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Citation for published version (APA):

Pinckaers, P. J. M., Hendriks, F. K., Hermans, W. J. H., Goessens, J. P. B., Senden, J. M., van Kranenburg, J. M. X., Wodzig, W. K. H. W., Snijders, T., & van Loon, L. J. C. (2022). Potato Protein Ingestion Increases Muscle Protein Synthesis Rates at Rest and during Recovery from Exercise in Humans. *Medicine and Science in Sports and Exercise*, *54*(9), 1572-1581. https://doi.org/10.1249/MSS.0000000000002937

Document status and date:
Published: 01/09/2022

DOI:
10.1249/MSS.0000000000002937

Document Version:
Publisher's PDF, also known as Version of record

Document license:
Taverne

Please check the document version of this publication:

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OPEN

Medicine & Science in Sports & Exercise

The Official Journal of the American College of Sports Medicine
www.acsm-msse.org

. . . Published ahead of Print

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[1]Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, Maastricht, THE NETHERLANDS; [2]Central Diagnostic Laboratory, Maastricht University Medical Centre+, Maastricht, THE NETHERLANDS

Accepted for Publication: 25 March 2022

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Medicine & Science in Sports & Exercise, Publish Ahead of Print
DOI: 10.1249/MSS.0000000000002937

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Corresponding author: Prof. Luc J.C. van Loon, Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, PO box 616, 6200 MD Maastricht, The Netherlands, Tel: +31 43 388 1397, E-mail: l.vanloon@maastrichtuniversity.nl

Clinical Trial Registry number: Nederlands Trial Register: NTR7152. https://www.trialregister.nl/

Conflicts of Interest and Funding Statement: This study was partially funded by APRE (Alliance for Potato Research & Education), Chicago, IL, and AVEBE, Veendam, The

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Netherlands. LJCvL has received research grants, consulting fees, speaking honoraria, or a combination of these from Friesland Campina, Tereos Syral, and Pepsico. PJMP, FKH, WJHH, JPBG, JMS, JMXvK, WKHW, and TS report no conflicts of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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ABSTRACT

Introduction: Plant-derived proteins have received considerable attention as an alternative to animal based proteins and are now frequently used in both plant-based diets and sports nutrition products. However, little information is available on the anabolic properties of potato-derived protein. This study compares muscle protein synthesis rates following the ingestion of 30g potato protein versus 30g milk protein at rest and during recovery from a single bout of resistance exercise in healthy, young males. **Methods:** In a randomized, double blind, parallel-group design, 24 healthy young males (24±4y) received primed continuous L-[*ring*-$^{13}C_6$]-phenylalanine infusions while ingesting 30g potato derived protein or 30g milk protein following a single bout of unilateral resistance exercise. Blood and muscle biopsies were collected for 5 hours following protein ingestion to assess post-prandial plasma amino acid profiles and mixed muscle protein synthesis rates at rest and during recovery from exercise. **Results:** Ingestion of both potato and milk protein increased mixed muscle protein synthesis rates when compared to basal post-absorptive values (from 0.020±0.011 to 0.053±0.017 $\%\cdot h^{-1}$ and from 0.021±0.014 to 0.050±0.012 $\%\cdot h^{-1}$, respectively ($P<0.001$)), with no differences between treatments ($P=0.54$). In the exercised leg, mixed muscle protein synthesis rates increased to 0.069±0.019 and 0.064±0.015 $\%\cdot h^{-1}$ after ingesting potato and milk protein, respectively ($P<0.001$), with no differences between treatments ($P=0.52$). The muscle protein synthetic response was greater in the exercised compared with the resting leg ($P<0.05$). **Conclusions:** Ingestion of 30g potato protein concentrate increases muscle protein synthesis rates at rest and during recovery from exercise in healthy, young males. Muscle protein synthesis rates following the ingestion of 30g potato protein do not differ from rates observed after ingesting an equivalent amount of milk protein.

Key Words: MUSCLE PROTEIN SYNTHESIS, MILK PROTEIN, RESISTANCE EXERCISE, SPORTS NUTRITION

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INTRODUCTION

Protein ingestion (1-3) and physical activity (4) stimulate muscle protein synthesis and are essential for the maintenance and accretion of skeletal muscle mass. Protein ingested during recovery from exercise further augments muscle protein synthesis rates (5-7) and supports the skeletal muscle adaptive response to more prolonged exercise training (8). The muscle protein synthetic response to protein ingestion is driven by the post-prandial increase in circulating essential amino acids (EAA) concentrations (9), with plasma leucine being of particular relevance (10-12). Post-prandial muscle protein synthesis rates at rest and during recovery from exercise have been reported to differ substantially following ingestion of different protein sources (13-15). The anabolic properties of a protein source is largely determined by its protein digestion and amino acid absorption kinetics, as well as the amino acid composition of the protein (9, 16-18).

Our habitual protein intake originates from both animal- and plant-based sources (19, 20). In general, plant-based proteins are considered to provide a lesser anabolic stimulus following ingestion when compared to animal based proteins. This is mainly attributed to their lower digestibility and incomplete amino acid (AA) profile, characterized by low leucine, lysine, and/or methionine contents (19, 20). Plant-based proteins already comprise a large part of our daily protein intake, but their contribution will become much greater due to the growing interest in consuming more plant-based diets and plant-based proteins (21). The trend of consuming a more plant-based diet has also reached the athletic community, where sports supplements containing whey or egg protein are now frequently traded in for supplements providing plant-derived protein isolates or concentrates. Despite their popularity, only few studies have actually compared the anabolic properties of animal *vs* plant-derived proteins (15, 22-24). Lesser anabolic properties have

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been reported following soy (15, 23, 24) and wheat (25) protein ingestion when compared to dairy protein both at rest and/or during recovery from exercise. However, these differences are not always apparent (15, 26, 27). As there is a large variety in plant-derived protein characteristics (28), more plant-derived proteins should be evaluated for their anabolic properties both at rest and during recovery from exercise.

Potatoes are the third most consumed crop worldwide (29, 30). Potatoes contain a mere ~1.5% protein based on their fresh weight (30). However, when potatoes are used for starch extraction, a residue remains (potato fruit juice) which is generally used for feed production or discarded as a waste product. From this residue a potato protein concentrate can be extracted (31). We previously identified the amino acid profile of potato derived protein along with various other plant-based protein sources (28). The analysis revealed an amino acid composition of potato protein that closely resembles milk protein. In contrast to most other plant-derived proteins, potato protein provides sufficient amounts of all individual essential amino acids according to the WHO/FAO/UNU amino acid requirements, with no apparent deficiencies (28). However, whether this favorable amino acid profile of potato derived protein also translates to strong anabolic properties upon ingestion remains to be established.

We hypothesize that ingestion of 30 g potato protein concentrate increases muscle protein synthesis rates both at rest and during recovery from exercise in healthy, young men. Furthermore, we hypothesize that the muscle protein synthetic response following the ingestion of 30 g potato protein does not differ from the ingestion of 30 g milk protein. To test our hypotheses, we assessed post-absorptive and post-prandial muscle protein synthesis rates following ingestion of either 30

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g potato or milk derived protein concentrate following a single bout of unilateral resistance exercise in 24 healthy, young males.

METHODS

Participants

Twenty-four healthy, recreationally active males (24±4 y; 1.79±0.07 m; 72.4±7.1 kg) volunteered to participate in this parallel group, double blind, randomized, controlled trial (subjects' characteristics are presented in Table 1). The trial was registered at the Netherlands Trial Register (NTR7152), and was conducted between April 2018 and February 2020 at Maastricht University in Maastricht, The Netherlands (See Supplemental Figure 1, Supplemental Digital Content, for the CONSORT (Consolidated Standards of Reporting Trials) flow diagram, http://links.lww.com/MSS/C572). All participants were informed on the purpose of the study, the experimental procedures, and possible risks before providing informed written consent to participate. The procedures followed were in accordance with the ethical standards of the medical ethics committee of the Maastricht University Medical Centre+ (METC 173053) on research involving human participants, and in accordance with the Helsinki Declaration of 1975 as revised in October 2013. The study was independently monitored by the Clinical Trial Centre Maastricht (CTCM).

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Preliminary screening

Participants aged 18-35 y, with BMI >18.5 and <27.5 kg·m^{-2} underwent an initial screening session to assess eligibility. For this purpose, height, weight, blood pressure and body composition (by dual-energy X-ray absorptiometry; Discovery A, Hologic) were determined. Participants were

deemed healthy based on their responses to a medical questionnaire, and were excluded from participation when smoking, performing progressive resistance exercise training, were using medication that affected protein metabolism, or were intolerant to the investigated protein products. Following initial screening, the participants were familiarized with the exercise testing protocol and the exercise equipment. Unilateral 1 repetition maximum (1-RM) strength was assessed for both legs separately, on the supine leg press (Technogym BV, Capelle aan den Ijssel, the Netherlands) and seated knee extension (Technogym BV) exercise using the multiple repetition testing procedure (32). Before testing, participants performed a unilateral warm-up at low resistance for 20 repetitions to become familiarized with the equipment and to have exercise technique assessed and adjusted. Working sets of 8 repetitions were then performed with progressively increased loads until failure, to perform a valid estimation within 1-8 repetitions of the set. A repetition was considered valid if the subject was able to complete it in a controlled manner. A 2-min rest period was allowed between successive sets. In between the screening session and the experimental trial, subjects reported to the laboratory for an additional visit to perform a true 1-RM strength test.

1-RM strength test

During this visit, the participant's unilateral 1-RM strength was determined for each leg separately, starting with the supine leg press, followed by the seated knee extension. The estimated 1-RM obtained during the screening visit was used to determine the initial load for the actual 1-RM test (33). Before testing each exercise, participants performed 2 sets of unilateral warm-up at low weight. First 20 repetitions at 25% of the estimated 1-RM followed by 8 repetitions at 50% of the estimated 1-RM. During these sets, the exercise technique was again closely assessed and adjusted

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when necessary. Following warm-up, the 1-RM was determined based on the protocol described by Kraemer and Fry (34). In short, for the first attempt, the load was set at 90% of the estimated 1-RM and was increased by 2.5-5% after each successful lift until failure. A 2-min rest period was allowed between successive attempts. A lift was deemed successful when performed in a controlled manner, without assistance, and for the full range of motion. The range of motion for the supine leg press started at a knee angle of 70° until full extension (without locking the knee), for the seated knee extension, the knee angle was set from 70° to 160°. The 1-RM testing and experimental trials were separated by at least 3 days.

Study design

Participants were randomly assigned to ingest a 400 mL beverage containing 30 g potato protein (POTATO) or 30 g milk protein (MILK). After beverage ingestion, the bottle was rinsed with 150 mL of water. Potato protein concentrate (Solanic 100) was supplied by AVEBE (Veendam, the Netherlands) and milk protein concentrate (MPC80) was obtained from FrieslandCampina (Wageningen, the Netherlands). Participants were allocated to a treatment according to a block randomization list performed using a computerized randomizer (http://www.randomization.com/). An independent researcher was responsible for random assignment (n=12 per group) and preparation of the study treatment beverages, which were sequentially numbered according to subject number. The beverages were prepared in non-transparent protein-shakers.

Diet and physical activity

Participants refrained from sports and strenuous physical activities (such as heavy lifting), and alcohol consumption for 3 days prior to the experimental trial. In addition, all participants filled

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out a food and activity diary for 3 days prior to the experimental trial. For the evening before the trial, all participants consumed the same standardized meal containing 2.3 MJ, with 20% energy provided as carbohydrate, 65% as fat, and 15% as protein, before 22:00 after which they remained fasted.

Experimental protocol

At ~7:30 AM, participants arrived at the laboratory in the overnight fasted state. A catheter was inserted into an antecubital vein for stable isotope amino acid infusion, while a second catheter was inserted retrogradely into a dorsal hand vein of the contralateral arm for arterialized blood sampling. To obtain arterialized blood samples, the hand was placed in a hot box (60°C) for 10 min prior to each blood sample collection (35).

After taking a baseline blood sample (t= -180 min), the plasma phenylalanine pool was primed with a single dose of L-[*ring*-$^{13}C_6$]-phenylalanine (2.25 µmol/kg). Thereafter, a continuous intravenous infusion of L-[*ring*-$^{13}C_6$]-phenylalanine (0.05 µmol/kg/min) was initiated (t= -180 min) using a calibrated IVAC 598 pump (San Diego, CA). While resting in a supine position, arterialized blood samples were collected in EDTA containing tubes 60 and 120 min (t= -120 and t= -60 respectively) following initiation of the tracer infusion. At 130 min (t= -50) the unilateral exercise session commenced. Following the exercise session (t= -10 min) the participants returned to the resting position. At t= 0 min an arterialized blood sample was obtained as well as bilateral muscle biopsy samples from the *M. vastus lateralis* of the rested and exercised leg. Immediately following the muscle biopsy, participants ingested the beverage corresponding to their randomized treatment allocation i.e.: POTATO (*n*=12) or MILK (*n*=12). To minimize dilution of the steady-

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state plasma L-[*ring*-$^{13}C_6$]-phenylalanine precursor pool, 3.85% L-[*ring*-$^{13}C_6$]-phenylalanine was added to the drinks. Arterialized blood samples were then collected at t= 15, 30, 45, 60, 90, 120, 150, 180, 210, 240 and 300 min into the postprandial period. A second and third muscle biopsy were collected at t= 120 and 300 min to determine postprandial muscle protein synthesis rates from 0-120, 120-300, and 0-300 min. Muscle biopsy collection was performed from both the rested and exercised leg during each time point, starting with the exercised leg. Blood samples were collected into EDTA-containing tubes and centrifuged at 1200*g* for 10 min at 4°C. Aliquots of plasma were frozen in liquid nitrogen and stored at -80°C. Biopsy samples were collected with the use of a 5 mm Bergström needle (36) custom-adapted for manual suction. Samples were obtained from separate incisions from the middle region of the *M. vastus lateralis*, ~15 cm above the patella and ~3 cm below entry through the fascia. Local anesthetic (1% Xylocaine with adrenaline 1:100,000) was applied to numb the skin and fascia. Muscle samples were freed from any visible non-muscle material, immediately frozen in liquid nitrogen, and stored at -80°C until further processing. When the experimental protocol was complete, cannulae were removed and participants were fed and assessed for ~30 min before leaving the laboratory. For a schematic representation of the infusion protocol, see Figure 1.

Exercise protocol

Participants began with a standardized warm-up on the supine leg press (20 repetitions at 25% 1-RM followed by 8 repetitions at 50% 1-RM) followed by 3 sets of 8 repetitions at ~80% 1-RM. For the 4th set, participants were instructed to perform as many repetitions as possible. Participants then carried out the same exercise protocol (i.e., same warm-up, number of sets and repetitions at percentage of estimated 1-RM) on the seated knee-extension machine. Each set was separated by

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2 min of passive recovery during which the participant remained seated. Strong verbal encouragement was provided by 1 of the study investigators during each set. Participants were randomly allocated to perform the exercise session with their dominant or non-dominant leg. The randomization scheme ensured an equal amount of participants performed the exercise with the dominant (n=6) as well as non-dominant (n=6) leg within each interventional group (n=12).

Dietary protein analysis

Batch specific nitrogen contents of both potato and milk were provided by the manufacturer. Milk protein content was determined as nitrogen content x6.38 (37, 38) and potato protein content as nitrogen content x6.25 (39). Amino acid contents in protein were determined by acid hydrolysis in triplicate. Specifically, the amino acids were liberated from the protein powders (~4 mg) by adding 2 mL of 6M HCl and heating to 110°C for 12 h. The hydrolysed proteins were subsequently dried under a nitrogen stream while heated to 120°C. Before analysis, the hydrolysate was dissolved in 5 mL of 0.1 M HCl and 20 µL of AccQ/Tag derivatizing reagent solution (Waters, Saint/Quentin, France) was added as described here below for the plasma amino acid concentration analysis. Amino acid composition of the proteins are presented in Table 2.

Plasma analysis

Plasma glucose and insulin concentrations were analyzed using commercially available kits (ref. no. A11A01667, Glucose HK CP, ABX Diagnostics, Montpellier, France; and ref. no. HI-14K, Millipore, St. Louis, MO, respectively). Plasma L-[*ring*-$^{13}C_6$]-phenylalanine, enrichments were determined by gas chromatography-mass spectrometry (GC-MS; Agilent 7890A GC/5975C MSD; Agilent Technologies). Specifically, the plasma was deproteinized on ice with dry 5-sulfosalicyclic

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acid. Free amino acids were purified using cation exchange AG 50W-X8 resin (mesh size: 100-200, ionic form: hydrogen (Bio-Rad Laboratories, Hercules, CA, USA)) columns. The free amino acids were converted to their tert-butyl dimethylsilyl (TBDMS) derivative before analysis by GC-MS using selected ion monitoring of masses 336 and 342 for unlabeled and labeled L-[*ring*-$^{13}C_6$]-phenylalanine, respectively. Standard regression curves were applied from a series of known standard enrichment values against the measured values to assess the linearity of the mass spectrometer and to account for any isotope fraction which may have occurred during the analysis.

In order to determine basal mixed muscle fractional synthetic rate (FSR), the single biopsy approach was applied as described by Burd *et al.* 2012 (40). In short, plasma protein obtained prior to tracer infusion (t= -180 min) was used to determine baseline L-[*ring*-$^{13}C_6$]-phenylalanine enrichments. For this purpose, the plasma sample was precipitated by adding perchloric acid. Subsequently, similarly as for the mixed muscle protein fraction, the denaturized plasma protein pellet was hydrolyzed, passed over a Dowex exchange resin, and the resulting amino acid samples were derivatized to their N(O,S)-ethoxycarbonyl-ethylesters before being measured by GC-IRMS, as explained below.

Plasma amino acid concentrations were determined by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS; ACQUITY UPLC H-Class with QDa; Waters, Saint-Quentin, France). Specifically, 50 µL blood plasma was deproteinized using 100 µL of 10% SSA with 50 µM of MSK-A2 internal standard (Cambridge Isotope Laboratories, Massachusetts, USA). Subsequently, 50 µL of ultra-pure demineralized water was added and samples were centrifuged. After centrifugation, 10 µL of supernatant was added to 70 µL of Borate reaction

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buffer (Waters, Saint-Quentin, France). In addition, 20 µL of AccQ/Tag derivatizing reagent solution (Waters, Saint/Quentin, France) was added after which the solution was heated to 55°C for 10 min. Of this 100 µL derivative 1 µL was injected and measured using UPLC-MS.

Muscle analysis

A piece of wet muscle (~50-70 mg) was freeze dried for 48 h. Collagen, excessive blood and other non-muscle materials were subsequently removed from the muscle fibers under a light microscope. The isolated muscle fiber mass was weighed and 35 volumes (7x wet weight of isolated muscle fibers x wet-to-dry ratio 5:1) of ice-cold 2% perchloric acid (PCA) was added. Thereafter, the tissue was homogenized by sonification, and centrifuged to separate the supernatant from the protein pellet. The supernatants containing the muscle tissue free amino acids were purified, and derivatized before analysis by GC-MS, similarly as for the plasma L-[*ring* $^{13}C_6$]-phenylalanine enrichments. The protein pellet was washed 3 times with 1 mL 2% PCA. The amino acids were liberated from the mixed muscle enriched protein fraction by adding 2 mL of 6M HCl and heating to 110°C for 15.5 h. The hydrolysed mixed muscle protein fractions were dried under a nitrogen stream while heated to 110°C. The dried mixed muscle protein fraction was dissolved in a 50% acetic acid solution. The amino acids from the mixed muscle protein fraction were passed over a Dowex exchange resin (AG 50W-X8, 100-200 mesh hydrogen form; Bio-Rad, Hercules, CA, USA) using 2M NH4OH. Subsequently, the purified amino acid solution was dried under a nitrogen stream at room temperature, followed by derivatization to their N(O,S)-ethoxycarbonyl-ethylesters. The ratio of $^{13}C/^{12}C$ of mixed muscle protein-bound phenylalanine was determined using gas chromatography-combustion-isotope ratio mass spectrometry (GC-IRMS; Delta V, Thermo Scientific, Bremen, Germany) by monitoring ion masses 44, 45 and 46. Standard

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regression curves were applied from a series of known standard enrichment values against the measured values to assess the linearity of the mass spectrometer and to account for any isotope fractionation which may have occurred during the analysis.

Calculations

The FSR ($\%\cdot h^{-1}$) of mixed muscle protein enriched fractions was calculated by the standard precursor-product equation (41):

$$FSR = \left(\frac{(E_{b2} - E_{b1})}{(E_{precursor} \cdot t)}\right) \cdot 100$$

Where E_b is the increment in mixed muscle protein bound L-[*ring*-$^{13}C_6$]-phenylalanine enrichment (mole % excess) during the tracer incorporation period, and t is the tracer incorporation time in h. Weighted mean plasma enrichments were calculated by taking the measured enrichment between consecutive time points and correcting for the time between these sampling time points ($E_{precursor}$). For calculation for postprandial FSR, biopsy samples at t= 0, 120 and 300 min were used. For the calculation of basal FSR, E_{b2} represented the protein bound L-[*ring*-$^{13}C_6$]-phenylalanine enrichments in the muscle of the rested leg at t= 0 min, and E_{b1} represented the protein bound L-[*ring*-$^{13}C_6$]-phenylalanine enrichments in plasma albumin at t= -180 min.

Net incremental area under curve (iAUC) was determined for plasma amino acid concentrations during the 5 h post-prandial period following protein ingestion. The iAUC was calculated using the trapezoid rule, with plasma concentrations before beverage ingestion (*t*= 0 min) serving as baseline.

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Outcome measures

The primary outcome measure is mixed muscle FSR over the aggregate (i.e. 0-300 min) postprandial period, comparing POTATO *vs* MILK in the rested and exercised leg. Secondary outcome measures were mixed muscle FSR changes from basal (i.e. -180 – 0 min and 0-300 min) and changes from basal to the early and late postprandial period (i.e. -180 – 0 min, 0 – 120 min, and 120 – 300 min), comparing POTATO *vs* MILK in the rested and exercised leg. Additional secondary outcome measures were plasma glucose, insulin and amino acid concentrations, and plasma amino acid iAUC. Plasma glucose, insulin, and amino acid peak concentrations and time to peak were tertiary outcomes.

Statistical analysis

A power calculation was performed with differences in postprandial muscle FSRs between the two interventional groups as primary outcome measure. A sample size of 12 participants per treatment, including a 10% dropout rate was calculated using a power of 80%, a significance level of 0.05, a standard deviation of 0.0065 $\%\cdot h^{-1}$, and a difference in FSR of 0.008 $\%\cdot h^{-1}$ between treatments (or ~20% when expressed as a relative difference). Participants' characteristics, were analyzed by an independent samples T-test. Plasma glucose, insulin, and amino acid concentrations and amino acid enrichments were analyzed by a 2-factor (*treatment x time*) repeated measures ANOVA. Plasma amino acid iAUC as well as plasma glucose, insulin, and amino acid peak concentrations and time to peak were analyzed by an independent samples T-test. Basal post-absorptive mixed muscle protein synthesis rates for the rested leg were analyzed by an independent samples T-test. Similarly, post-prandial mixed muscle protein synthesis rates during the early (0-120 min) and entire (0-300 min) postprandial period were analyzed by independent samples T-test for MILK *vs*

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POTATO in the rested leg as well as exercised leg. Changes is muscle protein synthesis rates over time (-180 – 0; 0-120; 120-300) were analyzed by a 2-factor repeated measures ANOVA. For the repeated measures ANOVA tests, Bonferroni post hoc analysis were performed whenever a significant F ratio was found to isolate specific differences. Statistical analyses were performed with a software package (IBM SPSS statistics for Windows, version 26.0, IBM Corp., Armonk, NY, USA). Means were considered to be significantly different for *P* values <0.05.

RESULTS

Plasma glucose and insulin concentrations

No significant changes in plasma glucose concentrations were observed following protein ingestion, with no differences between interventions (*time x treatment*: *P*=0.12, Figure 2a). Plasma insulin concentrations increased following MILK but not following POTATO ingestion (*time x treatment:* *P*<0.001), with a modest peak value of 26±12 $mU \cdot L^{-1}$ achieved 30 min after MILK ingestion (Figure 2b).

Plasma AA concentrations

Plasma EAA concentrations increased following protein ingestion (Figure 3a), with a delayed and smaller post-prandial rise following POTATO compared with MILK ingestion (*time x treatment:* *P*<0.001). Overall, plasma EAA concentrations were 16% lower following POTATO *vs* MILK protein ingestion (incremental area under curve (iAUC): 108±20 *vs* 129±29 $mmol \cdot 300\ min \cdot L^{-1}$ respectively, *P*=0.04, Figure 3b). The lower post-prandial EAA availability was also accompanied by 22% lower peak EAA concentrations (1402±118 *vs* 1788±250 $\mu mol \cdot L^{-1}$ respectively, *P*<0.001), that were reached 143±54 and 48±27 min following POTATO *vs* MILK ingestion (*P*<0.001).

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The post-prandial rise in circulating plasma leucine (Figure 3c), lysine (Figure 3e) and methionine (Figure 3g) concentrations was delayed and smaller following POTATO when compared with MILK ingestion (*time x treatment:* all P<0.001). Post-prandial plasma leucine (Figure 3d), lysine (Figure 3f), and methionine (Figure 3h) availability were respectively 23, 21, and 67% lower for POTATO when compared with MILK (iAUC: 27±4 *vs* 35±8, 15±4 *vs* 19±5, and 1±1 *vs* 3±2 mmol·300 min·L^{-1} , respectively; all P<0.05). Peak values were also respectively 26, 29 and 41% lower for POTATO *vs* MILK (252±23 *vs* 341±65, 247±34 *vs* 347±43, and 34±3 *vs* 58±11 µmol·L^{-1}, respectively; all P<0.001). Time to reach peak values was significantly longer for POTATO when compared to MILK ingestion (153±50 *vs* 48±27, 100±35 *vs* 40±20, and 103±41 *vs* 40±23 min, respectively; all P<0.001).

In general, all post-prandial plasma amino acid concentrations revealed similar differences between treatments (see Supplemental Figure 2, Supplemental Digital Content, Post-prandial plasma amino concentrations, http://links.lww.com/MSS/C572, *time x treatment:* all P<0.05). The overall proline and valine concentrations (iAUC) were lower for POTATO *vs* MILK, while the overall glycine concentrations were higher for POTATO *vs* MILK (Supplemental Figure 2, Supplemental Digital Content, http://links.lww.com/MSS/C572). Collectively, when evaluating the total sum of all amino acids (TAA), the post-prandial increase over time differed significantly between protein sources (*time x treatment*: P<0.001), with a trend towards overall lower plasma amino acid availability following POTATO *vs* MILK ingestion (iAUC: 115±43 *vs* 147±47 mmol·300 min·L^{-1} respectively; P=0.095). In line, peak TAA concentrations were 37% lower for POTATO *vs* MILK (2884±230 *vs* 3626±440 µmol·L^{-1} respectively; P<0.001) and were reached 118±56 and 43±24 min after protein ingestion, respectively (P<0.001).

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Plasma and muscle L-[*ring*-$^{13}C_6$]-phenylalanine enrichments

Plasma phenylalanine concentrations and L-[*ring*-$^{13}C_6$]-phenylalanine enrichments over time are presented in Figure 4a and 4b, respectively. Plasma L-[*ring*-$^{13}C_6$]-phenylalanine enrichments over time were higher during the first 60 min and lower during the last 150 min following POTATO *vs* MILK ingestion (*time x treatment:* $P<0.001$). Weighted mean plasma L-[*ring*-$^{13}C_6$]-phenylalanine enrichments averaged 6.63±0.46 and 6.75±0.55 MPE during the basal post-absorptive period ($P=0.55$), and 6.26±0.41 and 6.59±0.48 MPE during the post-prandial period ($P=0.09$) for POTATO and MILK respectively.

In the rested leg, mixed muscle protein-bound L-[*ring*-$^{13}C_6$]-phenylalanine enrichments increased following ingestion of POTATO and MILK from 0.0046±0.0028 and 0.0046±0.0029 MPE (at t=0 min, $P=0.99$), to 0.0135±0.0058 and 0.0124±0.0041 MPE (at t= 120 min, $P=0.61$) reaching 0.0230±0.0076 and 0.0220±0.0052 MPE, respectively, at 300 min after protein ingestion (at t=300 min, $P=0.72$; *time x treatment:* $P=0.70$).

In the exercised leg, mixed muscle protein-bound L-[*ring*-$^{13}C_6$]-phenylalanine enrichments increased following POTATO and MILK ingestion from 0.0050±0.0036 and 0.0044±0.0026 MPE (at t=0 min, $P=0.68$), to 0.0156±0.0063 and 0.0128±0.0035 MPE (at t= 120 min, $P=0.20$) reaching 0.0280±0.0096 and 0.0262±0.0049 MPE, respectively, at 300 min after protein ingestion (at t=300 min, $P=0.56$; *time x treatment:* $P=0.50$). Collectively, 300 min after protein ingestion, the mixed muscle protein-bound L-[*ring*-$^{13}C_6$]-phenylalanine enrichments were higher in the exercised compared with the rested leg (both treatments; $P<0.05$).

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Muscle protein synthesis rates

In the rested leg, post-absorptive fractional mixed muscle protein synthesis rates averaged 0.020±0.011 and 0.021±0.014 $\%\cdot h^{-1}$ in the POTATO and MILK trial, respectively, with no differences between groups (*P*=0.88; Figure 5). POTATO and MILK ingestion both strongly increased mixed muscle protein synthesis rates (main effect of time *P*<0.001), with no *time x treatment* interaction (*P*=0.52) from the basal post-absorptive to 5 h post-prandial period. No differences in post-prandial mixed muscle protein synthesis rates were observed between POTATO and MILK ingestion during the early (e.g. 0-120 min; 0.051±0.019 and 0.055±0.017 $\%\cdot h^{-1}$ respectively; *P*=0.55) late (e.g. 120-300 min; 0.055±0.023 and 0.046±0.017 $\%\cdot h^{-1}$ respectively; *P*=0.33) or entire post-prandial period (e.g. 0-300 min; 0.053±0.017 and 0.050±0.012 $\%\cdot h^{-1}$ respectively; *P*=0.54).

In the exercised leg, post-absorptive mixed muscle protein synthesis rates averaged 0.023±0.015 and 0.023±0.017 $\%\cdot h^{-1}$ for POTATO and MILK respectively, with no differences between groups (*P*=0.97; Figure 5). POTATO and MILK ingestion both strongly increased mixed muscle protein synthesis rates following exercise (main effect of time *P*<0.001), with no *time x treatment* interaction (P=0.58) from the basal post-absorptive to 5 h post-prandial period. No differences in post-prandial muscle protein synthesis rates were observed following POTATO and MILK ingestion during the early (0.060±0.021 and 0.058±0.021 $\%\cdot h^{-1}$ respectively; *P*=0.74), late (0.071±0.031 and 0.065±0.021 $\%\cdot h^{-1}$ respectively; *P*=0.25), and entire post-prandial period (0.069±0.019 and 0.064±0.015 $\%\cdot h^{-1}$ respectively; *P*=0.52). Post-prandial muscle protein synthesis rates over the 5 h period following exercise were significantly higher in the exercised versus rested leg, for both treatments (*P*<0.05).

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Mixed muscle protein synthesis rates determined with the intra-cellular L-[*ring*-$^{13}C_6$]-phenylalanine enrichments used as precursor pool (see Supplemental Figure 3, Supplemental Digital Content, Muscle intracellular L-[*ring*-$^{13}C_6$] phenylalanine enrichments, http://links.lww.com/MSS/C572) resulted in similar findings with no differences between treatments (see Supplemental Figure 4, Supplemental Digital Content, Mixed muscle fractional synthetic rate, http://links.lww.com/MSS/C572).

DISCUSSION

The present study shows that ingestion of 30 g potato derived protein strongly increases muscle protein synthesis rates at rest and during recovery from exercise in healthy, young males. Despite the observation of a lesser and more delayed post-prandial rise in plasma essential amino acid availability following potato when compared to milk protein ingestion, post-prandial mixed muscle protein synthesis rates did not differ between protein sources at rest or during recovery from exercise.

The anabolic properties of plant-derived proteins are generally considered to be lower when compared to animal-derived proteins (19, 20). This has been, at least partly, attributed to plant-derived proteins providing overall less essential amino acids and the prevalence of one or more specific amino acid deficiencies in these proteins (19, 20). In contrast to many plant-derived proteins (28), we observed that potato derived protein provides sufficient amounts of all essential amino acids according to the WHO/FAO guidelines for protein requirements. In fact, 30 g of the applied potato derived protein was shown to provide similar amounts of essential amino acids (10.5 *vs* 10.7 g), leucine (2.6 *vs* 2.6 g), lysine (1.8 *vs* 2.1 g), and methionine (0.6 *vs* 0.6 g) when

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compared to the equivalent amount of milk protein (Table 2). Despite similar amino acid composition, the post-prandial rise in circulating (essential) amino acids was attenuated following the ingestion of potato compared with milk protein (Figure 3), resulting in lower peak essential amino acid, leucine, lysine, and methionine concentrations (-22, -26, -29, and -41%) that were reached at a much later point in time (+200, +221, +150, and +156 min, respectively). Consequently, post-prandial plasma amino acid availability was substantially lower throughout the 5 h post-prandial period following potato when compared with milk protein ingestion (Figure 3). Based on the phenylalanine tracer kinetics (Figure 4), we attribute this to a more delayed protein digestion and amino acid absorption, an increased amino acid retention in splanchnic tissues, and/or a less efficient digestion of potato compared with milk protein. As the intrinsically labeled protein approach (42) simply can't be applied in the case of plant-derived proteins, it is impossible to directly quantify the exact amount of potato protein derived amino acids that were released in the circulation, as we have done previously for milk (2) and mealworm derived protein (43).

Despite the attenuated postprandial rise in circulating amino acids following the ingestion of potato derived protein we observed a strong increase in muscle protein synthesis rates (Figure 5). A response that did not differ from the response observed after ingesting an equivalent amount of milk protein (Figure 5). Clearly, the provided potato derived protein is capable of strongly stimulating muscle protein synthesis *in vivo* in humans. Whether the absence of any differences in the anabolic response to potato versus milk protein ingestion can be attributed to the favorable amino acid profile of potato protein when compared to other plant-derived proteins remains unclear, as previous work (26, 27) but certainly not all studies (15, 23-25) have reported no differences in the anabolic response to the ingestion of similar amounts of plant- versus animal

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derived protein. Obviously, the observed post-prandial rise in circulating amino acids following the ingestion of 30 g potato protein concentrate was sufficient to elevate muscle protein synthesis rates. The more sustained release of amino acids throughout the latter stages of the post-prandial period may have compensated for a potential lesser initial increase in post-prandial plasma amino acid availability, allowing a post-prandial muscle protein synthetic response that did not differ from the ingestion of 30 g of milk protein. However, our data did not show an early attenuated post-prandial increase in muscle protein synthesis rates following potato protein when compared with milk protein ingestion, with FSR values calculated using plasma (Figure 5) and tissue free enrichments (Supplemental figure 3, Supplemental Digital Content, http://links.lww.com/MSS/C572) as precursor pools. As there was some initial disbalance between L-[*ring*-$^{13}C_6$]-phenylalanine release and overall phenylalanine kinetics (Figure 4B), we cannot exclude that this may have caused a minor overestimation of the early post-prandial FSR in the potato group.

Exercise has previously been shown to sensitize skeletal muscle tissue to the anabolic properties of protein ingestion (44). In the current study we applied a unilateral exercise design to allow assessment of post-prandial muscle protein synthesis rates both at rest as well as during recovery from exercise. We observed a strong increase in muscle protein synthesis rates in the exercised leg following both potato as well as milk protein ingestion (Figure 5), with responses that were greater when compared with the rested leg. Again, no differences were observed in post-exercise muscle protein synthesis rates following ingestion of 30 g potato versus 30 g milk protein. These data imply that plant-derived protein concentrates can be applied effectively to support post-exercise muscle conditioning in athletes. These findings are in contrast to some (15, 23, 24) but

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certainly not all (15, 26) studies comparing post-exercise muscle protein synthesis rates following soy compared with dairy protein ingestion. The apparent discrepancy may be, at least partly, explained by the amount of protein provided. In the present study we provided 30 g potato or milk protein, which is more than the amount of egg or milk protein (20 g) that has previously been suggested to be required to maximize post-exercise muscle protein synthesis rates (45). Though we can only speculate on the impact of ingesting smaller amounts of potato derived protein on post-prandial muscle protein synthesis rates, our data imply that a maximal post-exercise muscle protein synthetic response can be obtained by ingesting up to 30 g of a high quality plant-derived protein concentrate.

There is an increasing interest in the consumption of food products and sports supplements containing alternative, more sustainable, sources of protein (20). The present study extends on prior work evaluating the post-prandial and/or post-exercise muscle protein synthetic responses following soy and wheat derived protein ingestion (15, 23-27), showing that potato protein ingestion can strongly increase muscle protein synthesis rates at rest and during recovery from exercise. In support, Oikawa and colleagues (46) observed increases in daily muscle protein synthesis rates following more prolonged potato protein supplementation in females during a period of exercise training. Furthermore, increases in muscle mass and strength gains have been reported following both plant as well as animal derived protein supplementation during prolonged resistance type exercise training (47-50). The present data clearly show that there are ample opportunities for the use of plant-derived proteins in sports nutrition, but more research will be needed to evaluate the anabolic properties of the various plant-derived proteins that are currently available and their potential blends (27).

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CONCLUSIONS

In conclusion, ingestion of 30 g potato derived protein concentrate strongly increases muscle protein synthesis rates at rest and during recovery from exercise *in vivo* in healthy, young males. The post-prandial muscle protein synthetic response following the ingestion of 30 g potato protein does not differ from the response following ingestion of an equivalent amount of milk protein. Plant-derived proteins may be applied effectively in vegan protein products and sports nutrition supplements to support skeletal muscle conditioning during recovery from exercise.

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Acknowledgements

We thank Lisanne H.P. Houben for her medical assistance, Francesco Recchia for this practical assistance, and Annemarie P. Gijssen, Wendy E. Sluijsmans, and Hasibe Aydeniz for their analytical work. We also extend our gratitude to all study participants for their time and commitment.

This study was partially funded by APRE (Alliance for Potato Research & Education), Chicago, IL, USA and AVEBE, Veendam, The Netherlands.

Conflict of Interest

LJCvL has received research grants, consulting fees, speaking honoraria, or a combination of these from Friesland Campina, Tereos Syral, and Pepsico. PJMP, FKH, WJHH, JPBG, JMS, JMXvK, WKHW, and TS report no conflicts of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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FIGURE LEGENDS

FIGURE 1

Schematic representation of the experimental design.

FIGURE 2

Post-prandial plasma glucose (Panel a) and insulin (Panel b) concentrations during the 300 min period following the ingestion of POTATO *vs* MILK in 24 healthy young males (*n*=12 per group). Time 0 min represents time of beverage intake. POTATO: 30 g potato derived protein, MILK: 30 g milk protein. Values represent means ± standard deviation; Repeated measures ANOVA with time as within-subject variable and interventional drink (treatment) as between-subject variable. *Time x treatment:* Panel a: P=0.12; Panel b: P<0.001.

FIGURE 3

Post-prandial plasma essential amino acid (EAA, Panel a), leucine (Panel c), lysine (Panel e), and methionine (Panel g) concentrations during the 300 min post-prandial period following the ingestion of POTATO *vs* MILK. Time 0 min represents time of beverage intake. Panels b, d, f and h represent the 0-5 h incremental area under the curve (iAUC) following protein ingestion. POTATO: 30 g potato derived protein, MILK: 30 g milk protein. Values represent means ± standard deviation; *significantly different for POTATO *vs* MILK (P<0.05). Repeated measures ANOVA with time as within-subject variable and interventional drink (treatment) as between-subject variable. *Time x treatment:* Panel a, c, e, and g, all P<0.001.

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FIGURE 4

Post-prandial plasma phenylalanine concentrations (Panel a) and plasma L-[*ring*-$^{13}C_6$]-phenylalanine enrichments (Panel b) during the 300 min period following the ingestion of POTATO *vs* MILK in healthy, young males (*n*=12 per group). Time 0 min represents time of protein ingestion. POTATO: 30 g potato protein, MILK: 30 g milk protein. Values represent means ± standard deviation; * significantly different for MILK *vs* WHEAT ($P<0.05$). Repeated measures ANOVA with time as within-subject variable and interventional drink (treatment) as between-subject variable. *Time x treatment:* Panel a: $P<0.001$, Panel b: $P<0.001$.

FIGURE 5

Mixed muscle fractional synthetic rate (FSR) in the basal post-absorptive and post-prandial period following ingestion of POTATO *vs* MILK in the rested and exercised leg. POTATO: 30 g potato derived protein, MILK: 30 g milk protein, REST: rested leg, EXERCISE: exercised leg. Values represent means ± standard deviation. *significantly different from basal, $P<0.05$. #significantly different from rested leg, $P<0.05$. Independent samples *t*-test POTATO *vs* MILK: REST: $P=0.88$, $P=0.55$, and $P=0.54$ for basal, 0-120, and 0-300 respectively. EXERCISE: $P=0.97$, $P=0.73$, and $P=0.52$ for basal, 0-120, and 0-300 respectively.

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SUPPLEMENTAL DIGITAL CONTENT

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Figure 1. CONSORT flow diagram.

Figure 2. Post-prandial plasma amino concentrations during the 300 min post-prandial period following the ingestion of POTATO vs MILK.

Figure 3. Muscle intracellular L-[*ring*-$^{13}C_6$] Phenylalanine enrichments at different time points following ingestion of POTATO vs MILK

Figure 4. Mixed muscle fractional synthetic rate determined with intra-cellular enrichments as precursor pool at different time points following ingestion of POTATO *vs* MILK

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Figure 1

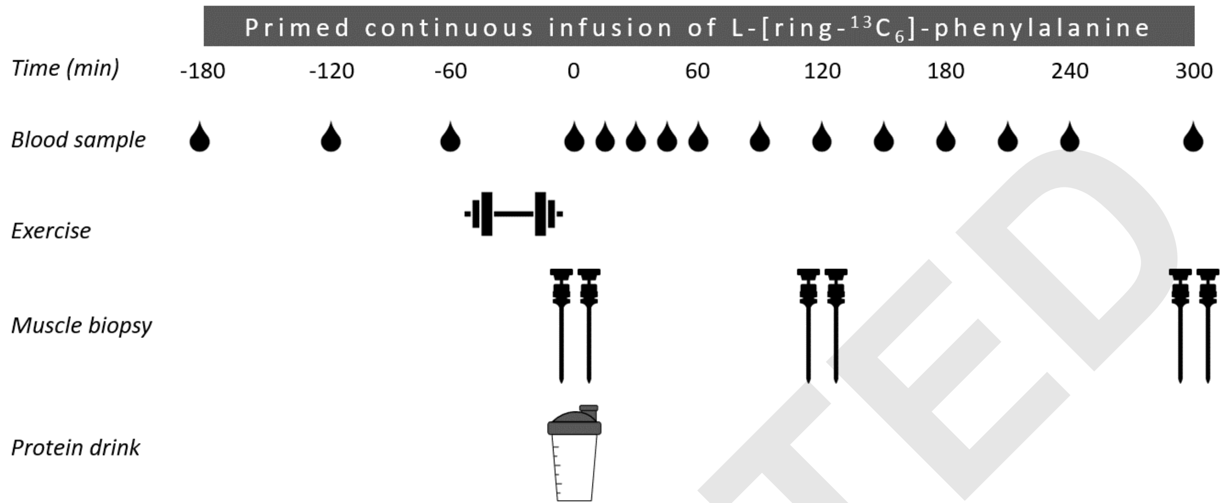

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Figure 2

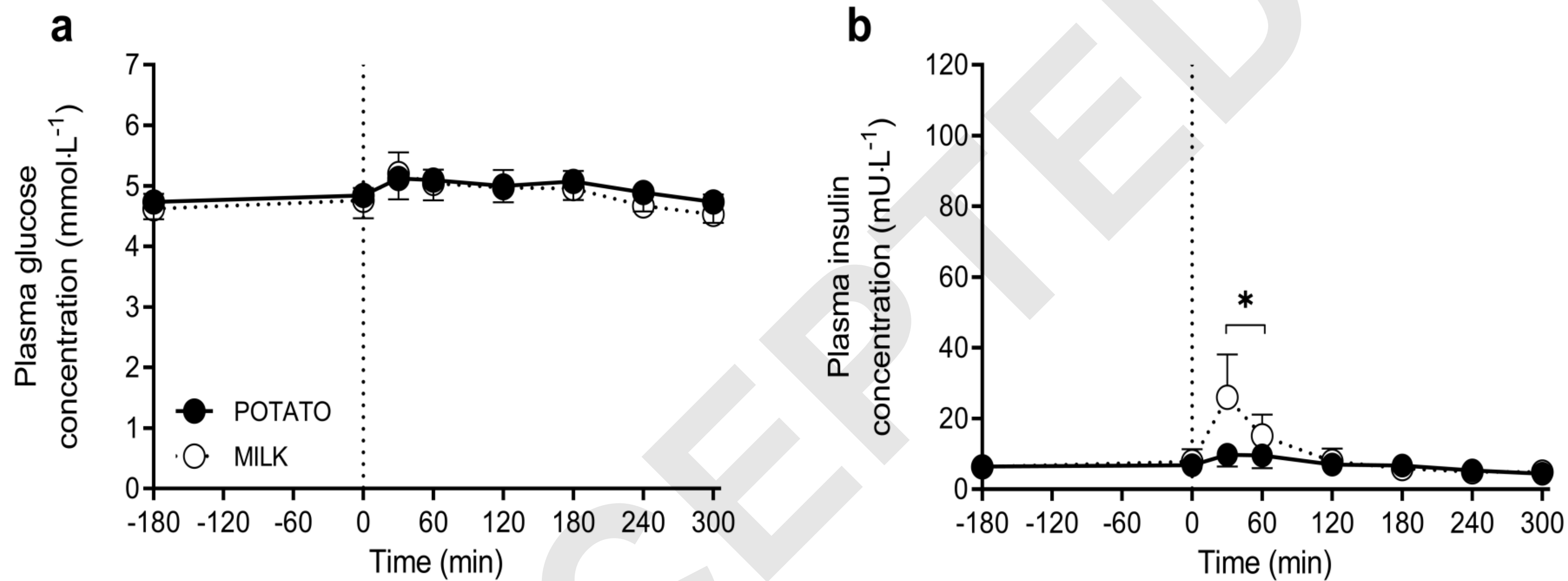

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Figure 3

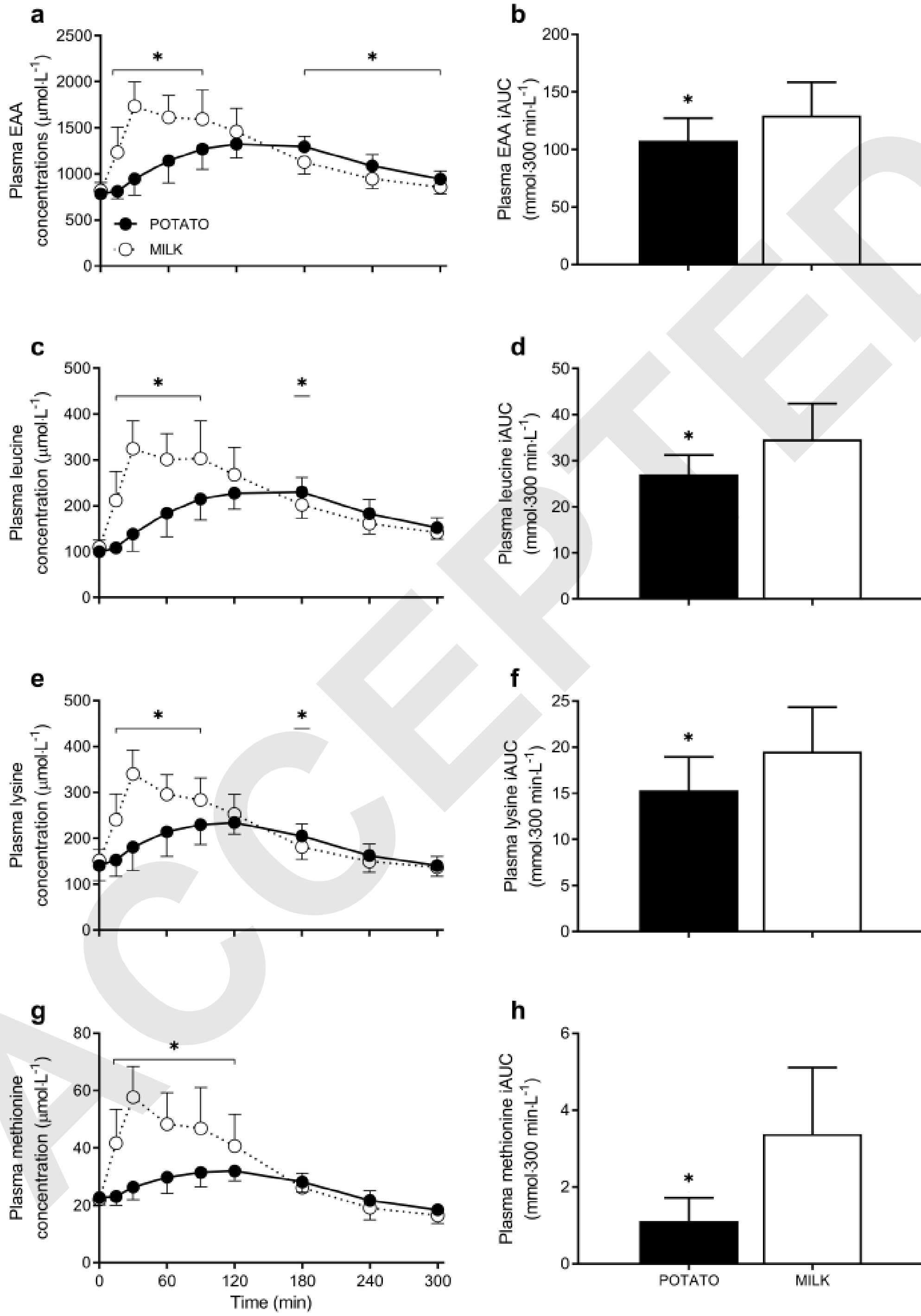

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Figure 4

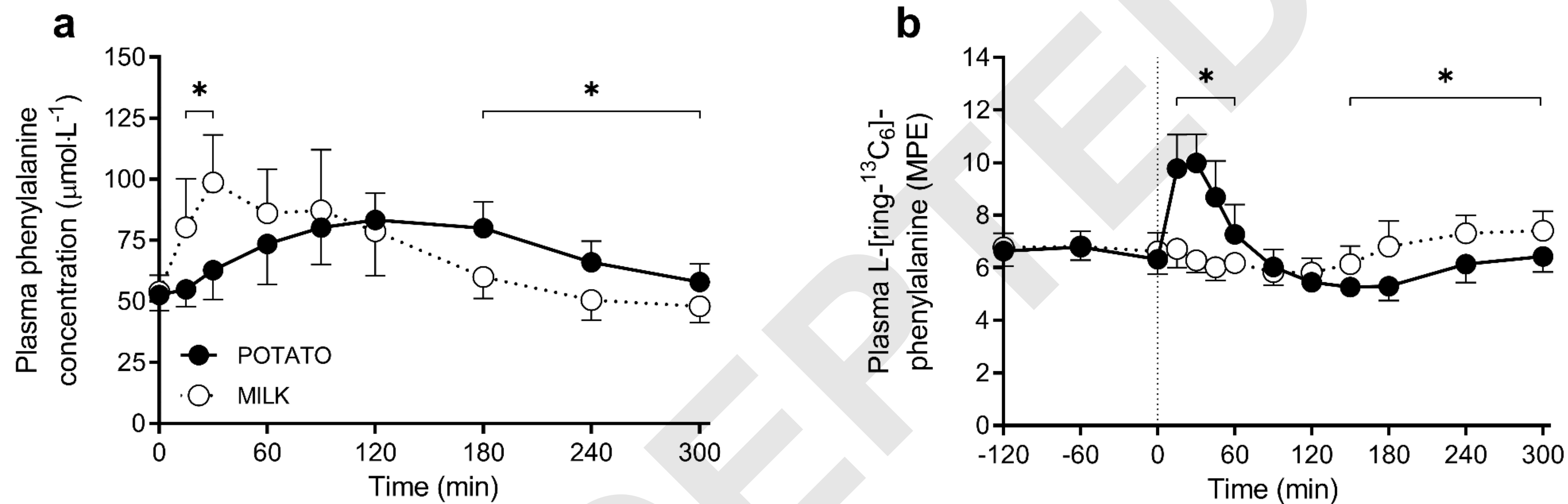

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Figure 5

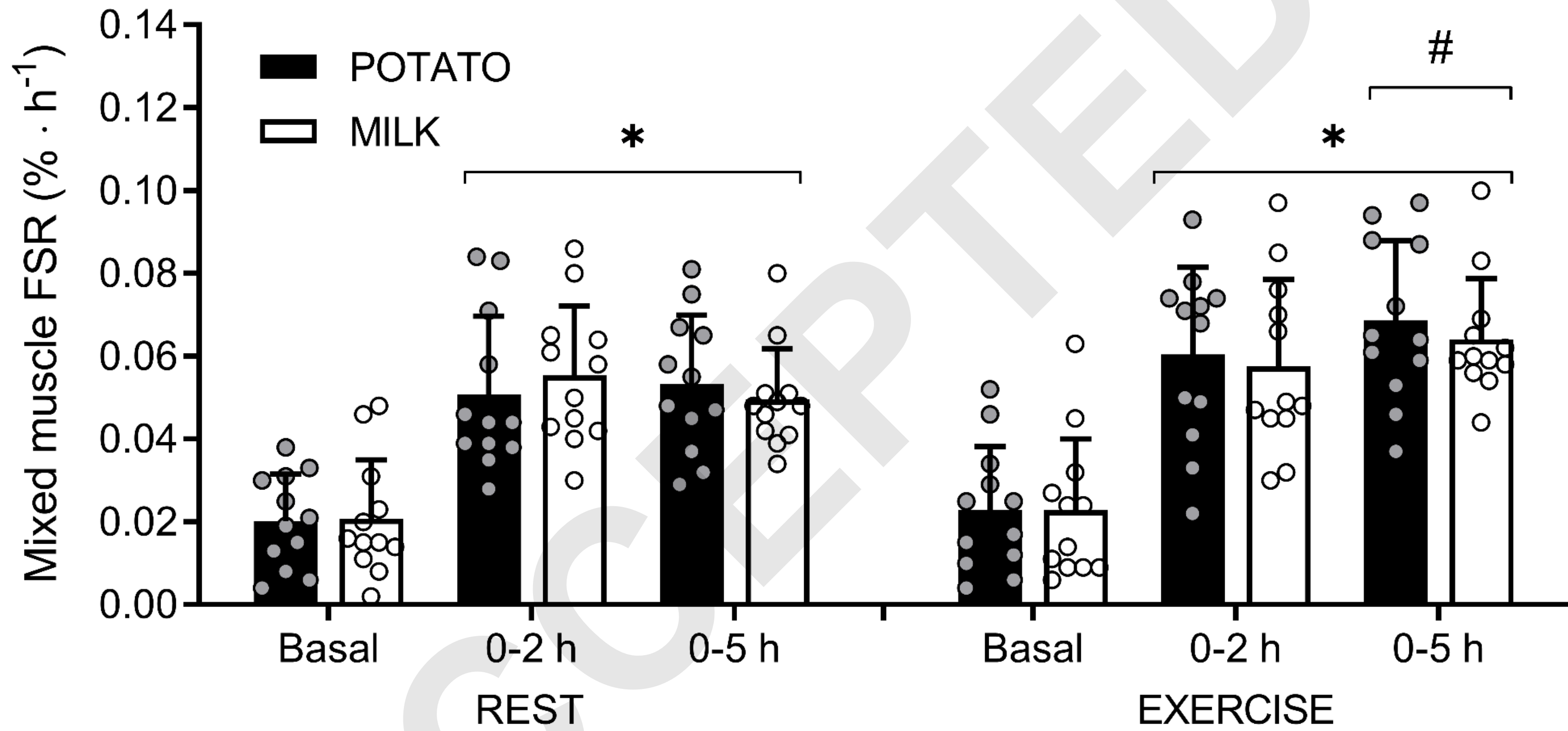

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TABLE 1. Participants' characteristics

	POTATO	MILK
Age (y)	23 ± 3	25 ± 4
Height (m)	1.81 ± 0.04	1.77 ± 0.8
Body mass (kg)	73.7 ± 6.4	71.2 ± 7.9
BMI (kg/m^2)	22.7 ± 1.4	22.7 ± 1.7
Systolic blood pressure (mmHg)	119 ± 11	119 ± 12
Diastolic blood Pressure (mmHg)	63 ± 8	68 ± 11
Resting heart rate (bpm)	63 ± 10	62 ± 8
Lean body mass (kg)	57.7 ± 6.1	52.6 ± 5.7
Body fat (%)	19.7 ± 3.1	22.8 ± 4.3
Leg press 1-RM (kg)	115 ± 26	98 ± 22
Knee extension 1-RM (kg)	61 ± 10	54 ± 9

Values represent mean ± standard deviation. n = 12 per nutritional intervention group. POTATO: 30 g of potato derived protein, MILK: 30 g milk protein. 1-RM: 1 repetition maximum of the exercised leg. Independent samples T-test for POTATO *vs* MILK all $P \geq 0.05$.

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TABLE 2. Amino acid composition of the protein concentrates

	POTATO	MILK
Alanine	1.4	1.0
Arginine	1.3	0.9
Aspartic acid	2.6	1.8
Cystine	0.2	0.1
Glutamic acid	2.5	5.5
Glycine	1.3	0.5
Histidine	0.5	0.7
Isoleucine	0.9	1.0
Leucine	2.6	2.6
Lysine	1.8	2.1
Methionine	0.6	0.6
Phenylalanine	1.5	1.3
Proline	1.4	2.9
Serine	1.4	1.3
Threonine	1.4	1.1
Tyrosine	0.7	0.7
Valine	1.1	1.2
TAA	23.2	25.3
EAA	10.5	10.7
BCAA	4.7	4.9
Nitrogen content (%)	13.1	12.8
Protein content (%)	81.9[1]	81.5[2]

Values for amino acid contents are in g per 30 g protein. [1]Protein as nitrogen * 6.25; [2]Protein as nitrogen content * 6.38; POTATO: 30 g potato derived protein, MILK: 30 g of milk protein. BCAA: Branched chain amino acids, EAA: Essential amino acids, TAA: Total amino acids.

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Online Supplemental Material

Medicine & Science in Sports & Exercise

Potato protein ingestion strongly increases muscle protein synthesis rates at rest and during recovery from exercise *in vivo* in humans

Philippe J.M. Pinckaers[1], Floris K. Hendriks[1], Wesley J.H. Hermans[1], Joy P.B. Goessens[1], Joan M. Senden[1], Janneau M.X. van Kranenburg[1], Will K.H.W. Wodzig[2], Tim Snijders[1], Luc J.C. van Loon[1]

[1]Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, Maastricht, The Netherlands

[2]Central Diagnostic Laboratory, Maastricht University Medical Centre+, Maastricht, The Netherlands

Conflicts of interest and Funding disclosure: This study was partially funded by APRE (Alliance for Potato Research & Education), Chicago, IL, USA and AVEBE, Veendam, The Netherlands. LJCvL has received research grants, consulting fees, speaking honoraria, or a combination of these from Friesland Campina, Tereos Syral, and Pepsico. PJMP, FKH, WJHH, JPBG, JMS, JMXvK, WKHW, and TS report no conflicts of interest.

Corresponding author: Prof. Luc J.C. van Loon, Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, PO box 616, 6200 MD Maastricht, The Netherlands, Tel: +31 43 388 1397, Email: l.vanloon@maastrichtuniversity.nl

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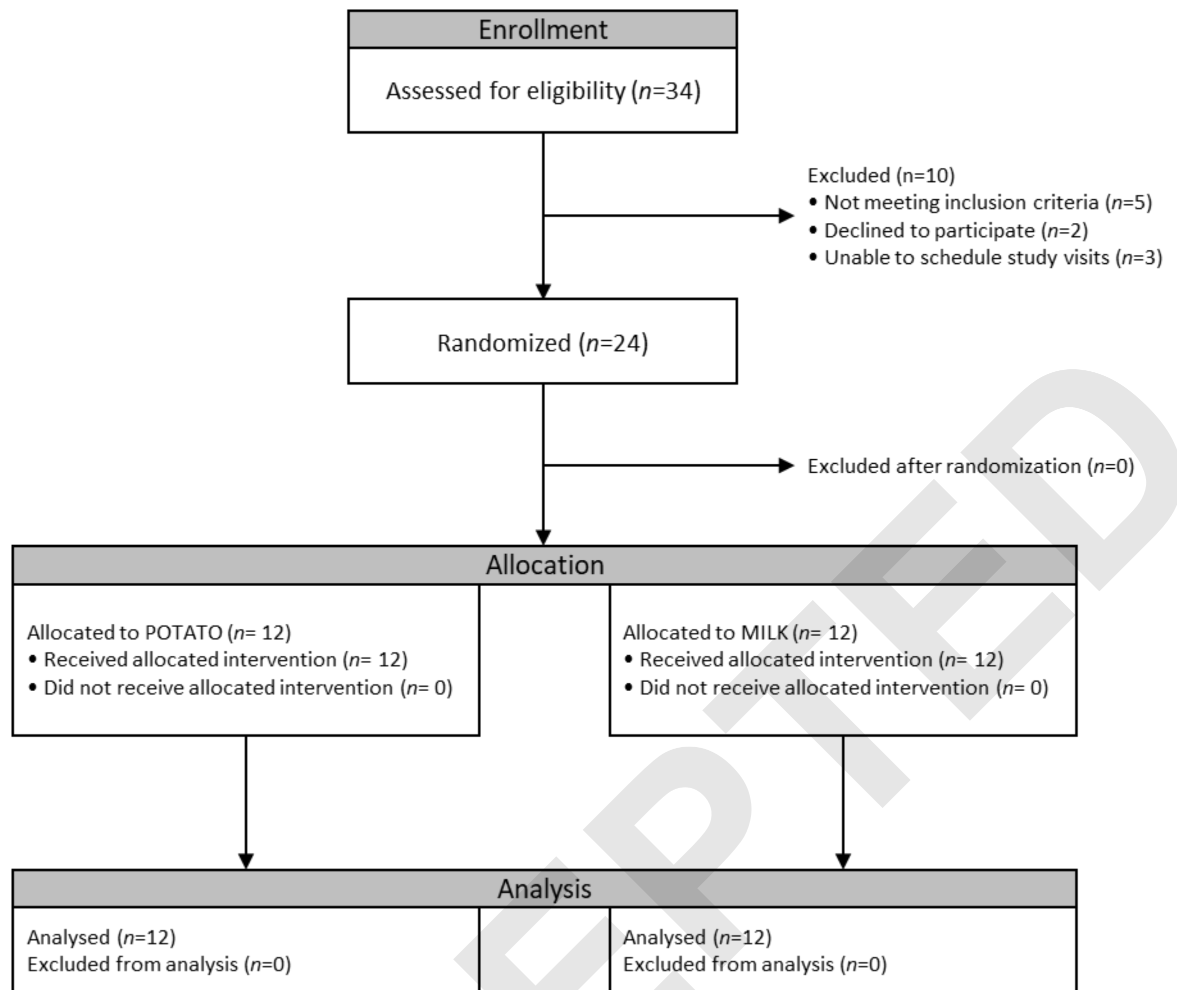

Supplemental Figure 1: CONSORT flow diagram. CONSORT, Consolidated Standards of Reporting Trials.

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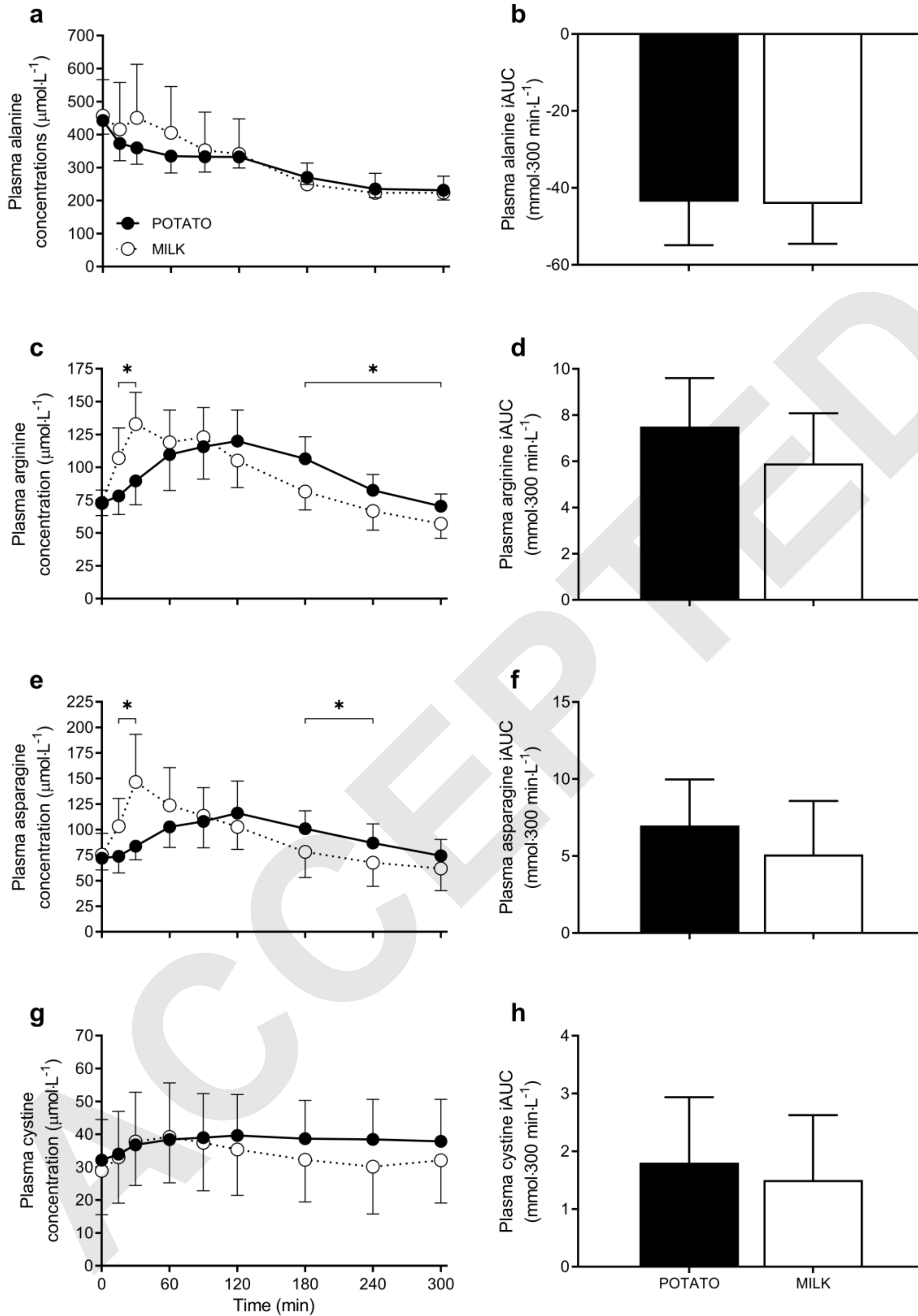

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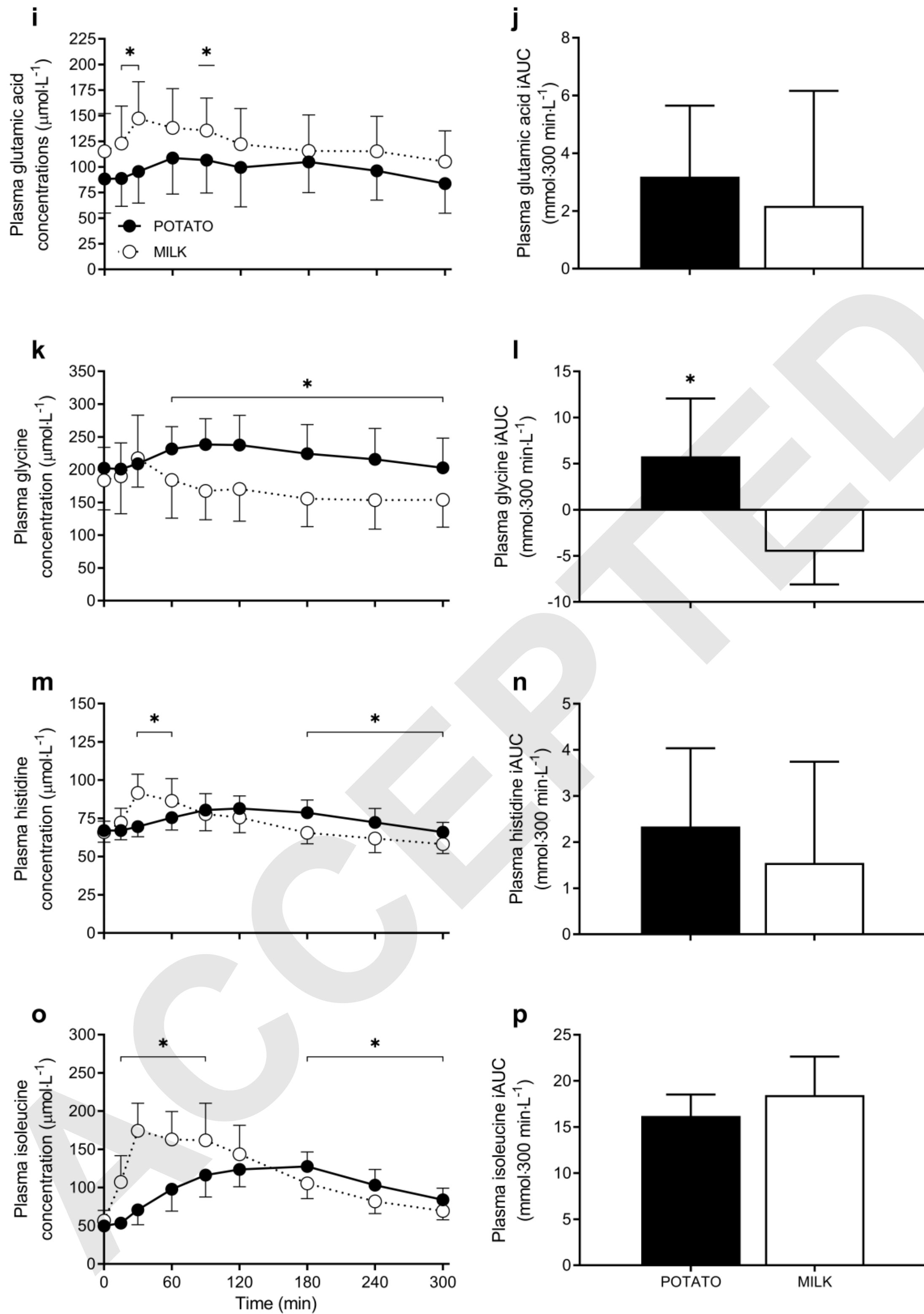

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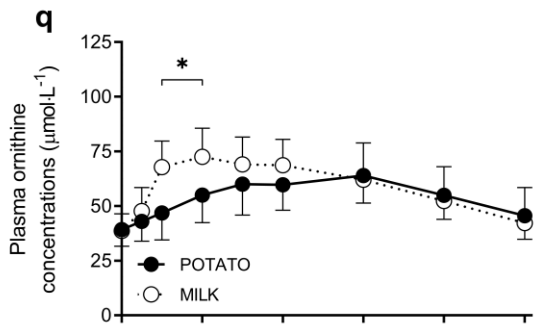

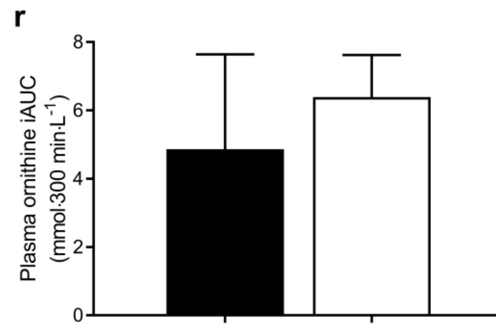

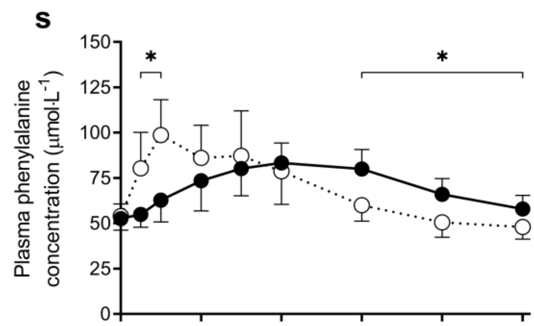

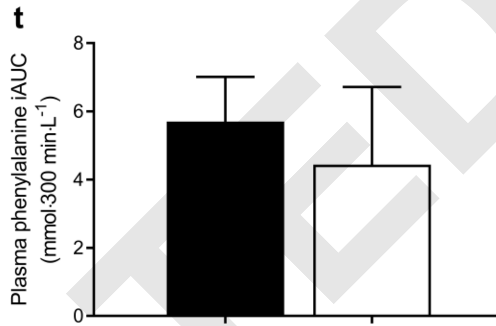

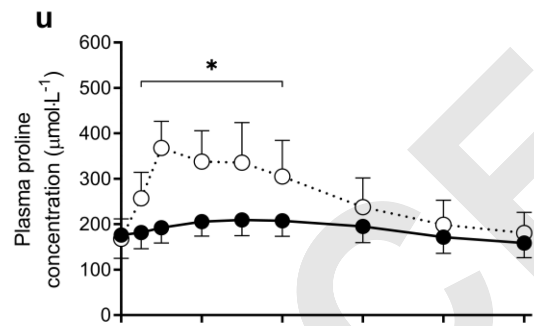

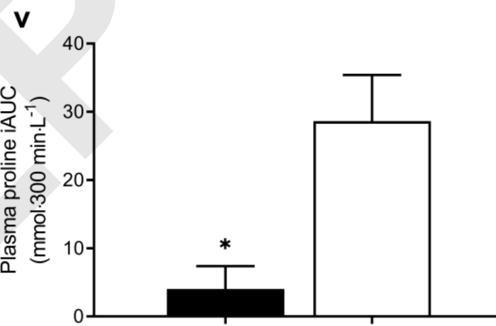

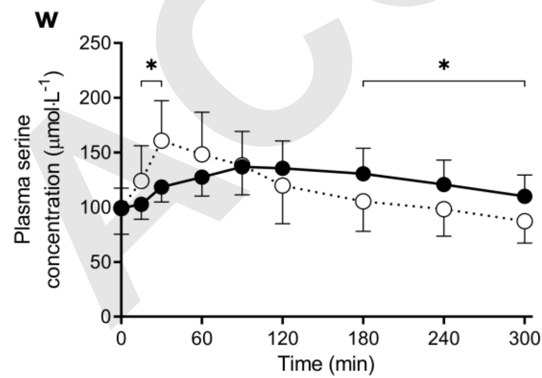

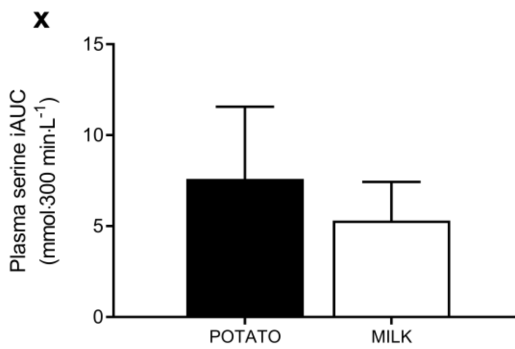

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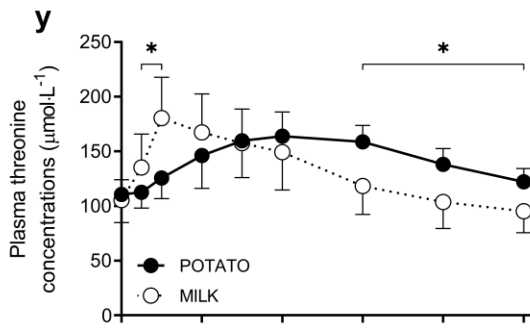

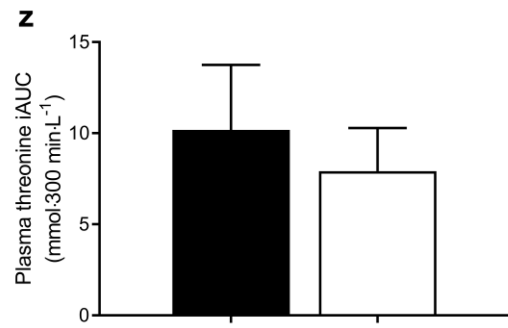

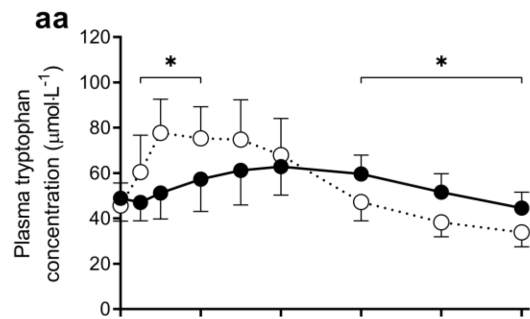

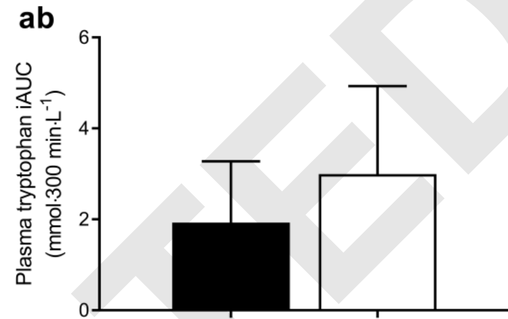

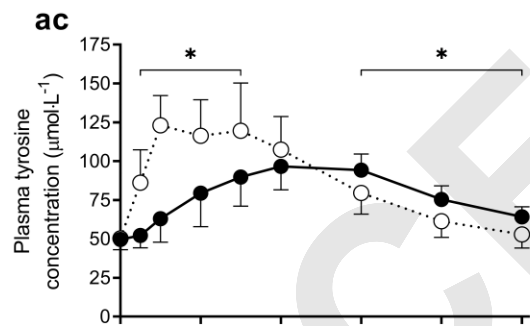

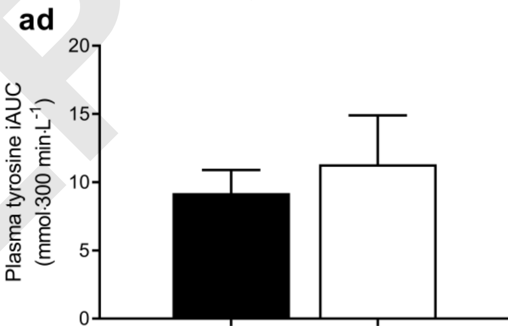

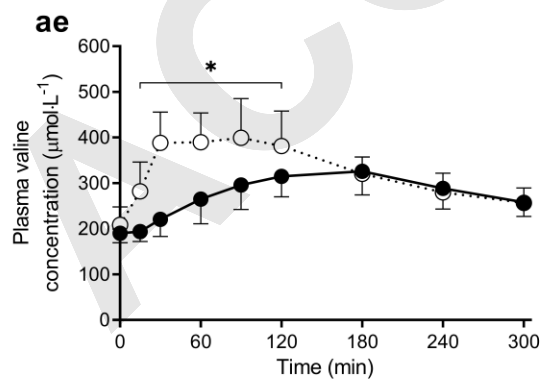

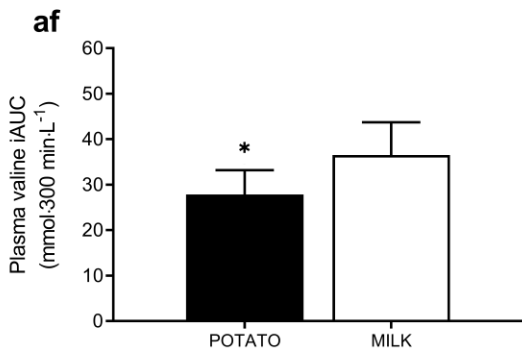

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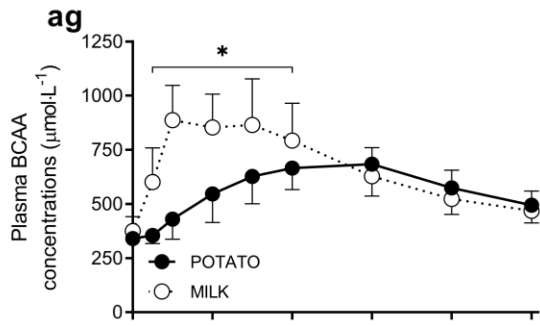

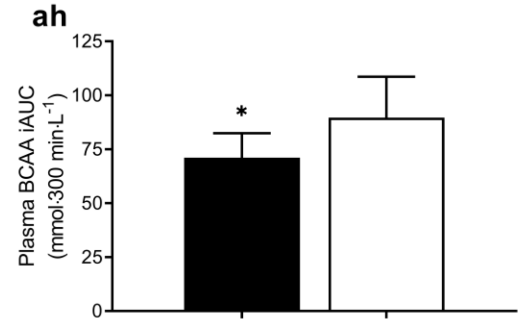

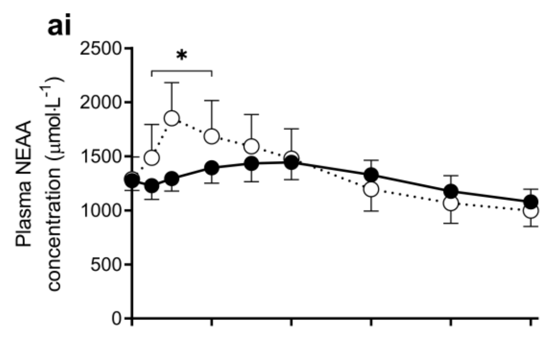

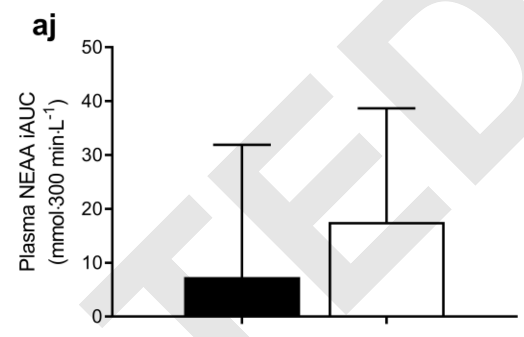

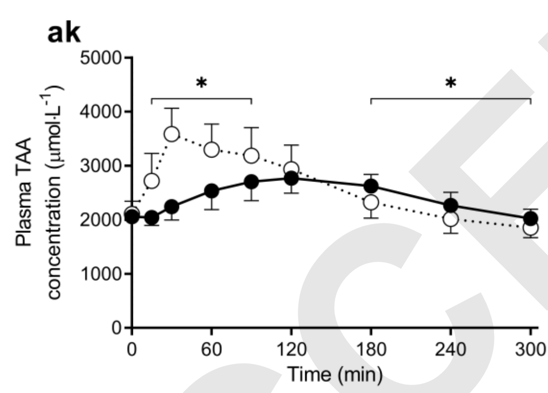

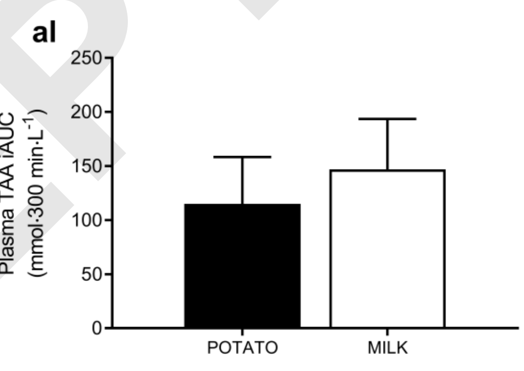

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Supplemental Figure 2: Post-prandial plasma amino concentrations during the 300 min post-prandial period following the ingestion of POTATO *vs* MILK. Time 0 min represents time of beverage intake. Panels b, d, f, h, j, l, n, p, r, t, v, x, z, ab, ad, af, ah, aj, al represent the 0-5 h incremental area under curve (iAUC) following protein ingestion. POTATO (30 g potato derived protein), MILK (30 g milk protein). Values represent means ± standard deviation; * significantly different for POTATO *vs* MILK (P<0.05). Repeated measures ANOVA with time as within-subject variable and interventional drink (treatment) as between-subject variable, and independent samples t-test were used to determine differences between groups. Values displaced below represent the P-values for the different panels.

Amino acid	**2-factor repeated measures ANOVA**		**Independent samples T-test**	
Alanine	a:	0.01	b:	0.90
Arginine	c:	<0.001	d:	0.08
Asparagine	e:	<0.001	f:	0.17
Cystine	g:	<0.001	h:	0.52
Glutamic acid	i:	0.001	j:	0.46
Glycine	k:	<0.001	l:	<0.001
Histidine	m:	<0.001	n:	0.33
Isoleucine	o:	<0.001	p:	0.11
Ornithine	q:	<0.001	r:	0.10
Phenylalanine	s:	<0.001	t:	0.11
Proline	u:	<0.001	v:	<0.001
Serine	w:	<0.001	x:	0.09
Threonine	y:	<0.001	z:	0.08
Tryptophane	aa:	<0.001	ab:	0.13
Tyrosine	ac:	<0.001	ad:	0.08
Valine	ae:	<0.001	af:	<0.01
BCAA	ag:	<0.001	ah:	<0.001
NEAA	ai:	<0.001	aj:	0.77
TAA	ak:	<0.001	al:	<0.001

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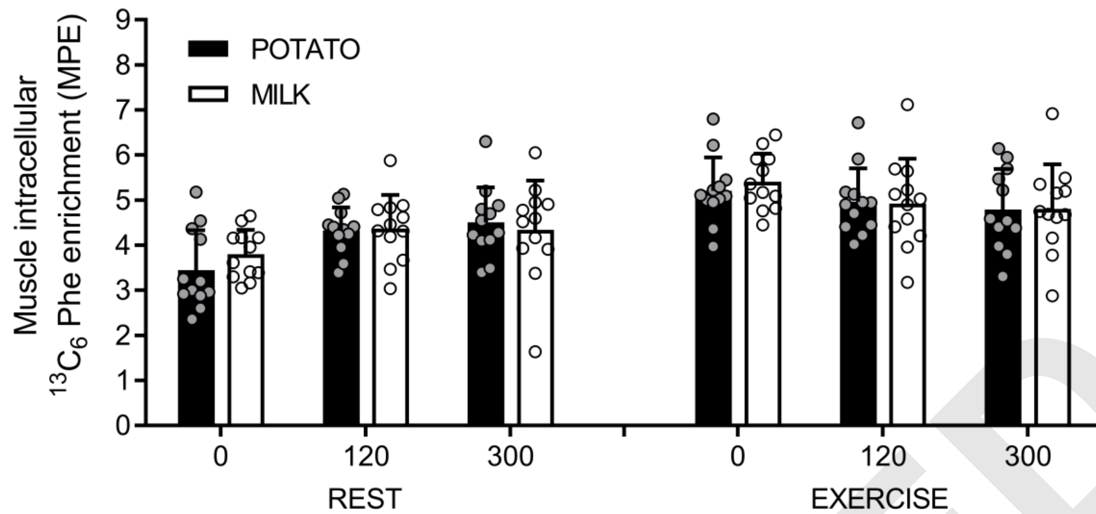
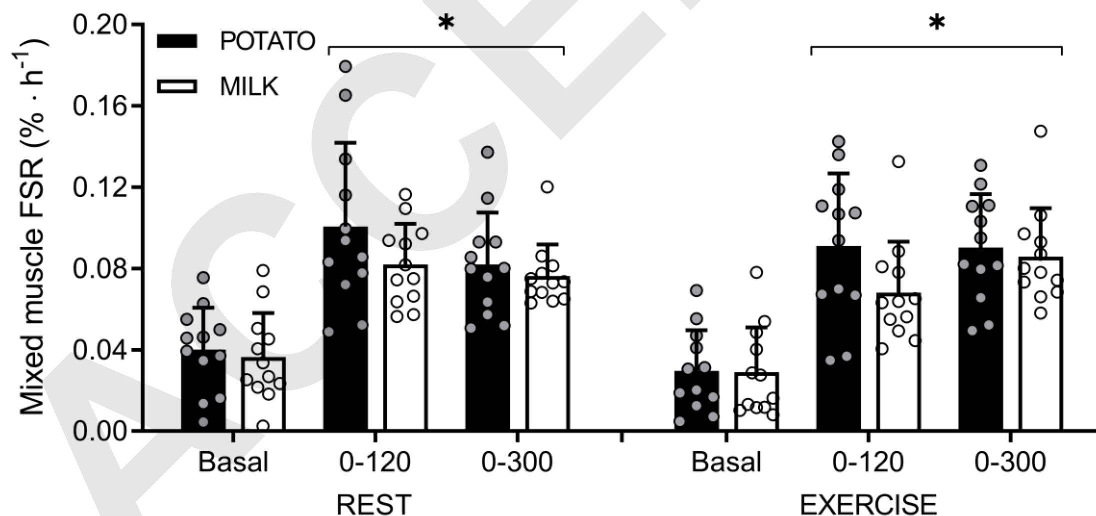


Supplemental Figure 3: Muscle intracellular L-[*ring*-$^{13}C_6$] Phenylalanine enrichments at different time points following ingestion of POTATO vs MILK during rest and during recovery following exercise in healthy, young males (*n*=12 per group). Phe: Phenylalanine, POTATO: 30 g potato protein, MILK: 30 g milk protein. Bars represent means ± standard deviation, dots represent individual values. Independent samples *t*-test: POTATO *vs* MILK: REST: *P*=0.26, *P*=0.86, and *P*=0.67 for basal, 0-120, and 0-300 min, respectively. EXERCISE: *P*=0.49, *P*=0.92, *P*=0.97 for basal, 0-120, and 0-300 min, respectively.



Supplemental Figure 4: Mixed muscle fractional synthetic rate (FSR) determined with intra-cellular enrichments as precursor pool at different time points following ingestion of POTATO *vs* MILK during rest and during recovery following exercise in healthy, young males (*n*=12 per group). POTATO: 30 g potato protein, MILK: 30 g milk protein. Bars represent means ± standard deviation, dots represent individual values. *significantly different from basal; *P*<0.05. Independent samples *t*-test: POTATO *vs* MILK: REST: *P*=0.67, *P*=0.17, and *P*=0.51 for basal, 0-120, and 0-300 min, respectively. EXERCISE: *P*=0.95, *P*=0.08, *P*=0.58 for basal, 0-120, and 0-300 min, respectively.

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