (-278.00-549.00) 0.1607^t	<0.0001^w
180 minutes post-ingestion	1332.14±187.19 1285.00 (947.00-1701.00)	1101.64±138.55 1103.50 (740.00-1383.00)	<0.0001^t
180 minutes post-ingestion - pre-ingestion	232.50±142.12 205.00 (0.00-574.00) <0.0001^w	-28.64±145.27 -10.00 (-352.00-210.00) 0.3657^t	<0.0001^w

Table 4: Plasma EAA concentration before and after administration of CKP and BFP.

Note: *Tested by the independent student t test (t) or by the non-parametric Wilcoxon rank sum test (w) if non-normally distributed. **Tested by the paired t test (t) or by the non-parametric Wilcoxon signed-ranks test (w) if non-normally distributed.

Variable/Time Point	Chik Pro (N=22)	Beef Protein (N=22)	p-value* (A vs B)
Pre-ingestion	32.05±5.55 31.50 (24.00-48.00)	32.95±6.15 32.00 (22.00-54.00)	0.5590^w
30 minutes post-ingestion	48.95±14.71 44.00 (33.00-86.00)	39.18±5.67 39.00 (29.00-49.00)	0.0170^w
30 minutes post-ingestion - pre-ingestion	16.91±13.40 13.50 (0.00-51.00) <0.0001^w	6.23±5.52 6.50 (-7.00-18.00) <0.0001^t	0.0006^w
60 minutes post-ingestion	57.36±9.54 58.50 (41.00-75.00)	37.23±5.27 37.00 (29.00-46.00)	<0.0001^t
60 minutes post-ingestion - pre-ingestion	25.32±10.03 26.00 (11.00-42.00) <0.0001^t	4.27±5.73 4.50 (-10.00-15.00) 0.0022^t	<0.0001^t
90 minutes post-ingestion	58.68±11.29 53.50 (45.00-85.00)	37.82±5.37 37.00 (30.00-51.00)	<0.0001^w
90 minutes post-ingestion - pre-ingestion	26.64±11.21 21.50 (15.00-54.00) <0.0001^w	4.86±6.58 6.00 (-11.00-20.00) 0.0023^t	<0.0001^w
120 minutes post-ingestion	53.45±9.46 49.50 (41.00-75.00)	35.82±5.47 35.50 (27.00-46.00)	<0.0001^w
120 minutes post-ingestion - pre-ingestion	21.41±9.54 21.00 (8.00-44.00) <0.0001^w	2.86±5.52 2.00 (-8.00-13.00) 0.0241^t	<0.0001^t
180 minutes post-ingestion	42.64±7.15 42.00 (31.00-58.00)	33.18±5.30 32.00 (25.00-43.00)	<0.0001^t
180 minutes post-ingestion - pre-ingestion	10.59±5.59 8.50 (4.00-22.00) <0.0001^t	0.23±5.35 0.50 (-12.00-12.00) 0.8441^t	<0.0001^w

Table 5: Plasma SAA concentrations before and after administration of CKP and BFP.

Note: *Tested by the independent student t test (t) or by the non-parametric Wilcoxon rank sum test (w) if non-normally distributed. **Tested by the paired t test (t) or by the non-parametric Wilcoxon signed-ranks test (w) if non-normally distributed.

Variable/Time Point	Chik Pro (N=22)	Beef Protein (N=22)	p-value* (A vs B)
Pre-ingestion	147.18±23.99 141.00 (85.00-203.00)	149.64±34.92 145.00 (99.00-252.00)	0.8620[w]
30 minutes post-ingestion	211.73±42.11 203.50 (150.00-328.00)	178.59±33.57 180.50 (127.00-271.00)	0.0061[t]
30 minutes post-ingestion - pre-ingestion	64.55±48.14 49.50 (3.00-184.00) <0.0001[w]	28.95±23.39 26.50 (-25.00-86.00) <0.0001[w]	0.0014[w]
60 minutes post-ingestion	235.23±25.86 236.00 (197.00-289.00)	164.68±33.35 162.50 (107.00-270.00)	<0.0001[t]
60 minutes post-ingestion - pre-ingestion	88.05±32.89 95.00 (20.00-140.00) <0.0001[w]	15.05±27.66 17.00 (-42.00-85.00) 0.0222[w]	<0.0001[t]
90 minutes post-ingestion	244.77±41.41 235.50 (155.00-343.00)	170.86±38.12 167.50 (113.00-297.00)	<0.0001[t]
90 minutes post-ingestion - pre-ingestion	97.59±39.39 84.00 (56.00-199.00) <0.0001[w]	21.23±33.77 30.00 (-51.00-112.00) 0.0047[w]	<0.0001[w]
120 minutes post-ingestion	220.41±39.82 217.00 (124.00-335.00)	156.05±30.59 158.50 (102.00-242.00)	<0.0001[w]
120 minutes post-ingestion - pre-ingestion	73.23±34.35 69.00 (16.00-172.00) <0.0001[w]	6.41±27.57 8.50 (-53.00-59.00) 0.2122[w]	<0.0001[w]
180 minutes post-ingestion	178.77±26.70 176.50 (118.00-227.00)	141.68±21.77 142.50 (92.00-180.00)	<0.0001[t]
180 minutes post-ingestion - pre-ingestion	31.59±23.36 28.00 (-5.00-85.00) <0.0001[w]	-7.95±23.92 -3.50 (-72.00-28.00) 0.2724[w]	<0.0001[t]

Table 6: Plasma leucine concentrations before and after administration of CKP and BFP.

Note: *Tested by the independent student t test (t) or by the non-parametric Wilcoxon rank sum test (w) if non-normally distributed. **Tested by the paired t test (t) or by the non-parametric Wilcoxon signed-ranks test (w) if non-normally distributed.

Both CKP and BFP resulted in significant elevations in plasma arginine at C30 min, C60 min, C90 min, C120 min, and C180 min ($p<0.05$). In addition, there was a significantly greater absorption in arginine at C60 min and C120 min with CKP compared to BFP ($p<0.05$) (Table 7). The Cmax for CKP comparable to BFP, but not significantly different ($p>0.05$); however, the AUC0-t CKP was significantly higher than BFP ($p<0.05$). The Tmax for CKP was significantly longer than BFP ($p<0.05$) (Table 3).

Discussion

In this study we sought to determine the relative bioavailability from two different animal protein sources in physically-active healthy men. This analysis included the PK of TAA, EAA, SAA, leucine, and arginine in response to CKP and BFP ingestion in healthy, physically-active, adult males. While not to diminish the overall importance of our findings, the results that are most impactful relative to exercise/sport nutrition are those for EAA, SAA, and leucine. Unlike BFP, we observed CKP to increase EAA and leucine concentrations at all time points up to 180 minutes following ingestion (C30 min-C180 min). In addition, the increases for CKP, along with Cmax, AUC0-t, and Tmax were greater than BFP. For SAA, we observed a similar response as with EAA and leucine, which was a greater impact than the CKP. The apparent bioavailability of EAA is greater in CKP by a factor of 3.4x greater (EAA AUC0-t, h·nmol/mL 1120 (457) 329 (297)) than BFP.

Incomplete, lower-quality proteins such as soy, pea, or wheat are low or lacking in one or more EAAs; therefore, they are less effective at stimulating MPS and increases in muscle mass than complete, higher-quality sources [5,10,11]. In this context, protein quality is defined by the amount and profile of EAA, as well as the ideal digestibility (PDCAAs) [12,13]. However, independent of the protein source/quality feeding-induced hyperaminoacidemia stimulates amino acid uptake across the sarcolemma [14]. Following an increase in plasma amino acid levels, there is an approximate 30-minute delay in the stimulation of MPS before it peaks at 2 hours [15,16]. Relative to the results of our study, particularly for leucine, EAA, and SAA, this is important because the hyperaminoacidemia-induced up-regulation in MPS appears to regress to basal levels after approximately 2-3 hours, despite a continued increase in plasma amino acid levels [16]. Therefore, in regard to leucine, EAA, and SAA, the ability of CKP to result in elevated amino acid levels for 180 minutes following ingestion indicates the ability of this protein source to have a more prolonged impact on MPS when compared to BFP.

A hyperaminoacidemia-induced increase in MPS appears to be primarily dependent on the EAA composition of protein [17]. Of these amino acids, leucine is considered to be the primary trigger for initiating MPS [18-20], and can do so in the absence of other amino acids. However, if the availability of other EAA is limited MPS will become limited, independent of leucine content [14]. Regarding the results of the present study, this is noteworthy since we observed greater increases in EAA and SAA (which involves the EAA methionine) for CKP at all time points up to 180 minutes following ingestion. This scenario implies that MPS could be more prolonged with CKP than BFP.

Variable/Time Point	Chik Pro (N=22)	Beef Protein (N=22)	p-value* (A vs B)
Pre-ingestion	77.09±21.47 77.50 (44.00-143.00)	76.23±18.09 76.50 (41.00-109.00)	0.8967^w
30 minutes post-ingestion	129.68±47.14 113.00 (73.00-244.00)	141.86±31.30 137.50 (91.00-190.00)	0.3184^t
30 minutes post-ingestion - pre-ingestion	52.59±39.22 45.00 (5.00-165.00) <0.0001^w	65.64±28.18 69.50 (26.00-129.00) <0.0001^t	0.2121^t
60 minutes post-ingestion	133.73±30.41 128.50 (97.00-220.00)	117.14±27.01 116.50 (71.00-172.00)	0.0626^t
60 minutes post-ingestion - pre-ingestion	56.64±26.08 55.50 (-2.00-115.00) <0.0001^w	40.91±20.66 37.50 (5.00-84.00) <0.0001^t	0.0321^t
90 minutes post-ingestion	143.73±48.74 127.00 (82.00-290.00)	123.59±28.53 125.00 (68.00-197.00)	0.3075^w
90 minutes post-ingestion - pre-ingestion	66.64±37.88 51.00 (35.00-171.00) <0.0001^w	47.36±23.22 48.50 (4.00-90.00) <0.0001^t	0.1160^w
120 minutes post-ingestion	121.32±30.82 119.50 (69.00-170.00)	100.91±20.94 99.00 (63.00-144.00)	0.0138^t
120 minutes post-ingestion - pre-ingestion	44.23±23.92 35.50 (11.00-98.00) <0.0001^w	24.68±16.73 23.00 (-3.00-58.00) <0.0001^t	0.0038^w
180 minutes post-ingestion	98.86±26.29 96.50 (53.00-168.00)	93.14±23.23 89.00 (50.00-141.00)	0.4481^t
180 minutes post-ingestion - pre-ingestion	21.77±14.24 19.00 (-1.00-52.00) <0.0001^w	16.91±13.37 18.00 (-9.00-44.00) <0.0001^t	0.4003^w

Table 7: Plasma arginine concentrations before and after administration of CKP and BFP.

Note: *Tested by the independent student t test (t) or by the non-parametric Wilcoxon rank sum test (w) if non-normally distributed. **Tested by the paired t test (t) or by the non-parametric Wilcoxon signed-ranks test (w) if non-normally distributed.

A previous study has shown considerably greater levels of EAA and BCAA for chicken protein isolate compared to beef protein isolate [1]. In this study they also found that the post-ingestion plasma amino acid response mirrored the amino acid composition for the protein sources. In agreement with Storcksdieck et al. [21], the amino acids found at the four highest levels (in descending order) were glutamic acid/glutamine, aspartic acid, lysine, and leucine for the chicken protein isolate, whereas for the beef protein isolate they were glycine, glutamic acid/glutamine, proline, and alanine. It was also shown that the beef protein contained only 4% leucine, which may conceivably be below the threshold needed to increase MPS [21]. Since leucine is one of the more abundant amino acids contained within chicken protein, this suggests this protein source to be more effective at inducing MPS than beef protein. This becomes an important consideration in exercise/sport nutrition since a protein source that is digested and absorbed more rapidly can improve the post-prandial availability in plasma amino acids, thereby resulting in greater increases in MPS [22,23] and perhaps overall exercise recovery.

In the present study we showed CKP to have a significantly greater Cmax and Tmax compared to BFP for leucine, EAA, and SAA. This suggests that CKP has a greater bioavailability, rate of delivery, and potential to prolong MPS than BFP. Our present results agree with those of Detzel et al. [1], who observed peak plasma amino acid levels to appear by 30 minutes following ingestion of chicken isolate protein, but not until 30-60 minutes for beef protein. A likely reason for this more favorable response of CKP is due to chicken protein isolate having over 85% of its total protein content consisting of free amino acids or peptides smaller than 5 kDa, whereas beef protein consists of larger peptides, ranging from 5-15 kDa [1].

Skeletal muscle is the primary target of dietary protein [24]. As a result, skeletal muscle is more responsive to variations in dietary intake than other tissues and organs [25]. The intramuscular anabolic impact of dietary protein intake is important since skeletal muscle is a primary fate of leucine and the EAA's absorbed from dietary protein [25]. Consumption of dietary protein stimulates MPS within an hour. The present study shows the bioavailability of EAA for both CKP and BFP potentially capable of being able to stimulate MPS within this period of time; however, the duration of BFP over the course of 180 minutes was less robust than the CKP. The ability of a dietary protein such as CKP to have a rapid and sustained response in circulation is important because a significant portion of amino acids absorbed from the ingested protein will be retained in the splanchnic area, mainly in the gut [26,27]. This is also a noteworthy consideration since hyper-aminoacidemia following protein intake can lead to the suppression of muscle proteolysis [28].

The MPS that occurs because of exercise, and extends into the post-exercise recovery period, can be augmented with sufficient intake of a dietary protein that has rapid absorption into the circulation and is able to be maintained over the course of several hours. In addition, the type of protein and the serving size ingested may impact MPS and the overall balance of muscle Protein Breakdown (PPB). While our results presented herein demonstrate both CKP and BFP to have significantly elevated levels of leucine, EAA, and SAA in circulation within an hour following ingestion, the response of CKP was

significantly greater than BFP. Therefore, based on the bioavailability and observed pharmacokinetics from the present study, we conclude that CKP is potentially a more effective protein source and is more bioavailable for increasing MPS and enhancing recovery as compared to BFP. This was a small study and future studies may consider a larger study population. Future studies should may also consider applications for a variety of populations, particularly the elderly and those at risk for muscle loss who may benefit from a rapidly absorbed and easily consumable protein source. In addition, comparisons to a variety of protein sources would add valuable information to the literature.

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Author Contributions

Douglas Kalman-study design, data interpretation, manuscript writing. Susan Hewlings-data interpretation, manuscript writing. Robin Lee-study design, study conduct, project management. Jacob Bentley-study design, clinical conduct. Richard Foster-study design, clinical conduct. Kayce Morton, study design, principal investigator. Darryn S. Willoughby - data interpretation, manuscript writing.

Conflicts of Interest

Douglas Kalman, Robin Lee, Jacob Bentley, Richard Foster were employed by QPS at the time of this study, the Contract Research Organization that received funding from the sponsor to conduct the study.

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