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Consumption of whole eggs promotes greater stimulation of postexercise muscle protein synthesis than consumption of isonitrogenous amounts of egg whites in young men

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ABSTRACT

Background: Protein in the diet is commonly ingested from whole foods that contain various macro- and micronutrients. However, the effect of consuming protein within its natural whole-food matrix on postprandial protein metabolism remains understudied in humans.

Objective: We aimed to compare the whole-body and muscle protein metabolic responses after the consumption of whole eggs with egg whites during exercise recovery in young men.

Design: In crossover trials, 10 resistance-trained men [aged 21 ± 1 y; 88 ± 3 kg; body fat: 16% ± 1% (means ± SEMs)] received primed continuous L-[*ring*-$^{2}H_5$]phenylalanine and L-[1-^{13}C]leucine infusions and performed a single bout of resistance exercise. After exercise, participants consumed intrinsically L-[5,5,5-$^{2}H_3$]leucine-labeled whole eggs (18 g protein, 17 g fat) or egg whites (18 g protein, 0 g fat). Repeated blood and muscle biopsy samples were collected to assess whole-body leucine kinetics, intramuscular signaling, and myofibrillar protein synthesis.

Results: Plasma appearance rates of protein-derived leucine were more rapid after the consumption of egg whites than after whole eggs ($P = 0.01$). Total plasma availability of leucine over the 300-min postprandial period was similar ($P = 0.75$) between the ingestion of whole eggs (68% ± 1%) and egg whites (66% ± 2%), with no difference in whole-body net leucine balance ($P = 0.27$). Both whole-egg and egg white conditions increased the phosphorylation of mammalian target of rapamycin complex 1, ribosomal protein S6 kinase 1, and eukaryotic translation initiation factor 4E–binding protein 1 during postexercise recovery (all $P < 0.05$). However, whole-egg ingestion increased the postexercise myofibrillar protein synthetic response to a greater extent than did the ingestion of egg whites ($P = 0.04$).

Conclusions: We show that the ingestion of whole eggs immediately after resistance exercise resulted in greater stimulation of myofibrillar protein synthesis than did the ingestion of egg whites, despite being matched for protein content in young men. Our data indicate that the ingestion of nutrient- and protein-dense foods differentially stimulates muscle anabolism compared with protein-dense foods. This trial was registered at clinicaltrials.gov as NCT03117127. *Am J Clin Nutr* 2017;106:1401–12.

Keywords: protein digestion, food protein quality, leucine, amino acid transporters, anabolic signaling, exercise

INTRODUCTION

Dietary patterns that include the regular ingestion of high-quality, protein-dense foods are important to optimize the stimulation of postprandial muscle protein synthesis rates to improve skeletal muscle remodeling. Protein quality in human nutrition can be at least partly determined from the bioavailability of dietary protein–derived amino acids in the circulation to support whole-body and muscle protein metabolic needs. Studies have shown that various meal characteristics, such as protein source (1, 2) and ingested protein quantity (3), can influence the amount of dietary protein–derived amino acids available in the circulation to stimulate postprandial muscle protein synthesis rates. However, most work has focused on the effects of consuming isolated protein fractions dissolved in liquid beverages (1, 2, 4–12). There is far less information available about the effects of ingesting nutrient- and protein-dense whole foods on the regulation of postprandial protein metabolism. This is noteworthy because nutrient-dense whole foods, compared with supplemental isolated protein sources, are more commonly consumed within a normal eating pattern to achieve daily protein recommendations and concomitant improvements in diet quality.

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Supplemental Table 1, Supplemental Figures 1–3, and Supplemental Methods are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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Abbreviations used: AMPKα, adenosine monophosphate–activated protein kinase α; ERK, extracellular signal–regulated kinase; FSR, fractional synthesis rate; GC-MS, gas chromatography–mass spectrometry; LAT1, large neutral amino acid transporter small subunit 1; MRM, multiple reaction monitoring; mTORC1, mammalian target of rapamycin complex 1; NOLD, nonoxidative leucine disposal; R_a, rate of appearance; R_d, rate of disappearance; SLC, solute carrier; SNAT2, sodium-coupled neutral amino acid transporter 2; TBST, Tris-buffered saline with 0.1% Tween 20; 10-RM, 10–repetition maximum.

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To better define whole-food protein quality in human nutrition, we developed intrinsically L-[5,5,5-$^{2}H_3$]leucine–labeled eggs to allow for the detailed assessment of postprandial whole-body and skeletal muscle protein metabolism in vivo in humans (13). Eggs are a nutrient-rich food source and are commonly consumed at breakfast by US adults (14). However, the removal of the yolk is often promoted for improved health when multiple eggs are consumed. This an unsubstantiated belief related to the cholesterol and fat content of the egg yolk (15). The yolk is nutrient dense and contains ~40% of the total protein contained in the egg, and its removal seems counterproductive for meeting protein recommendations. Because nutrient-dense protein foods are often recommended to achieve protein recommendations (16), it is important to define how the ingestion of more nutrient- and protein-dense foods modulates protein metabolism under a setting that includes other components of a healthy lifestyle, such as the incorporation of regular exercise.

Therefore, the purpose of this study was to compare whole-body leucine kinetics and postprandial myofibrillar protein synthesis rates after the ingestion of whole eggs and isonitrogenous amounts of egg whites during recovery from resistance exercise in young men. In addition, we examined skeletal muscle amino acid transporter protein content and the phosphorylation status of protein signaling molecules that may regulate changes in myofibrillar protein synthesis rates (17). We hypothesized that whole-egg ingestion, due to its fat content, would delay the appearance rates of postprandial protein-derived amino acids in circulation but would not modulate plasma dietary amino acid availability or myofibrillar protein synthesis rates throughout a 0- to 300-min recovery period when compared with egg white ingestion in healthy young men.

METHODS

Participants and ethical approval

Ten healthy young men (mean ± SEM age: 21 ± 1 y) who were regularly engaged in structured resistance exercise training (mean ± SEM training years: 5 ± 1 y) volunteered for the study. Participant characteristics are presented in **Table 1**. All of the participants were deemed to be healthy on the basis of responses to a routine medical screening questionnaire and had no previous history of participating in stable-isotope amino acid tracer experiments. All of the participants were informed about the experimental procedures to be used, the purpose of the study, and all potential risks before giving written consent. The study was approved by the Institutional Review Board at the University of Illinois at Urbana-Champaign and conformed to standards for the use of human participants in research as outlined in the seventh revision of the Declaration of Helsinki. The study was registered at clinicaltrials.gov as NCT03117127.

TABLE 1
Participant characteristics[1]

Variable	Value
Age, y	21 ± 1
Weight, kg	88 ± 3
Fat, %	16 ± 1
Lean body mass, kg	72 ± 2
Systolic BP, mm Hg	121 ± 4
Diastolic BP, mm Hg	70 ± 2
Fasting glucose, mg/dL	76 ± 1
10-RM leg press, kg	242 ± 32
10-RM leg extension, kg	112 ± 10

[1] Values are means ± SEMs; n = 10. BP, blood pressure; 10-RM, 10–repetition maximum.

Experimental design

A within-subject crossover design was used for this study. At least 1 wk before the first infusion trial, participants reported to the laboratory for familiarization with the exercise equipment and for maximum strength testing as determined by their 10–repetition maximum (10-RM) for the leg press and leg extension. In addition, body weight and height were measured, as was body composition by dual-energy X-ray absorptiometry (Hologic QDR 4500A). On a separate occasion, ≥3 d after initial maximum strength testing and ≥3 d before the first infusion trial, participants re-visited the laboratory to confirm their 10-RM for the leg press and leg extension. The highest obtained 10-RMs for the leg press and leg extension (mean ± SEM: 233 ± 32 and 112 ± 10 kg, respectively) were used to set the workload for the infusion trials. Participants were instructed to refrain from vigorous physical activity for 3 d before each trial and to record their dietary intake by using an online food tracker (MyFitnessPal) for 2 d before each trial. Participants were subsequently instructed to follow their food diary as closely as possible during the 2 d leading into the second infusion trial while again recording their dietary intake. The average 2-d macronutrient intake was similar between the whole-egg and egg white trials (P = 0.16) (**Supplemental Table 1**). Participants were counterbalanced in random fashion so that half of the participants would consume whole eggs or egg whites for their first infusion trial. The time between crossover trials was 7–14 d (8 ± 1 d). A CONSORT (Consolidated Standards of Reporting Trials) flowchart of the study is presented in **Supplemental Figure 1**.

Infusion protocol

A schematic overview of the infusion protocol is shown in **Figure 1**. For both infusion trials, participants reported to the laboratory at 0700 after an overnight fast. After collecting a baseline breath sample, a polytetrafluorethylene catheter was inserted into an antecubital vein for baseline blood sample collection (t = −210 min) and participants received priming doses of $NaH^{13}CO_2$ (2.35 μmol/kg), L-[1-^{13}C]leucine (7.6 μmol/kg), and L-[*ring*-$^{2}H_5$]phenylalanine (2.0 μmol/kg). Subsequently, a continuous intravenous infusion of L-[1-^{13}C]leucine (0.10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and L-[ring-$^{2}H_5$]phenylalanine (0.05 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was initiated ($t$ = −210 min) and maintained over the infusion trials. A second Teflon catheter was inserted into a heated dorsal hand vein for repeated arterialized blood sampling and remained patent by a 0.9% saline drip. In the postabsorptive state of infusion trial 1, muscle biopsy samples were collected at t = −150 and −30 min of infusion to determine fasted myofibrillar protein synthesis rates, relative skeletal muscle amino acid transporter content, and anabolic-related signaling. In the subsequent crossover trial, only one muscle biopsy was collected at t = −30 min for anabolic-related

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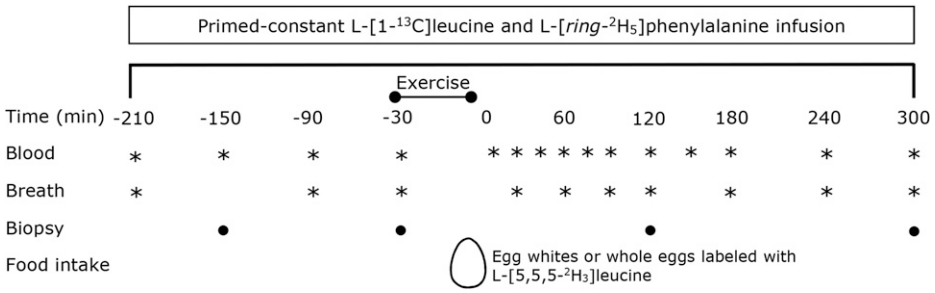

FIGURE 1 Schematic of the experimental infusion protocol. Participants consumed whole eggs or egg whites in a randomly assigned order (n = 10 for both trials as a crossover design). Two pre-exercise muscle biopsy samples were collected at the first trial only. In trial 2, one pre-exercise muscle biopsy sample was collected at $t = -30$ min. Exercise consisted of 4 sets of 10 repetitions for the leg press and leg extension exercises. Asterisks indicate blood and breath sampling; filled circles indicate muscle biopsies.

signaling analysis and fasted myofibrillar protein–bound tracer enrichment. After collection of the resting muscle biopsy sample at $t = -30$ for both trials, the participants performed resistance exercise that consisted of 4 sets of 10 repetitions at 80% of 10-RM for both the leg press and leg extension exercises. The exercise external work (repetitions × load) was matched between the whole-egg (9896 ± 52 kg) and egg white (9893 ± 52 kg) trials (P = 0.34). Immediately after completion of the exercise bout, participants consumed 3 whole eggs or an equivalent amount of protein from egg whites ($t = -5$ min). Participants also finished 300 mL water with each meal. The completion of the meal marked the start of the postprandial phase (t = 0 min), and additional muscle biopsy samples were collected at t = 120 and 300 min. To reduce the number of muscle biopsy samples collected and to minimize participant discomfort during the infusions, biopsy samples were not collected immediately after the completion of exercise for both the whole-egg and egg white treatments ($t = -5$ min). As such, it is assumed that the brief resistance exercise period (~25 min) did not significantly affect muscle protein synthesis rates expressed over 2 and 5 h of postexercise recovery, as we explained in detail in previous work (18). Biopsy samples were collected from the middle region of the vastus lateralis (15 cm above the patella) with a Bergström needle modified for suction under local anesthesia (2% Lidocaine; Hospira Worldwide). The postabsorptive muscle biopsy samples in trial 1 were collected from the same incision with the needle pointed to distal and proximal directions, respectively. The muscle biopsy samples obtained at t = 120 and 300 min of postexercise recovery were collected from the contralateral leg through separate incisions (2–3 cm apart) for both trials. All of the muscle biopsy samples were freed from any visible blood, adipose, and connective tissue; immediately frozen in liquid nitrogen; and stored at −80°C until subsequent analysis. Breath samples and arterialized blood samples were collected every 30 or 60 min during the postabsorptive and postprandial states. Total carbon dioxide production rates were measured with a metabolic cart (TrueOne 2400; ParvoMedics) at regular intervals throughout the infusion trials. The blood samples were immediately analyzed for whole-blood glucose concentrations (2300 Stat Plus; YSI Life Sciences) and subsequently centrifuged at 3000 × g for 10 min at 4°C. Aliquots of plasma were frozen and stored at −80°C until subsequent analysis. The breath samples were collected in 10-mL evacuated tubes and stored at 20°C until subsequent determination of $^{13}CO_2$ enrichment by isotope ratio mass spectrometry (IDmicro Breath; Compact Science Systems Ltd.).

Meal composition

The intrinsically L-[5,5,5-$^2H_3$]leucine–labeled eggs were produced by supplementing the diet of laying hens (Lohmann LSL Whites) with 0.3% L-[5,5,5-$^2H_3$]leucine as described previously (13). A portion of the collected eggs had the yolks removed before storage at −20°C in aliquots of 18 g protein. The other portion of eggs had the yolks and whites completely mixed before storage at −20°C in aliquots of isonitrogenous amounts. The L-[5,5,5-$^2H_3$]leucine enrichments of the whole-egg and egg white aliquots were determined by gas chromatography–mass spectrometry (GC-MS) and averaged 28.6 and 26.1 mole percent excess, respectively. Proximate analyses for protein, lipid, and carbohydrate concentrations were determined by using the combustion method (method 990.03; AOAC International, 2000; TruMac; LECO Corporation) (13). Leucine contents of the whole-egg and egg white aliquots were determined by GC-MS with integration of amino acid peak areas compared with an internal standard (DL-p-chlorophenylalanine) by using the AMDIS software package (version 2.71; National Institute of Standards and Technology) (19). Before the infusions, the egg aliquots were thawed overnight in a refrigerator at 4°C. On the morning of the experiment, the whole eggs or egg whites were scrambled in a skillet until solid with no visible liquid remaining. The macronutrient composition and energy content were 18 g protein (1.57 g leucine), 17 g fat, and 226 kcal for the whole eggs and 18 g protein (1.60 g leucine), 0 g fat, and 73 kcal for the egg white treatments.

Plasma analyses

Plasma insulin concentrations were determined by using a commercially available ELISA (Alpco Diagnostics). Plasma triglyceride concentrations were determined by using a point-of-care chemistry analyzer (Piccolo Xpress Chemistry Analyzer; Abaxis). Plasma amino acid concentrations and enrichments were determined by GC-MS analysis (Agilent 7890A GC/5975C; MSD) as described in our previous work (13). Briefly, plasma samples were prepared for amino acid analysis with the use of a mixture of isopropanol:acetonitrile:water (3:3:2, vol:vol:vol) and centrifuged at 20,000 × g for 10 min at 4°C. Subsequently, the supernatant was dried and the amino acids converted into tert-butyldimethylsilyl derivatives before GC-MS analysis. Plasma L-[1-^{13}C]leucine and L-[5,5,5-$^2H_3$]leucine enrichments were determined by ion monitoring at m/z 302 (m + 0), 303 (m + 1), and 305 (m + 3), with m + 0 representing the lowest

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molecular weight of the ion or unlabeled leucine. For L-[*ring*-$^2H_5$]phenylalanine, *m/z* 336 (*m* + 0) and 341 (*m* + 5) were monitored for unlabeled and labeled phenylalanine, respectively. Plasma enrichments of the tert-butyldimethylsilyl derivative of α-[^{13}C]-ketoisocaproate were measured by GC-MS analysis by ion monitoring at *m/z* 232 and 233. The plasma leucine concentrations were determined by integrating amino acid peak areas in comparison to U-[$^{13}C_6$]leucine as an internal standard with the use of the AMDIS software package (version 2.71; National Institute of Standards and Technology) (19).

Muscle analysis

Myofibrillar protein–enriched fractions were isolated from ~50 mg wet tissue as described previously (13). Myofibrillar-enriched protein pellets were hydrolyzed overnight in 6 M HCl at 110°C. The resultant free amino acids were purified by using cation exchange chromatography (Dowex 50W-X8-200 resin; Acros Organics) and dried under vacuum. Free amino acids were resuspended in 60% methanol and centrifuged before the myofibrillar protein–bound enrichments were determined by 5500 QTRAP liquid chromatography–tandem mass spectrometry (Sciex). The myofibrillar protein–bound L-[ring-$^2H_5$]phenylalanine enrichments were determined by multiple reaction monitoring (MRM) at *m/z* 166.0→103.0 and 171.0→106.0 for unlabeled and labeled L-[ring-$^2H_5$]phenylalanine, respectively. Muscle intracellular free amino acids were extracted from a separate piece of wet muscle (~30 mg) by using a polytetrafluorethylene-coated pestle and ice-cold 2% perchloric acid and then centrifuged at 10,000 × *g* at 4°C for 10 min. The supernatant was then collected, and this process was repeated 2 more times. The supernatant was taken as the muscle intracellular free amino acids and subsequently purified by using cation exchange chromatography (Dowex 50W-X8-200 resin; Acros Organics) before analysis by liquid chromatography–tandem mass spectrometry. Muscle intracellular free L-[1-^{13}C]leucine and L-[5,5,5-$^2H_3$]leucine enrichments were measured by MRM at *m/z* 132.0→86.0, 133.0→87.0, and 135.0→89.0 for unlabeled and labeled leucine. For L-[*ring*-$^2H_5$]phenylalanine enrichments, MRM at171.0→106.0 for unlabeled and labeled phenylalanine was measured. Sciex Software Analyst 1.6.2 was used for data acquisition and analysis.

Western blotting

A portion of whole-muscle homogenates isolated during the myofibrillar protein extractions was used for Western blotting analysis. The protein concentrations of the homogenates were determined by bicinchoninic acid assay protein assay (Thermo Fisher Scientific) and then used to prepare working samples of equal concentrations in Laemmli buffer. Working samples were heated to 95°C for 5 min, and equal amounts of protein (10 μg) were subsequently separated by SDS-PAGE and transferred to nitrocellulose membrane (wet transfer, 100 V for 60 min) for antibody incubation. Membranes were blocked in 5% fat-free milk at room temperature for 60 min, washed in Tris-buffered saline with 0.1% Tween 20 (TBST), and incubated in primary antibody (1:1000; Cell Signaling Technology) overnight at 4°C to determine phosphorylation status of adenosine monophosphate–activated protein kinase α (AMPKα) at Thr172 (catalog no. 2535), mammalian target of rapamycin complex 1 (mTORC1) at Ser2448 (catalog no. 2971), extracellular signal–regulated kinase (Erk1/2) at Thr202/Tyr204 (catalog no. 4377), eukaryotic translation initiation factor 4E–binding protein 1 (4E-BP1) at Thr37/46 (catalog no. 9459), and eukaryotic translation elongation factor 2 (eEF2) at Thr56 (catalog no. 2331). The antibodies (1:1000; Abcam) used to determine the relative protein content of skeletal muscle amino acid transporters were solute carrier family 7 member 5/large neutral amino acid transporter small sub unit 1 (SLC7A5/LAT1), solute carrier family 3 member 2/CD98 (SLC3A2/CD98), and solute carrier family 38 member 2/sodium-coupled neutral amino acid transporter 2 (SLC38A2/SNAT2). After overnight incubation of the primary antibody (4°C), membranes were then washed (3 × 5 min TBST), incubated in secondary antibody for 60 min at room temperature (1:20,000 in 3% fat-free milk), and washed (3 × 5 min TBST) before detection with chemiluminescence (catalog no. WBKLS0500; Millipore). To determine the phosphorylation status of 70-kDa S6 protein kinase (p70S6K1) at Thr389 (catalog no. 9205), 25 μg protein was loaded followed by incubation in primary (overnight, 1:1000 in 5% bovine serum albumin) and secondary antibody (1:5000 in 5% fat-free milk). Bands were imaged by using FluorChem E Imaging System (Protein Simple; Alpha Innotech). Bands were quantified by using AlphaView SA software (Protein Simple) and normalized to Ponçeau S staining; this approach has been validated as an alternative loading control (e.g., α-actin) for Western blot analysis (20, 21).

Calculations

Whole-body leucine kinetics were assessed under non–steady state conditions by the ingestion of L-[5,5,5-$^2H_3$]leucine eggs combined with intravenous infusion of L-[1-^{13}C]leucine. Leucine oxidation was calculated from the appearance of the ^{13}C-label in the expired carbon dioxide by using the α-[^{13}C]ketoisocaproate reciprocal pool model with fractional bicarbonate retention factors of 0.7 and 0.83 for postabsorptive and postprandial states, respectively (22). For the other leucine fluxes, calculations were performed by using the plasma L-[5,5,5-$^2H_3$]leucine and L-[1-^{13}C]leucine enrichments and leucine concentrations. Total, exogenous, and endogenous leucine rates of appearance (R_a) and total leucine rates of disappearance (R_d) were calculated with the use of modified Steele equations (23, 24). Furthermore, plasma protein-derived leucine availability, total leucine oxidation, nonoxidative leucine disposal (NOLD), whole-body leucine net balance, and whole-body leucine retention were calculated (see **Supplemental Methods** for more details on calculations) (23, 24). Myofibrillar protein fractional synthesis rates (FSRs) were calculated by using the standard precursor-product equation by dividing the increment in L-[*ring*-$^2H_5$]phenylalanine enrichment in the myofibrillar protein pool by the weighted average of L-[*ring*-$^2H_5$]phenylalanine enrichment in the plasma or intracellular muscle free precursor pool over time.

Statistical analysis

A within-subject crossover design was used for this study. A power analysis based on previous research (2, 12) showed that

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n = 8/condition was sufficient to detect differences in postprandial muscle protein synthesis between conditions when using a 2-sided statistical test ($P < 0.05$, 80% power, $f = 1.2$; G*power version 3.1.9.2). Considering a potential dropout rate of 20% during the protocol, the final number of participants recruited was 10/condition. Differences in time-dependent blood and muscle measurements were tested by 2-factor (time × condition) repeated-measures ANOVA. Differences in self-reported dietary intakes, exercise external loads, and the fraction of dietary protein–derived leucine that appeared into plasma were tested by paired t test. When significant interaction effects were identified in the ANOVA, Bonferroni post hoc tests were performed to determine the differences between means for all significant main effects and interactions. For all analysis, differences were considered significant at $P < 0.05$. All of the calculations were performed by using IBM SPSS Statistics (version 24) unless otherwise designated. All data are expressed as means ± SEMs.

RESULTS

Plasma metabolites and enrichments

Plasma leucine concentrations increased after egg ingestion (time: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.76$). Peak plasma leucine concentrations were observed at 150 min (210 ± 14 μmol/L) and 240 min (200 ± 11 μmol/L) after the ingestion of egg whites and whole eggs, respectively (**Figure 2**A). Plasma glucose concentrations tended to decrease transiently at 60 min of the postprandial period ($P = 0.06$) with no differences between conditions (time × condition: $P = 0.73$) (Figure 2B). Plasma insulin concentrations rapidly increased after egg ingestion (time: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.48$) (Figure 2C). Plasma triglyceride concentrations increased at 180 min after the ingestion of whole eggs but not egg whites (time effect: $P = 0.007$; time × condition: $P = 0.03$) (Figure 2D).

Plasma L-[5,5,5-$^2H_3$]leucine enrichment rapidly increased after egg ingestion ($P < 0.001$) with no differences between conditions (time × condition: $P = 0.37$) (**Figure 3**A). Plasma L-[1-^{13}C]leucine enrichments decreased after whole-egg and egg white ingestion (time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.86$) (Figure 3B). Plasma α-[^{13}C]ketoisocaproate enrichments increased after egg ingestion (time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.68$) (Figure 3C). Plasma L-[*ring*-$^2H_5$]phenylalanine enrichments declined after egg ingestion

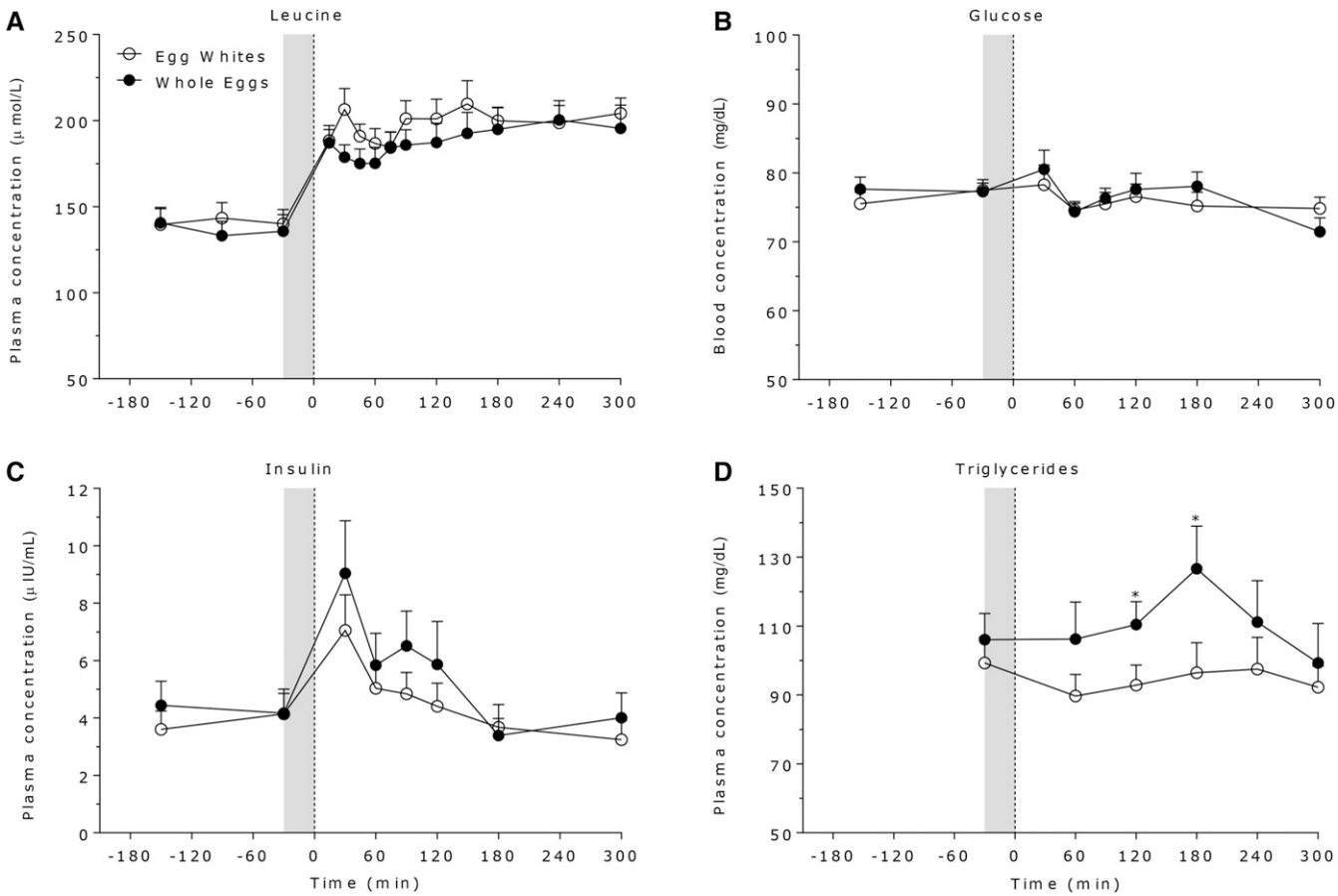

FIGURE 2 Plasma leucine (A), blood glucose (B), plasma insulin (C), and plasma triglyceride (D) concentrations in the fasted state and after consumption of egg whites or whole eggs in young men (n = 10/condition). Values are means ± SEMs. Gray-shaded areas correspond to the exercise bout; the dashed lines refer to time of egg ingestion. Data were analyzed with 2-factor ANOVA with repeated measures on time. A Bonferroni post hoc test was used to locate differences between means for all significant interactions. Leucine: time effect, $P < 0.001$; time × condition, $P = 0.76$. Glucose: time effect, $P = 0.06$; time × condition, $P = 0.73$. Insulin: time effect, $P < 0.001$; time × condition, $P = 0.484$. Triglycerides: time effect, $P = 0.007$; time × condition, $P = 0.03$. *Different between egg conditions, $P < 0.05$.

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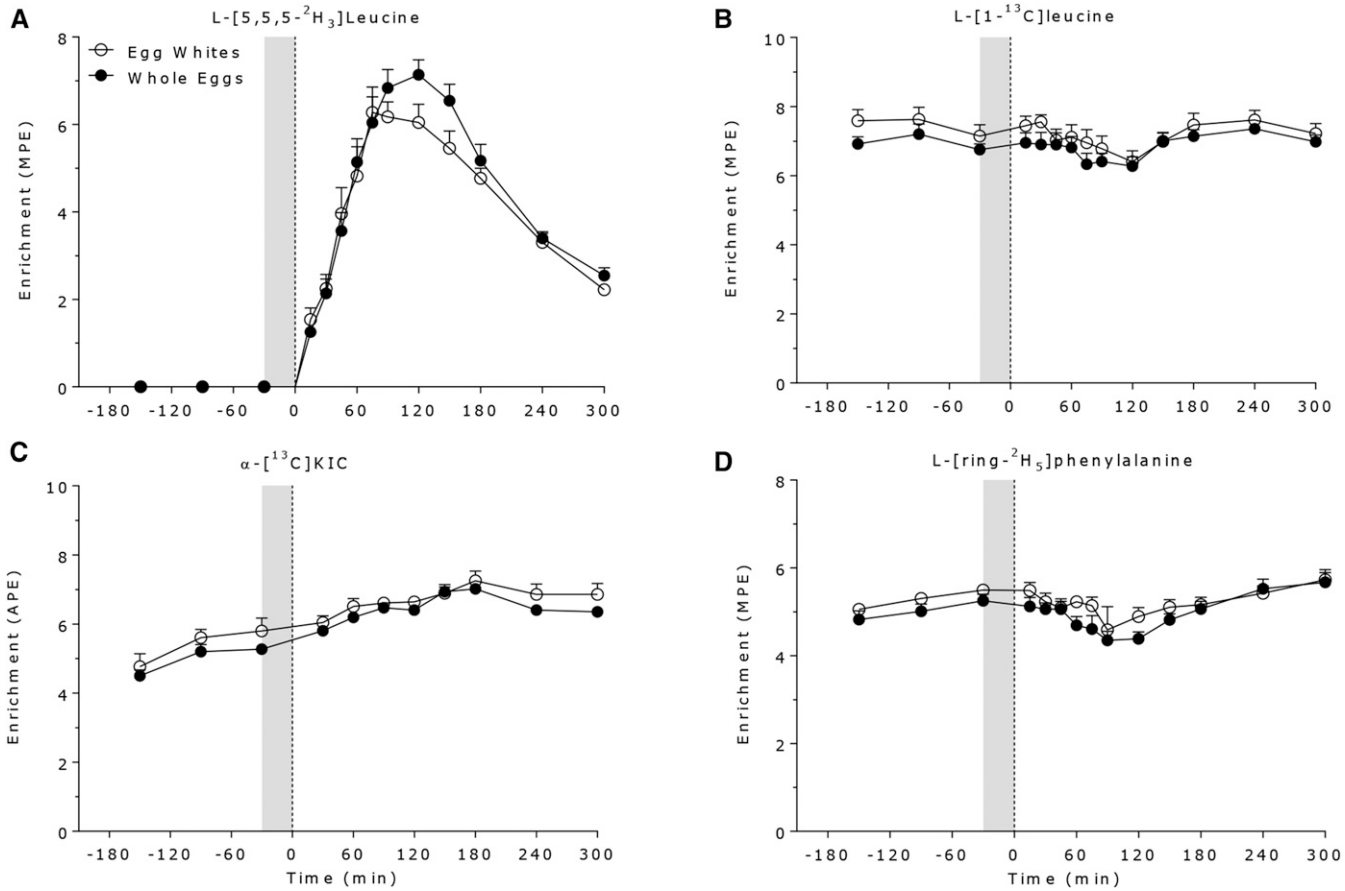

FIGURE 3 Plasma L-[5,5,5-$^2H_3$]leucine (A), L-[1-^{13}C]leucine (B), α-[^{13}C]KIC (C), and L-[*ring*-$^2H_5$]phenylalanine (D) enrichments in the fasted state and after consumption of egg whites or whole eggs in young men (n = 10/condition). Values are means ± SEMs. Gray-shaded areas correspond to the exercise bout; dashed lines refer to time of egg ingestion. Data were analyzed with 2-factor ANOVA with repeated measures on time. A Bonferroni post hoc test was used to locate differences between means for all significant interactions. L-[5,5,5-$^2H_3$]leucine: time effect, $P < 0.001$; time × condition, $P = 0.37$. L-[1-^{13}C]leucine: time effect, $P < 0.001$; time × condition, $P = 0.86$. α-[^{13}C]KIC: time effect, $P < 0.001$; time × condition, $P = 0.78$. L-[ring-$^2H_5$]phenylalanine: time effect, $P < 0.001$; time × condition, $P = 0.78$. APE, atom percent excess; KIC, ketoisocaproate; MPE, mole percent excess.

(time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.78$) (Figure 3D).

Whole-body leucine kinetics

Exogenous leucine rates of appearance (representing the appearance of dietary protein–derived leucine into circulation) increased after whole-egg and egg white ingestion (time effect: $P < 0.001$) (**Figure 4**A). Exogenous leucine appearance rates were higher between 15 and 75 min after the ingestion of egg whites than after whole eggs, whereas exogenous leucine rates of appearance were higher between 150 and 300 min after whole-egg ingestion than after egg whites (time × condition: $P = 0.001$). Thus, more rapid peak exogenous leucine appearance rates were observed at 75 min (529 ± 56 nmol · kg^{-1} · min^{-1}) than after 120 min (496 ± 38 nmol · kg^{-1} · min^{-1}) of the postprandial period after the ingestion of egg whites and whole eggs, respectively. The fraction of dietary protein–derived leucine that appeared in the circulation during the first 120 min of the postprandial period was greater after egg white (34% ± 2%) than after whole-egg (25% ± 3%) ingestion ($P = 0.02$). However, the cumulative fraction of dietary protein–derived leucine that appeared in the circulation throughout the 300-min postprandial period was similar between the egg white (68% ± 1%) and whole-egg (66% ± 2%) conditions ($P = 0.75$). Endogenous leucine rates of appearance (representing the appearance of leucine derived from whole-body protein breakdown into the circulation) decreased after egg ingestion (time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.17$) (Figure 4B). Total leucine rates of appearance increased after egg ingestion (time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.83$) (Figure 4C). Total leucine rates of disappearance increased after egg ingestion (time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.84$) (Figure 4D).

AUCs of fasted and fed rates of whole-body protein breakdown [Endo (endogenous) R_a], synthesis (NOLD), oxidation (Total Ox), and net balance (NOLD − Endo R_a) are presented in **Figure 5**. Regardless of condition, egg ingestion decreased whole-body protein breakdown rates (time effect: $P < 0.001$; time × condition: $P = 0.31$) and increased whole-body synthesis rates ($P = 0.031$; time × condition: $P = 0.87$), thereby resulting in an improved net protein balance when compared with fasted conditions (time effect: $P < 0.001$; time × condition: $P = 0.27$). Whole-body leucine oxidation rates decreased (time effect: $P = 0.008$) after egg ingestion with no differences between conditions (time × condition: $P = 0.74$).

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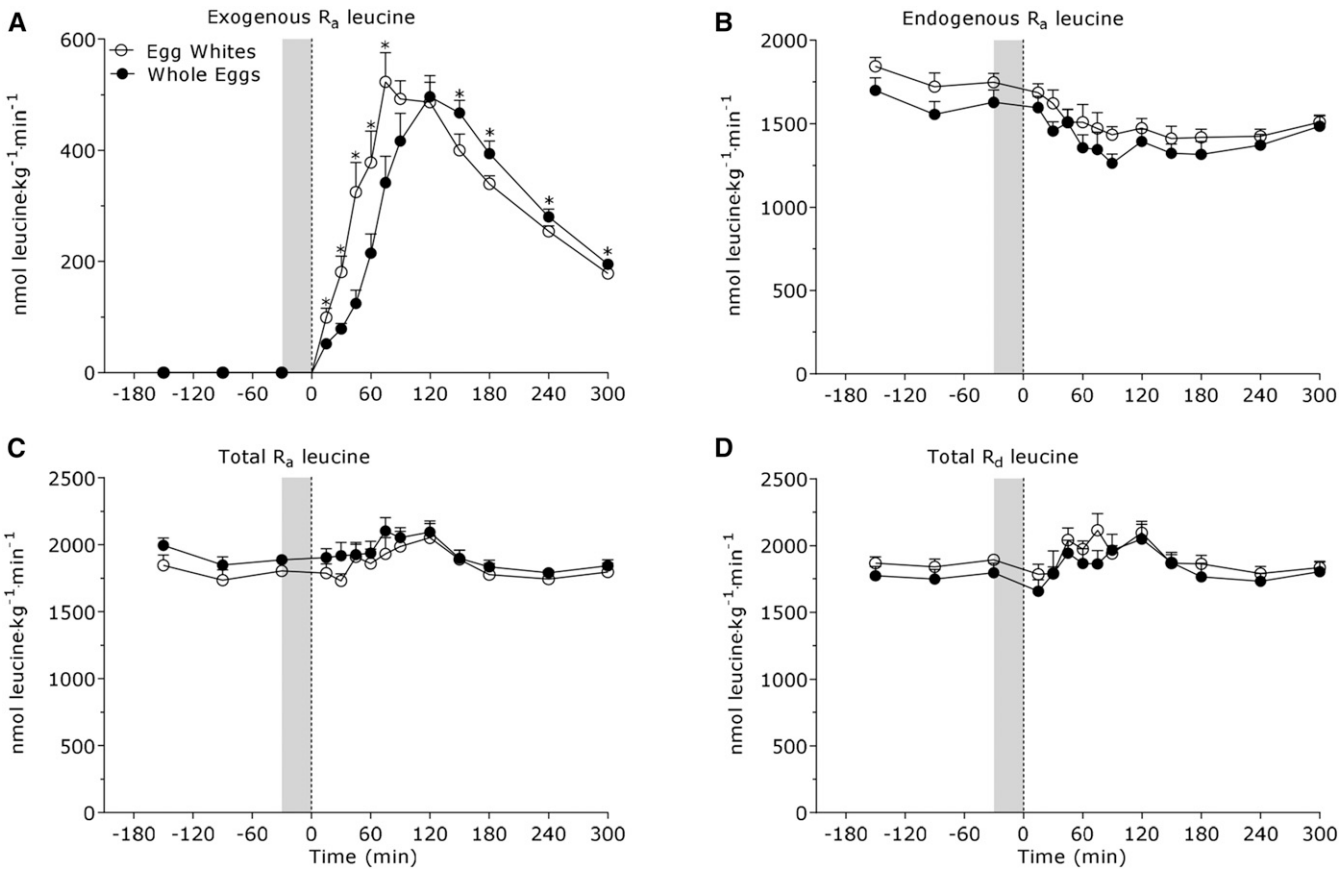

FIGURE 4 Whole-body leucine kinetics over time in the fasted state and after resistance exercise and consumption of egg whites or whole eggs in young men (n = 10/condition). Exogenous leucine R_a (A), endogenous leucine R_a (B), total leucine R_a (C), and total leucine R_d (D) are shown. Values are means ± SEMs. Gray-shaded areas correspond to the exercise bout; dashed lines refer to time of egg ingestion. Data were analyzed with 2-factor ANOVA with repeated measures on time. A Bonferroni post hoc test was used to locate differences between means for all significant interactions. Exogenous R_a: time effect, $P < 0.001$; time × condition, $P = 0.001$. Endogenous R_a: time effect, $P < 0.001$; time × condition, $P = 0.17$. Total R_a: time effect, $P < 0.001$; time × condition, $P = 0.83$. Total R_d: time effect, $P < 0.001$; time × condition, $P = 0.84$. *Different between egg conditions, $P < 0.05$. R_a, rate of appearance; R_d, rate of disappearance.

Whole-body net leucine retention improved after egg ingestion when compared with fasted values (time effect: $P < 0.001$) with no difference between conditions (time × condition: $P = 0.70$). Whole-body leucine retention improved from negative fasted values of −354 ± 29 and −347 ± 20 to positive fed values of 131 ± 33 and 123 ± 22 nmol leucine · kg^{-1} · min^{-1} for egg whites and whole eggs, respectively.

Intramuscular signaling and amino acid transporters

There was no change in the relative protein content of skeletal muscle amino acid transporters (SLC7A5/LAT1, SLC3A/CD98, and SLC38A2/SNAT2) throughout postexercise recovery (all time effect: $P > 0.05$) (**Figure 6**). AMPKα phosphorylation decreased during postexercise recovery (time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.52$) (**Figure 7**A). Phosphorylation of mTORC1 increased by 0.9- ± 0.3-fold and 0.6- ± 0.3-fold above fasted values at 300 min of postexercise recovery ($P = 0.01$) after whole-egg and egg white ingestion, respectively, with no differences between egg conditions (time × condition: $P = 0.58$) (Figure 7B). Erk1/2 phosphorylation decreased during postexercise recovery (time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.64$) (Figure 7C). Phosphorylation of p70S6K1 was elevated above the fasted state throughout postexercise recovery (time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.49$) (Figure 7D). 4E-BP1 phosphorylation was elevated throughout postexercise recovery (time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.23$) (Figure 7E). There was no change in the phosphorylated state of eEF2 phosphorylation, regardless of egg condition, during recovery from exercise (time effect: $P = 0.13$) (Figure 7F). Representative blots are shown in **Supplemental Figures 2** and **3**.

Muscle intracellular free enrichments

Muscle intracellular enrichments are presented in **Table 2**. Muscle intracellular L-[5,5,5-$^2H_3$]leucine enrichments increased after egg ingestion (time effect: $P < 0.001$) with no differences between conditions (time × condition: $P = 0.61$). Muscle intracellular L-[1-^{13}C]leucine enrichments remained steady over time (time effect: $P = 0.30$) with no differences between conditions (time × condition: $P = 0.62$). Muscle intracellular L-[ring-$^2H_5$]phenylalanine enrichments were lower at $t = -150$ min than at time points $t = -30$, 120, and 300 min (all $P < 0.05$), with

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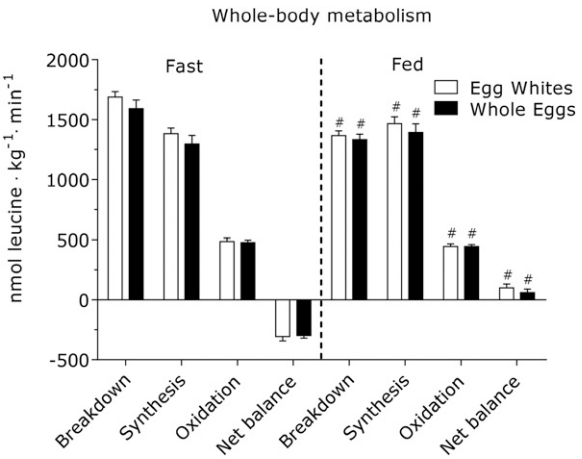

FIGURE 5 Whole-body leucine metabolism shown as the AUC of endogenous R_a (marker of protein breakdown), NOLD (marker of protein synthesis), leucine oxidation (oxidation), and net leucine balance in the fasted state and after resistance exercise and consumption of egg whites or whole eggs in young men (n = 10/condition). Values are means ± SEMs. Data were analyzed with 2-factor ANOVA with repeated measures on time. A Bonferroni post hoc test was used to locate differences between means for all significant interactions. Endogenous R_a: time effect, $P < 0.001$; time × condition, $P = 0.31$. NOLD: time effect, $P = 0.031$; time × condition, $P = 0.87$. Oxidation: time effect, $P = 0.008$; time × condition, $P = 0.74$. Net balance: time effect, $P < 0.001$; time × condition, $P = 0.27$. #Different from fasting, $P < 0.05$. NOLD, nonoxidative leucine disposal; R_a, rate of appearance.

no differences between conditions ($P = 0.63$) or other time points (all $P > 0.05$).

Myofibrillar protein synthesis rates

With the use of plasma L-[*ring*-$^2H_5$]phenylalanine enrichments as the precursor, myofibrillar FSRs increased above fasted values (0.015% ± 0.002%/h) after egg white and whole-egg ingestion (time effect: $P = 0.015$), with a greater cumulative response (0–300 min) after whole-egg ingestion (0.034% ± 0.004%/h; 2.7- ± 0.5-fold above fasted) when compared with egg white ingestion (0.024% ± 0.002%/h; 1.9- ± 0.4-fold above fasted) (time × condition: $P = 0.04$) (**Figure 8**). The temporal pattern of change in the early (0–120 min) and late (120–300 min) myofibrillar protein FSR after egg ingestion was not different between the whole-egg and egg white conditions (time × condition: $P = 0.54$); however, both egg conditions were elevated above the fasted state during the early and late recovery phases (all $P < 0.05$) (Figure 8, inset).

Similarly, by using the intracellular L-[*ring*-$^2H_5$]phenylalanine enrichment as the precursor, the cumulative (0–300 min) myofibrillar protein FSR increased above fasted values (0.07% ± 0.011%/h) to a greater extent after whole-egg ingestion (0.13% ± 0.017%/h; 2.1- ± 0.3-fold above fasted) than after egg white ingestion (0.086% ± 0.006%/h; 1.6- ± 0.3-fold above fasted) (time effect: $P = 0.001$; time × condition: $P = 0.05$). However, by using intracellular L-[*ring*-$^2H_5$]phenylalanine enrichments as the precursor, the temporal pattern of increase in myofibrillar protein FSR did not show time- or condition-dependent differences in the response (time effect: $P = 0.12$; time × condition: $P = 0.53$). Specifically, postexercise myofibrillar protein FSRs between 0 and 120 min were 0.140% ± 0.035%/h (2.5- ± 0.8-fold above fasted) and 0.098% ± 0.017%/h (1.8- ± 0.6-fold above fasted) after whole-egg and egg white ingestion, respectively. Between 120 and 300 min, myofibrillar protein FSRs were 0.123% ± 0.027%/h (1.8- ± 0.3-fold above fasted) and

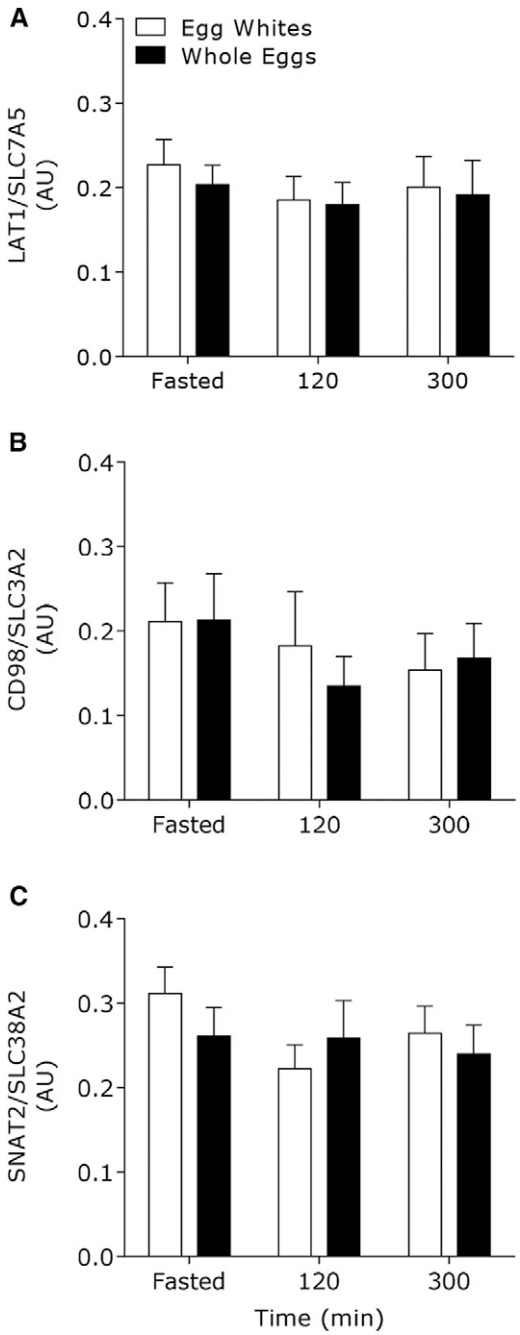

FIGURE 6 Skeletal muscle protein content of LAT1/SLC7A5 (A), CD98/SLC3A2 (B), and SNAT2/SLC38A2 (C) in the fasted state and after resistance exercise and consumption of egg whites or whole eggs in young men (n = 10/condition). Values are means ± SEMs. Data were analyzed with 2-factor ANOVA with repeated measures on time. A Bonferroni post hoc test was used to locate differences between means for all significant interactions. LAT1/SLC7A5: time effect, $P = 0.18$; time × condition, $P = 0.81$. CD98/SLC3A2: time effect, $P = 0.10$; time × condition, $P = 0.49$. SNAT2/SLC38A2: time effect, $P = 0.12$; time × condition, $P = 0.16$. AU, arbitrary unit; LAT1, large neutral amino acid transporter small subunit 1; SLC3A2, solute carrier family 3 member 2; SLC7A5, solute carrier family 7 member 5; SLC38A2, solute carrier family 38 member 2; SNAT2, sodium-coupled neutral amino acid transporter 2.

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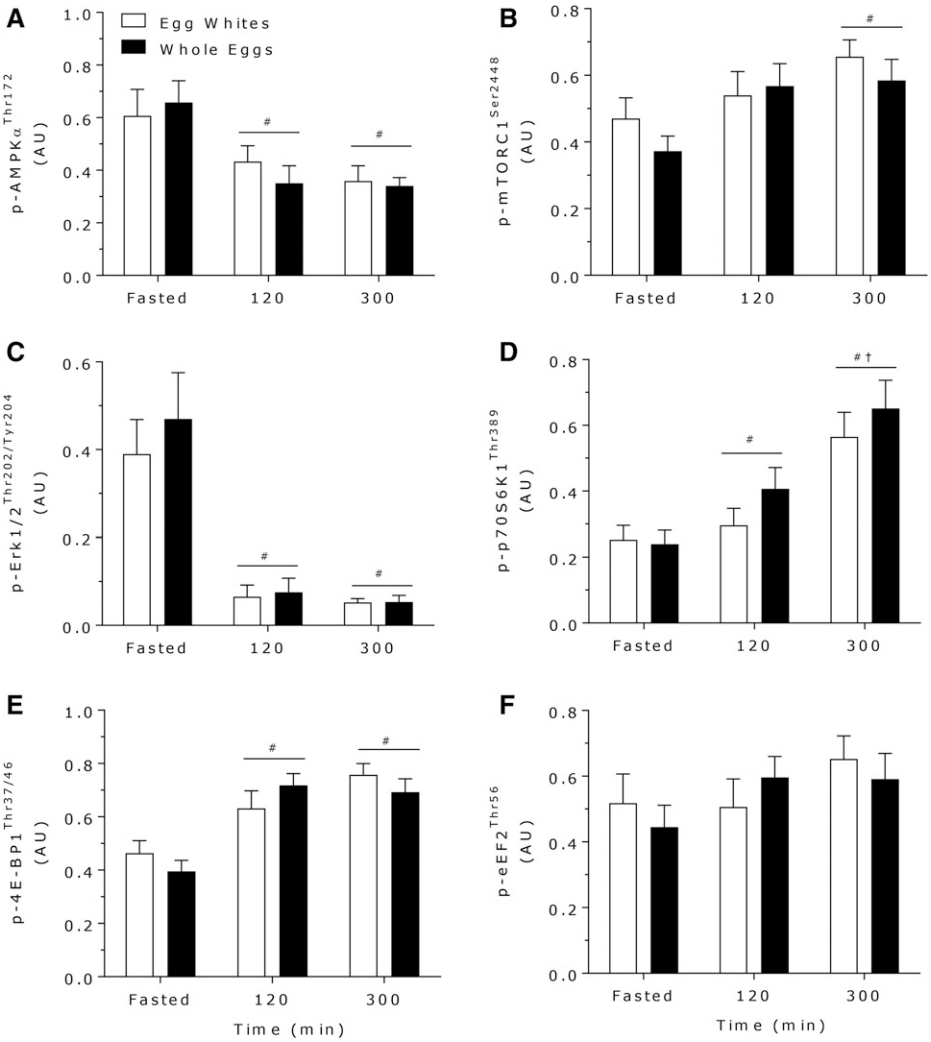

FIGURE 7 Phosphorylation status of AMPKα^{Thr172} (A), mTORC1^{Ser2448} (B), Erk1/2$^{Thr202/Tyr204}$ (C), p70S6K1^{Thr389} (D), 4E-BP1$^{Thr37/46}$ (E), and eEF2^{Thr56} (F) in the fasted state and after resistance exercise and consumption of egg whites or whole eggs in young men (n = 10/condition). Values are means ± SEMs. Data were analyzed with 2-factor ANOVA with repeated measures on time. A Bonferroni post hoc test was used to locate differences between means for all significant interactions. p-AMPKα^{Thr172}: time effect, $P < 0.001$; time × condition, $P = 0.52$. p-mTORC1^{Ser2448}: time effect, $P = 0.010$; time × condition, $P = 0.577$. p-Erk1/2$^{Thr202/Tyr204}$: time effect, $P < 0.001$; time × condition, $P = 0.64$. p-p70S6K1^{Thr389}: time effect, $P < 0.001$; time × condition, $P = 0.49$. p-4E-BP1$^{Thr37/46}$: time effect, $P < 0.001$; time × condition, $P = 0.23$. p-eEF2^{Thr56}: time effect, $P = 0.13$; time × condition, $P = 0.41$. $^{\#}$Different from fasted, $P < 0.05$; †different from 120 min, $P < 0.05$. AMPKα, AMP-activated protein kinase α; AU, arbitrary unit; eEF2, eukaryotic translation elongation factor 2; Erk, extracellular signal–regulated kinase; mTORC1, mammalian target of rapamycin complex 1; p-, phosphorylated; p70S6K1, p70-kDa S6 protein kinase 1; 4E-BP1, eukaryotic translation initiation factor 4E–binding protein 1.

0.078% ± 0.013%/h (1.4- ± 0.4-fold above fasted) after whole-egg and egg white ingestion, respectively.

DISCUSSION

To our knowledge, we report for the first time the potentiation of exercise-mediated stimulation of postprandial myofibrillar protein synthesis rates in response to the ingestion of whole eggs compared with isonitrogenous amounts of egg whites in healthy young men. Our work points to the concept that dietary protein may show differential anabolic properties on skeletal muscle tissue when consumed within its natural whole-food matrix.

Similar to isolated protein sources (e.g., whey and casein) (5, 24), we also showed that the consumption of protein-dense foods in the form of whole eggs and egg whites improves whole-body net protein balance (Figure 5). Although insight into whole-body protein kinetics can provide valuable information on whole-body protein remodeling and net anabolism, it is not uncommon that whole-body protein kinetics may "mask" important changes within specific tissues such as skeletal muscle (2). In particular, the observed potentiation of postexercise myofibrillar protein synthesis rates in response to whole-egg compared with egg white ingestion was not observed on a whole-body level in which there was a similar stimulation of whole-body protein synthesis rates in both conditions.

We studied various upstream factors that are often assumed to be regulatory for postprandial muscle anabolism in an attempt to underpin how the ingestion of whole eggs supported a greater postexercise myofibrillar protein synthetic response. In particular, we showed more rapid appearance rates of protein-derived leucine into the circulation and higher peak leucinemia after egg white than after whole-egg ingestion (Figure 4). The plasma

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TABLE 2
Muscle intracellular free tracer enrichments before and after egg ingestion[1]

Tracer	−150 min (n = 5)	−30 min (n = 10)	120 min (n = 10)	300 min (n = 10)
L-[5,5,5-$^2H_3$]leucine				
Egg whites	0	0	3.5 ± 0.5$^{\#}$	1.4 ± 0.2$^{\#}$
Whole eggs	0	0	4.0 ± 0.4$^{\#}$	1.8 ± 0.1$^{\#}$
L-[1-^{13}C]leucine				
Egg whites	5.1 ± 0.6	5.1 ± 0.3	5.5 ± 0.5	4.8 ± 0.3
Whole eggs	4.1 ± 0.5	4.9 ± 0.3	5.1 ± 0.5	5.2 ± 0.4
L-[*ring*-$^2H_5$]phenylalanine				
Egg whites	0.8 ± 0.1	1.4 ± 0.1$^{\#}$	1.5 ± 0.2$^{\#}$	1.3 ± 0.1$^{\#}$
Whole eggs	0.7 ± 0.1	1.3 ± 0.1$^{\#}$	1.3 ± 0.0$^{\#}$	1.3 ± 0.1$^{\#}$

[1] Values are mean ± SEM muscle free L-[5,5,5-$^2H_3$]leucine, L-[1-^{13}C]leucine, and L-[*ring*-$^2H_5$]phenylalanine enrichments (in mole percent excess) in the fasted state and after consumption of egg whites or whole eggs in young men. Muscle biopsies at t = −150 and −30 min represent the fasted state and t = 120 and 300 min represent the postprandial state. Data were analyzed with 2-factor ANOVA with repeated measures on time. A Bonferroni post hoc test was used to locate differences between means for all significant interactions. L-[5,5,5-$^2H_3$]leucine: time effect, $P < 0.001$; time × condition, $P = 0.61$. L-[1-^{13}C]leucine: time effect, $P = 0.30$; time × condition, $P = 0.62$. L-[*ring*-$^2H_5$]phenylalanine: time effect, $P < 0.001$; time × condition, $P = 0.54$. $^{\#}$Different from t = −150 min, $P < 0.05$.

leucine profiles were similar in pattern to previous studies that used either isolated egg protein (25) or whole eggs (26). However, there were no differences between the total amount of dietary protein–derived leucine that became available in circulation throughout the 5-h postprandial period between the egg white (68% ± 1%) and whole-egg (66% ± 2%) conditions. Similarly, there were no differences in the relative protein content of skeletal muscle amino acid transporters (e.g., SLC7A5/LAT1, SLC3A/CD98, and SLC38A2/SNAT2) (Figure 6) or muscle free L-[5,5,5-$^2H_3$]leucine enrichments during recovery from exercise between the egg conditions (Table 2). Collectively, these data indicate that muscle amino acid sensing, amino acid uptake, or both likely did not contribute to the differential stimulation of postexercise myofibrillar protein synthesis rates between the egg conditions.

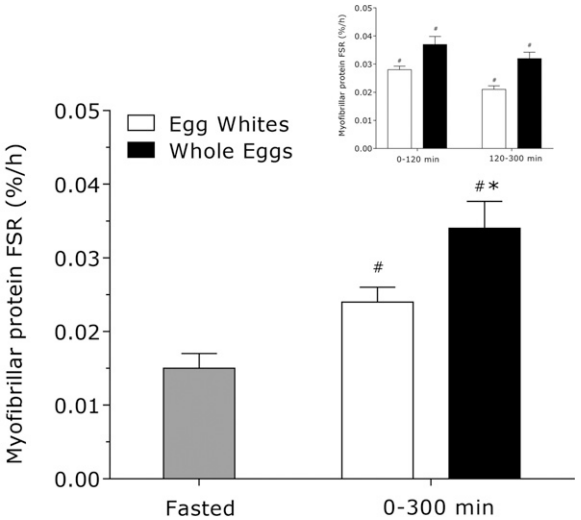

FIGURE 8 Myofibrillar protein synthesis rates in the fasted state and after resistance exercise and consumption of egg whites or whole eggs in young men (n = 10/condition). Values are means ± SEMs. The inset shows the temporal responsiveness of myofibrillar protein synthesis rates during the early (0–120 min) and late (120–300 min) periods after resistance exercise and egg ingestion. Data were analyzed with 2-factor ANOVA with repeated measures on time. A Bonferroni post hoc test was used to locate differences between means for all significant interactions. FSR: time effect, $P = 0.02$; time × condition, $P = 0.04$. Inset FSR: time effect, $P = 0.04$; time × condition, $P = 0.50$. $^{\#}$Different from fasted, $P < 0.05$; *different between egg conditions, $P < 0.05$. FSR, fractional synthesis rate.

In addition, the temporal assessment of various metabolic and molecular readouts often associated with the control of translation initiation and elongation, such as mitogen-activated protein kinase (MAPK)–related and mTORC1-related signaling pathways, did not show significant differences in phosphorylation between the egg conditions (Figure 7). The lack of differences in the phosphorylated state of mTORC1-mediated signaling between the egg conditions could imply that this anabolic pathway was maximized from the previous performance of resistance exercise and food ingestion (2, 9, 10, 25), thereby obscuring any subtle nonprotein nutritive influences of whole-egg consumption on mTORC1 phosphorylation and its downstream targets. We also examined metabolic regulatory pathways and energy-sensing protein phosphorylation. Similar to the other assessed molecular readouts, there were no observed differences in the phosphorylated states of AMPK or Erk1/2.

The essential amino acid compositions, and leucine in particular, were nearly identical between the egg conditions (27). Thus, differences in the amino acid composition of the ingested egg sources likely did not have an influential role in the muscle anabolic response. In addition, the greater overall energy content, and subsequent insulinemia, within the whole-egg (256 kcal) and egg white (73 kcal) conditions likely did not influence the differential stimulation of the postprandial myofibrillar protein synthetic response. For example, it has been shown that only relatively low plasma insulin concentrations (5 μU/mL) are required to maximize the muscle anabolic potential of elevated plasma amino acid availability in humans (28, 29). Moreover, the additional substrate (i.e., fat) for energy production with whole-egg ingestion did not spare the use of amino acids for oxidative "fuel" (Figure 5). Also, previous reports have shown that providing additional energy with isolated protein sources does not amplify the anabolic properties of dietary amino acids to stimulate postprandial muscle protein synthesis rates when compared with the ingestion of protein or amino acids alone (6–8, 30, 31).

Interestingly, research has shown that other food components, beyond dietary amino acids, may have a supporting role in modulating postprandial muscle anabolism during recovery from exercise. For example, Elliot et al. (32) previously showed that whole-milk ingestion immediately after resistance exercise resulted in greater amino acid uptake across the leg than after the consumption of isonitrogenous or isoenergetic amounts of skim milk in healthy young adults. In the present study, we showed a greater early (0–120 min) postprandial dietary-derived leucine availability after the ingestion of egg whites (34% ± 2%) than after whole eggs (25% ± 3%). The greater postprandial plasma leucine availability after egg white than after whole-egg ingestion, however, did not result in a greater early stimulation of the postprandial (0–120 min) myofibrillar protein synthetic response after the ingestion of egg whites when compared with whole eggs. Hence, it seems that "extra" nutritional food constituents, and not simply a rapid aminoacidemia or leucinemia

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(1, 5, 11, 12), may also have a role in modulating the postprandial muscle protein synthetic response in healthy adults. For example, as part of its whole-food matrix, the egg yolk contains various nonprotein food components that may have anabolic properties, such microRNAs (33), vitamins (34, 35), minerals (36), and lipids [e.g., phosphatidic acid (37), palmitic acid (38), and DHA (39)] by modifying pathways related to transcriptional or protein translational control. However, more work is required to systematically assess the role of such food components on modulating postprandial muscle protein synthesis rates in vivo in humans.

It is important to note that despite recent modifications of dietary guidelines to reflect that total dietary cholesterol intake is often misrepresented as a risk factor for cardiovascular disease (40, 41), popular practice may still dictate the discarding of the yolk when multiple eggs are consumed in a meal. However, the yolk is nutrient dense and may contain a variety of important bioactive compounds such as lipids, micronutrients, antioxidant carotenoids, and microRNAs (42, 43). The removal of the yolk and its associated nutrients from eggs may limit the stimulation of muscle protein synthesis rates as well as overall human health (42, 44, 45). Overall, this work supports recommendations that nutrient- and protein-dense foods are cornerstones to meeting daily protein requirements to optimize muscle protein synthesis rates with exercise.

In conclusion, we show that postexercise myofibrillar protein synthesis rates are stimulated to a greater extent after the consumption of whole eggs than after the consumption of egg whites in healthy young men, despite being matched for protein content. We observed no differences between egg conditions in the commonly assumed regulators of postexercise muscle protein synthesis rates, such as total postprandial plasma leucine availability, whole-body leucine oxidation rates, skeletal muscle amino acid transport protein content, or molecular readouts associated with metabolic and anabolic protein signaling. Future work is required to identify the potential role of nonprotein food components to contribute to the stimulation of postprandial muscle protein synthesis rates in humans. This information is important because other nutritional components may contribute to food protein requirements and particularly when dietary protein is consumed in moderate amounts ($\sim$15–20 g protein/meal).

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The authors' responsibilities were as follows—SvV and NAB: contributed to the conception and design of the experiment, contributed to drafting or revising the intellectual content of the manuscript, and had primary responsibility for the final content; and all authors: contributed to the collection, analysis, and interpretation of data and read, edited, and approved the final version of the manuscript. None of the authors reported a conflict of interest related to the study.

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