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Quality of meal protein determines anabolic response in older adults

Il-Young Kim[a,c,*], **Yun-A. Shin**[b], **Scott E. Schutzler**[a], **Gohar Azhar**[a], **Robert R. Wolfe**[a], **Arny A. Ferrando**[a]

[a]Department of Geriatrics, The Center for Translational Research in Aging & Longevity, Donald W. Reynolds Institute on Aging, University of Arkansas for Medical Sciences, Little Rock, AR, USA

[b]Department of Prescription & Rehabilitation of Exercise, Dankook University, Cheonan, Republic of Korea

[c]Department of Molecular Medicine, Lee Gil Ya Cancer and Diabetes Institute Gachon University School of Medicine, Incheon, Republic of Korea

SUMMARY

Background & aims: It has been demonstrated that the relative content and profile of essential amino acids (EAA) play a determining role for stimulation of muscle protein synthesis (MPS) following intake of pure EAA or protein alone.

Methods: To test if this also holds in the context of mixed meals at both whole body and muscle levels, twelve older subjects (57–74 yrs) received primed continuous infusion of L-[ring-$^2H_5$]phenylalanine and L-[ring-$^2H_2$]tyrosine over a 9-h experimental period to determine whole body protein kinetics and MPS in the fasted state and following consumption of egg-based (EGG) or cereal-based (CEREAL) isocaloric and isonitrogenous breakfast. A standardized lunch, primarily consisting of beef protein was also consumed by each group. Whole body protein kinetics [protein synthesis (PS), breakdown (PB), and net balance (NB)] were expressed as changes from basal fasted period.

Results: We found that EGG breakfast resulted in a greater NB through a greater suppression of PB compared with the CEREAL breakfast. The greater NB during the post-breakfast period with the EGG was normalized following the standard lunch despite the sustained elevations in plasma EAA concentrations. However, the EGG breakfast stimulated both PS and PB compared with the CEREAL breakfast during the post-lunch period. MPS was not different between meals despite larges differences in the plasma EAA responses.

*Corresponding author. Department of Molecular Medicine, Lee Gil Ya Cancer and Diabetes Institute Gachon University School of Medicine, 155, Gaetbeol-ro, Yeonsu-ku, Incheon, 21999, Republic of Korea., iykim@gachon.ac.kr (I.-Y. Kim).

Authors contributions

I.-Y.K., Y.-A.S., R.R.W., and A.A.F. analyzed data and interpreted results of experiments; I.-Y.K. performed calculations of protein kinetics. I.-Y.K. and Y.-A.S. performed statistical analysis; I.-Y.K. prepared tables and figures and drafted manuscript; I.-Y.K., and S.E.S. performed experiments; G.A. provided medical supervision; I.-Y.K., R.R.W., and A.A.F. research conception and design of experiments. All authors read and approved the final manuscript.

Conflict of interest

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Conclusions: We conclude that in the context of mixed meals, quality of protein affects NB through changes in protein breakdown and affects protein turnover following subsequent meal intake.

Keywords

Protein quality; Sarcopenia; Aging; Stable isotope tracer

1. Introduction

Maintenance of body protein is the primary nutritional goal of dietary protein. This is particularly pertinent in aging individuals to prevent the development of sarcopenia owing to loss of muscle mass and function. It is well known that protein/amino acid ingestion promotes muscle anabolism [1–3], thereby countering the development of sarcopenia. The net anabolic response (protein synthesis - breakdown) to dietary protein consumption is primarily determined by two factors: 1) quantity and 2) quality of protein. The role of these factors with respect to MPS has been well described following ingestion of pure protein/amino acid [1–3], but not in the context of mixed meal intake [1,2]. In terms of the role of protein quantity, it has been demonstrated that there is a dose-response relation between MPS and amount of protein intake up to ~30 g of “high quality” protein, or 0.4 g protein/kg body weight/meal, above which no further stimulation of MPS occurs in older adults [1,4]. Consequently, it has been postulated that exceeding 30 g of protein in a meal provides no further anabolic benefit. However, this assertion has been based upon the determination of only protein synthesis, without consideration of a potential role for changes in protein breakdown in achieving a greater anabolic response. In line with this notion, our recent studies have shown that the net anabolic response is greater with increasing amounts of protein intake in mixed meals through reductions in protein breakdown, without any sign of plateau with protein intake even above 70 g [5,6]. Second, it has been shown that the quality of protein plays an important role, in that for a given amount of protein intake, “higher quality” protein results in a greater anabolic response [7,8]. Protein quality can be assessed by two factors: 1) fractional content and profile of EAA in the protein source and 2) fractional EAA absorption to systemic circulation for a given quantity of protein intake (i.e., digestibility) [9]. Protein quality varies among protein sources. Animal proteins generally are higher in EAA content than plant-based proteins, and they also have a greater digestibility. In addition, plant-based proteins are also deficient in one or more specific EAAs [10]. Accordingly, it has been shown that ingestion of animal-based protein resulted in a greater MPS response compared with that of plant-based protein [11]. However, few studies have accessed the role of protein quality in the context of mixed meals, or considered the importance of protein balance at the whole-body level. Therefore, in this study we have compared two common breakfast formats whereby protein is consumed in a plant-based cereal format versus an animal-based egg format. We hypothesized that 1) an egg-based breakfast will result in a greater anabolic response versus an isonitrogenous and isocaloric cereal-based breakfast, and 2) the greater anabolic response after the egg-based breakfast will persist after a standard lunch, due in large part to a greater increase in plasma essential amino acid concentrations.

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2. Materials and methods

2.1. Subjects

Fourteen healthy male and female older adults [57–74 yrs] were recruited from the Little Rock area using local newspaper advertisements and flyers. One subject failed screening, and one subject dropped out. Subjects were included if they aged between 50 an 75 yrs and with BMI between 21 and 30 kg/m^2. Subjects were excluded with anemia (hemoglobin <11.0 g/dl), diabetes, active malignancy within the past 6 months, lactose intolerance or dairy allergy, gastrointestinal bypass surgery, a chronic inflammatory or other chronic disease such as HIV/AIDS, low hematocrit or hemoglobin concentration, low platelets, concomitant use of corticosteroids, any unstable medical conditions, and pregnant females. Written informed consent was obtained from all subjects, and the Institutional Review Board at the University of Arkansas for Medical Sciences approved the study. A sample size calculation of 12 subjects was estimated to have approximately 80% power based on the power analysis (two-sample equal variance t-test) of blood leucine delivery between the 2 breakfast treatments resulting in a standard deviation estimate of 15 g protein/750 min for net protein balance.

2.2. Experimental protocol

During the screening for subject eligibility, body composition was determined by dual-energy X-ray absorptiometry (DEXA, QDR-4500A; Hologic, Waltham, MA) (Table 1). After screening, a standardized diet was consumed 2 days prior to each metabolic study. In a crossover design, subjects (n = 12 each) completed the following treatments consisting of an isonitrogenous (≈26 g protein) and isocaloric (≈500 kcal) breakfast and a standardized lunch (≈25 g (primarily) beef protein) in random order. In treatment 1 (EGG), subjects consumed a breakfast with egg as the primary protein source and a standardized lunch. In treatment 2 (CEREAL), subjects consumed a breakfast of common complementary/cereal proteins and a standardized lunch. Intended to be isonitrogenous and isocaloric, the complementary/cereal protein breakfast had an EAA content of approximately 6.9 g, while the egg-protein breakfast had an EAA content of approximately 9.2 g (See Table 2 for detailed macronutrient info). The meals were designed by our Registered Dietitian and made prior to each study in our research kitchen. Subjects were asked to eat each meal fully and to refrain from consuming protein supplements during the term of their participation in this study. They were permitted to continue taking non-protein supplements (e.g. vitamins/minerals).

2.3. Stable isotope tracer infusion protocol

Subjects reported to the Reynolds Institute on Aging (RIOA) after an overnight (after 2200 h) fast. During each metabolic study (Fig. 1), a catheter was placed into each lower arm; one for the infusion of stable isotope tracers and the other for “arterialized” blood sampling via a heating box or using a heating pad [12]. Prior to the infusion of tracers, a baseline blood sample was collected to determine background isotopic enrichments and blood chemistry. For determination of in vivo protein kinetics (rates of protein synthesis, breakdown, and net balance) at the whole body level, primed continuous infusions of L-[ring-$^2H_5$]phenylalanine (prime, 3.92 μmol/kg; infusion rate, 4.60 μmol/kg/h) and L-[ring-$^2H_2$] tyrosine (prime, 1.57

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μmol/kg; infusion rate, 0.95 μmol/kg/h) were performed. To appropriately reach isotopic equilibrium of L-[ring-$^2H_4$]-tyrosine enrichment derived from L-[ring-$^2H_5$]phenylalanine tracer infused, a priming dose of L-[ring-$^2H_4$]tyrosine was also injected (prime, 0.33 μmol/kg). All isotope tracers were purchased from Cambridge Isotope Laboratories (Andover, MA). Blood samples were taken at 0, 100, 120, 150, 165, 180, 195, 225, 255, 285, 315, 375, 390, 405, 420, 450, 480, 510, 540 min to determine tracer enrichment. Glucose and insulin were also measured. A total of 19 blood samples were taken during the study (approx.100 ml). Meals were provided at 135 and 360 min. The muscle biopsy samples were collected from the vastus lateralis at 120 min after the start of tracer infusion (before the first meal). The second and third muscle biopsies were taken at 315 min and 540 min, respectively. To mitigate the potential catabolic effects of the 9-h bed rest during the metabolic study, subjects walked on a treadmill at 3.22 km/h for 15 min (345–400 min) as in our previous studies [6,13].

2.4. Analytic methods

Plasma and muscle samples were processed before determinations of enrichment for calculations of protein kinetics or of amino acid concentrations as previously described [5,6,13]. Enrichments of phenylalanine and tyrosine tracers and plasma amino acid concentrations were determined by using liquid-chromatography mass spectrometry (QTrap 5500 MS; AB Sciex) (Fig. 2). Plasma glucose concentrations were determined spectrophotometrically on a Cobas c 111 analyzer (Roche, F. HoffmaneLa Roche Ltd, Basel, Switzerland). Plasma insulin concentrations were determined by using commercially available human insulin ELISA kit (Alpco Diagnostics, Salem, USA).

2.5. Calculations of protein kinetics

Whole body protein kinetics [protein synthesis (PS), protein breakdown (PB), and net protein balance (NB)] following breakfast or lunch were calculated and expressed as changes from basal, fasted to fed states, based on the determinations of the rate of appearance (R_a) into the plasma of phenylalanine and tyrosine, and the fractional R_a of endogenous tyrosine converted from phenylalanine as previously described [5,6,13].

$$\text{Total rate of appearance into plasma}(R_a) = F/E \quad (1)$$

$$\text{Fractional}R_a\text{of Tyr from Phe} = E_{TyrM+4}/E_{PheM+5} \quad (2)$$

$$\text{HydX} = \text{Fractional}R_a\text{of Tyr from Phe} \times R_a\text{Tyr} \quad (3)$$

$$PS = [(R_a\text{Phe} - \text{Phe hydroxylation rate}) \times 25] \quad (4)$$

$$PB = [(R_a\text{Phe} - F_{Phe} - EXO_{Phe}) \times 25] \quad (5)$$

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$$NB = PS - PB \quad (6)$$

$$MPS\left(\% \cdot h^{-1}\right) = [(E_{B2} - E_{B1})/(E_{PL} \times t)] \times 60 \times 100 \quad (7)$$

Where enrichment (E) is expressed as tracer to tracee ratio (TTR) for calculation of PB or mole percent excess (MPE = TTR/(TTR+1)) for calculation of PS. Phe and Tyr are phenylalanine and tyrosine, respectively. F is respective tracer infusion rate into a venous side: F_{Phe} for phenylalanine tracer. $E_{Tyr\ M+4}$ and $E_{Phe\ M+5}$ are plasma enrichments of tyrosine and phenylalanine tracers at M+4 and M+5 relative to M+0, respectively. 25 is the conversion factor from kinetics at amino acid (i.e., phenylalanine) to protein levels based on the assumption that contribution of phenylalanine to protein is 4% (100/4 = 25) [14]. EXO_{Phe} is the rate of appearance of exogenous phenylalanine in the meals in the circulation, accounting for digestibility of amino acids [15]. HydX is the rate of appearance of tyrosine derived from phenylalanine via hydroxylation. To account for differences in digestibility and splanchnic extraction of amino acids in the kinetic calculations with different protein sources, we assumed that 90.9% (for EGG breakfast), 66.7% (for CEREAL breakfast), and 91% (for the standardized lunch) of the amount of exogenous protein in the meals appeared in the circulation [16–18]. Muscle protein fractional synthesis rate (MPS) was determined using the precursor-product method [19] as previously described [5,6]. E_{B2} and E_{B1} are the enrichments of bound L-[ring-$^2H_5$] phenylalanine from muscle samples at t_2 and t_1, respectively, and E_{PL} is the average plasma enrichment of L-[ring-$^2H_5$]phenylalanine between t_1 and t_2. *t* is the time in minutes elapsed between muscle biopsies. To express MPS in percent per hour, appropriate factors (60 and 100) were used, respectively.

2.6. Statistical analysis

To evaluate the effect of meal (EGG vs. CEREAL) and time (breakfast vs. lunch meals) on measures of whole body protein kinetics (i.e., NB, PS, and PB) and muscle protein fractional synthesis rate (MPS) as well as time-course responses and area under the curves of plasma amino acids, glucose, and insulin, two-way repeated measures of ANOVA were performed, followed by post-hoc analysis using 2 tailed t-test. Statistical significance was declared when the *p*-value was less than the 5% level. This analysis was using PASW Statistic Package software version 18 for Mac (SPSS, Chicago, IL).

3. Results

3.1. Whole body protein kinetics

Whole body PS, PB, and NB were expressed as changes from the fasted to fed states (i.e., 165 min for each post-meal period: post-breakfast and post-lunch period) (Fig. 3). Statistical comparisons were directed towards the anabolic responses to dietary protein intake, as we have demonstrated previously [5,6,13]. For PS, there were no significant effects for meals ($p = 0.210$), time ($p = 0.370$), and the meal-by-time interaction ($p = 0.106$). For PB, there were significant effects of meal ($p = 0.001$) and the meal-by-time interaction ($p < 0.001$); however, not for time ($p = 0.838$). The magnitude of reduction in PB was greater in the EGG compared to the CEREAL during the post-breakfast time period ($p < 0.001$), whereas

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it was greater in the CEREAL compared to the EGG during the post-lunch time period (p = 0.014). Suppression of PB was slightly attenuated in the EGG while slightly increased in the CEREAL during the post-lunch meal period versus the post-breakfast meal period (for both $p < 0.005$). For NB, there were significant effects for meal ($p < 0.001$) and time ($p = 0.004$), as well as the meal-by-time interaction ($p < 0.001$). Due to the differential responses in PB following each meal, NB was significantly higher in the EGG compared to the CEREAL during the post-breakfast time period ($p < 0.001$), but the standardized lunch mitigated these differences ($p = 0.867$). The post-lunch meal decreased NB in EGG, while increasing it slightly in CEREAL $p < 0.005$).

3.2. MPS

There was no meal effect ($p = 0.941$) or meal-by-time interaction ($p = 0.949$) for MPS; however, there was a significant time effect ($p = 0.006$). Post-hoc analysis revealed no significant difference but a tendency of increases in MPS in the post-lunch time period vs. post-breakfast time period in both EGG ($p = 0.108$) and CEREAL ($p = 0.095$) (Fig. 4).

3.3. Plasma amino acid responses

Plasma EAA, nonessential amino acids (NEAA), and leucine responses following meals are depicted in Fig. 5. For plasma responses of EAA, NEAA, and leucine, there were significant effects for meal, time, and meal-by-time interaction (for all, $p < 0.001$). Areas under the curve (AUC) for EAA and leucine were significantly higher in the EGG compared to the CEREAL during post-breakfast and post-lunch time periods (for all, $p < 0.01$). However, NEAA AUC was significantly higher in the CEREAL compared to EGG during post-breakfast and post-lunch time periods (for all, $p < 0.01$) (Fig. 5).

3.4. Plasma glucose and insulin responses

Plasma glucose and insulin responses are depicted in Fig. 6. For the time course responses of plasma insulin and glucose, there were significant main effects for meal (for both, $p < 0.005$) and time (for both, $p < 0.001$), as well as meal-by-time interaction (for both, $p < 0.001$). Plasma insulin AUC was significantly higher in the CEREAL compared to the EGG during the post-breakfast meal period ($p < 0.001$) but not during post-lunch meal period ($p = 0.622$). Similarly, plasma glucose AUC during the post-breakfast meal period was significantly higher in the CEREAL. Glucose AUC was significantly higher in the EGG compared to the CEREAL in the post-lunch meal period ($p < 0.001$), although the magnitude of the difference was small ($p < 0.01$).

4. Discussion

In the present study, we assessed the anabolic response to consumption of iso-caloric, isonitrogenous protein breakfast meals differing in quality of protein (i.e., relative EAA contents) and the anabolic response with a standard lunch meal. The “higher quality” egg-based (EGG) breakfast resulted in a greater whole-body NB compared to the cereal-based (CEREAL) breakfast through a greater suppression of whole-body PB. Neither whole-body PS or MPS were different despite greater increases in plasma EAA concentrations with the EGG compared with CEREAL breakfast. Following the standardized lunch, plasma

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EAA responses remained higher in the EGG compared with the CEREAL breakfast, which resulted in increases in protein turnover (i.e., relatively higher PS and PB in the EGG vs. the CEREAL) without net effects on NB or MPS.

After ingestion of the breakfast meals, NB became positive in the CEREAL and to a greater extent in the EGG. The greater whole-body NB with the EGG breakfast in the context of mixed meals was due to a greater reduction in PB. This result is consistent with previous findings [5,6,13,20] indicating the critical role for PB in determining anabolic response to protein intake in mixed meals. It is generally accepted that elevations in plasma insulin concentrations following a mixed meal result in a suppression of protein breakdown [21]. Consistent with this finding, we observed that a breakfast meal ingestion resulted in plasma elevations of insulin concentrations and suppression of PB with both meals. However, the EGG breakfast, with a lower insulin response, resulted in a greater suppression of PB compared with the CEREAL breakfast. This finding suggests that factor(s) other than insulin play a role in the greater reduction in PB. It is likely that in each group the insulin-mediated suppression of protein breakdown was maximized while an EAA effect on suppression of protein breakdown above the insulin effect may play an additional role. In line with the notion, Greenhaff et al. [22] demonstrated suppression of leg muscle protein breakdown with increasing insulin concentration up to 30 μIU/ml, with no further suppression at higher insulin concentrations. The plasma insulin concentrations with the EGG breakfast approximated those reported by Greenhaff et al. (i.e., ~30 μIU/ml) [22]. While the insulin effect is intact, it is not sufficient to explain the discrepancy in suppression of PB. In this regard, we have previously postulated that a rise in intracellular EAA concentrations due to hyperaminoacidemia following a higher protein intake leads to further suppression of PB [5,31]. We observed that plasma elevations of EAA concentrations were much greater with the EGG breakfast compared with the CEREAL breakfast. Although not directly determined in the present study, it is likely that the elevated plasma EAA concentrations with EGG breakfast would stimulate EAA influx into intracellular compartment [23]. Thus, with adequate intracellular EAA precursors to support a given rate of MPS via inward transport, provision of additional intracellular precursors from protein breakdown is no longer required. Consistent with this finding, we have previously shown that higher protein intake (70 g) resulted in a greater reduction in protein breakdown compared to lower protein intake (40 g), despite similar insulin responses [5]. Although protein intake in the breakfast meals was only 26 g, plasma elevations of EAA with the EGG breakfast were actually higher than those achieved with the moderate protein (40 g), and approximated those observed with higher protein intake (70 g) [5]. The elevation of plasma EAA following a lower protein intake in the present study may be due in part to the fact that 1) the protein quality (i.e., relative EAA contents) was higher and 2) content of other macronutrients (i.e., fat and carbohydrate) in the meals was much smaller (~385 kcal vs. ~730 kcal in the previous study). Taken together, the EGG breakfast resulted in a greater NB compared with the CEREAL breakfast, which was entirely due to a greater suppression of PB. The greater PB suppression can be explained by a greater rise in EAA concentrations, given similar insulin responses.

The stimulation of MPS is the main driver for the anabolic response when pure protein/AA is consumed, with no apparent changes in muscle protein breakdown (MPB) [24–26].

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However, in the context of mixed meals or intact meat, stimulation of protein synthesis at both the whole-body and muscle levels appears to play a minor role in achieving anabolic response [5,6,13,20]. For example, it has been shown that 135 g of lean beef (containing ~26 g protein) failed to stimulate MPS above the basal state [20]. The lack of stimulation of MPS above the basal state is due in part to the fact that plasma elevations of EAA concentrations (particularly leucine) was far smaller when compared to ingestion of similar amount of pure protein (e.g., 20 g whey protein) [27]. This phenomenon is more pronounced in the context of mixed meals where plasma EAA and leucine responses are even smaller [5,6]. Thus, in the context of a mixed meal, higher protein ingestion is required to stimulate MPS as compared to protein/AA alone. Due to the lack of measurement in the fasted state in the present study, we do not know if the same response occurred (i.e., failure of stimulation of MPS in response to protein intake). However, we found that breakfast feeding in the present study elevated plasma EAA concentrations above basal fasted states and in turn resulted in a stimulation of PS in both meals above basal fasted states. At the muscle level, we did not observe differences in the stimulation of MPS despite a greater elevation of plasma EAA concentration with the EGG. This may seem contrary to the idea that plasma EAAs, particularly leucine, are key factors in the translation [28] and regulation of PS [2]. However, this finding is not at odds if one realizes the close relationship between rates of protein synthesis and breakdown. Quantitatively, amino acids derived from protein breakdown are the major source of precursors for synthesis of new proteins, whereas the inward transport of amino acids from extracellular fluid constitute a secondary source [29]. Thus, the increased plasma EEA availability with the EGG breakfast facilitates inward EAA transport, which in turn serves to reduce EAA requirement from protein breakdown to maintain the total intracellular availability of EAA. On the other hand, it is likely that the lower plasma EAA response with the CEREAL breakfast may reduce inward EAA transport. This reduced contribution from inward EAA transport may force reliance upon protein breakdown to maintain total intracellular EAA availability.

Many Americans consume a cereal-based meal containing incomplete proteins for breakfast, while the lunch meal often contains higher quality proteins. We hypothesized that by adopting a high-quality protein breakfast meal (e.g., egg-based meal) instead of a cereal-based meal, the enhanced anabolic response following the EGG breakfast would be extended after the ingestion of a standardized lunch, in large part due to a persistent elevation in plasma EAA concentrations. Consistent with this hypothesis, we observed a persistent elevation in plasma EAA concentrations and a correspondingly greater PS in the EGG after a standardized lunch. Despite the difference in EAA concentrations, NB was not different between meals. However, we found that protein turnover rate was relatively greater with EGG compared to the CEREAL, as both PS and PB were higher in the EGG compared with the CEREAL. This may be an important finding as it has been shown that protein turnover rate is positively associated with muscle function [30]. Thus, the prolonged elevation of EAA with EGG and the standard lunch may promote PS through both increased inward transport-derived EAA and EAA derived from PB.

In conclusion, the quality of protein in a mixed meal plays an important role in determining whole body anabolic responses. Anabolism in response to a mixed meal is achieved through

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a decrease in whole-body PB, facilitated in part by both increased circulating/available EAA, and an insulin response.

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References

[1]. Moore DR, Churchward-Venne TA, Witard O, Breen L, Burd NA, Tipton KD, et al. Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. J Gerontol Biol Sci Med Sci 2015;70:57–62. 10.1093/gerona/glu103.

[2]. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr 2003;78: 250–8. [PubMed: 12885705]

[3]. Tipton KD, Gurkin BE, Matin S, Wolfe RR. Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. J Nutr Biochem 1999;10:89–95. [PubMed: 15539275]

[4]. Symons TB, Sheffield-Moore M, Wolfe RR, Paddon-Jones D. A moderate serving of high-quality protein maximally stimulates skeletal muscle protein synthesis in young and elderly subjects. J Am Diet Assoc 2009;109:1582–6. 10.1016/j.jada.2009.06.369. [PubMed: 19699838]

[5]. Kim I-Y, Schutzler S, Schrader A, Spencer HJ, Azhar G, Ferrando AA, et al. The anabolic response to a meal containing different amounts of protein is not limited by the maximal stimulation of protein synthesis in healthy young adults. Am J Physiol Endocrinol Metab 2016;310:E73–80. 10.1152/ajpendo.00365.2015. [PubMed: 26530155]

[6]. Kim I-Y, Schutzler S, Schrader A, Spencer H, Kortebein P, Deutz NEP, et al. Quantity of dietary protein intake, but not pattern of intake, affects net protein balance primarily through differences in protein synthesis in older adults. Am J Physiol Endocrinol Metab 2015;308:E21–8. 10.1152/ajpendo.00382.2014. [PubMed: 25352437]

[7]. Pennings B, Boirie Y, Senden JMG, Gijsen AP, Kuipers H, van Loon LJC. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. Am J Clin Nutr 2011;93: 997–1005. 10.3945/ajcn.110.008102. [PubMed: 21367943]

[8]. Devries MC, Phillips SM. Supplemental protein in support of muscle mass and health: advantage whey. J Food Sci 2015;80(Suppl 1):A8–15. 10.1111/1750-3841.12802. [PubMed: 25757896]

[9]. Wolfe RR, Rutherfurd SM, Kim I-Y, Moughan PJ. Protein quality as determined by the digestible indispensable amino acid score: evaluation of factors underlying the calculation. Nutr Rev 2016;74:584–99. 10.1093/nutrit/nuw022. [PubMed: 27452871]

[10]. Wolfe RR. Update on protein intake: importance of milk proteins for health status of the elderly. Nutr Rev 2015;73(Suppl 1):41–7. 10.1093/nutrit/nuv021. [PubMed: 26175489]

[11]. van Vliet S, Burd NA, van Loon LJC. The skeletal muscle anabolic response to plant- versus animal-based protein consumption. J Nutr 2015;145:1981–91. 10.3945/jn.114.204305. [PubMed: 26224750]

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[12]. Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. Metab Clin Exp 1981;30:936–40. [PubMed: 7022111]

[13]. Kim I-Y, Schutzler S, Schrader AM, Spencer HJ, Azhar G, Wolfe RR, et al. Protein intake distribution pattern does not affect anabolic response, lean body mass, muscle strength or function over 8 weeks in older adults: a randomizedcontrolled trial. Clin Nutr 2017;37:488–93. [PubMed: 28318687]

[14]. Biolo G, Declan Fleming RY, Wolfe RR. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. J Clin Invest 1995;95:811–9. 10.1172/JCI117731. [PubMed: 7860765]

[15]. Volpi E, Mittendorfer B, Wolf SE, Wolfe RR. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction 1999;277:E513–20.

[16]. Evenepoel P, Geypens B, Luypaerts A, Hiele M, Ghoos Y, Rutgeerts P. Digestibility of cooked and raw egg protein in humans as assessed by stable isotope techniques. J Nutr 1998;128:1716–22. [PubMed: 9772141]

[17]. Rutherfurd SM, Fanning AC, Miller BJ, Moughan PJ. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. J Nutr 2015;145: 372–9. 10.3945/jn.114.195438. [PubMed: 25644361]

[18]. Silvester KR, Cummings JH. Does digestibility of meat protein help explain large bowel cancer risk? Nutr Cancer 1995;24:279–88. 10.1080/01635589509514417. [PubMed: 8610047]

[19]. Baumann PQ, Stirewalt WS, O'Rourke BD, Howard D, Nair KS. Precursor pools of protein synthesis a stable isotope study in a swine model 1994;267: E203–9.

[20]. Pennings B, Groen BBL, van Dijk J-W, de Lange A, Kiskini A, Kuklinski M, et al. Minced beef is more rapidly digested and absorbed than beef steak, resulting in greater postprandial protein retention in older men. Am J Clin Nutr 2013;98:121–8. 10.3945/ajcn.112.051201. [PubMed: 23636241]

[21]. Atherton PJ, Smith K. Muscle protein synthesis in response to nutrition and exercise. J Physiol (Lond) 2012;590:1049–57. 10.1113/jphysiol.2011.225003. [PubMed: 22289911]

[22]. Greenhaff PL, Karagounis LG, Peirce N, Simpson EJ, Hazell M, Layfield R, et al. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. Am J Physiol Endocrinol Metab 2008;295:E595–604. 10.1152/ajpendo.90411.2008. [PubMed: 18577697]

[23]. Biolo G, Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein 1997;273:E122–9.

[24]. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. Am J Clin Nutr 2005;82: 1065–73. [PubMed: 16280440]

[25]. Tipton KD, Borsheim E, Wolf SE, Sanford AP, Wolfe RR. Acute response of net muscle protein balance reflects 24-h balance after exercise and amino acid ingestion. Am J Physiol Endocrinol Metab 2003;284:E76–89. 10.1152/ajpendo.00234.2002. [PubMed: 12388164]

[26]. Volpi E, Ferrando AA, Yeckel CW, Tipton KD, Wolfe RR. Exogenous amino acids stimulate net muscle protein synthesis in the elderly. J Clin Invest 1998;101: 2000–7. 10.1172/JCI939. [PubMed: 9576765]

[27]. Pennings B, Groen B, de Lange A, Gijsen AP, Zorenc AH, Senden JMG, et al. Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. Am J Physiol Endocrinol Metab 2012;302:E992–9. 10.1152/ajpendo.00517.2011. [PubMed: 22338070]

[28]. Kimball SR, Shantz LM, Horetsky RL, Jefferson LS. Leucine regulates translation of specific mRNAs in L6 myoblasts through mTOR-mediated changes in availability of eIF4E and phosphorylation of ribosomal protein S6. J Biol Chem 1999;274:11647–52. [PubMed: 10206976]

[29]. Wolfe RR, Chinkes DL. Isotope tracers in metabolic research. 2nd ed. Hoboken, New Jersey: John Wiley & Sons, Inc; 2005.

[30]. Fitts RH, Romatowski JG, Peters JR, Paddon-Jones D, Wolfe RR, Ferrando AA. The deleterious effects of bed rest on human skeletal muscle fibers are exacerbated by hypercortisolemia and

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ameliorated by dietary supplementation. Am J Physiol Cell Physiol 2007;293:C313–20. 10.1152/ajpcell.00573.2006. [PubMed: 17409123]

[31]. Kim I-Y, Deutz NEP, Wolfe RR. Update on maximal anabolic response to dietary protein. Clin Nutr 2017;37:411–8. [PubMed: 28807333]

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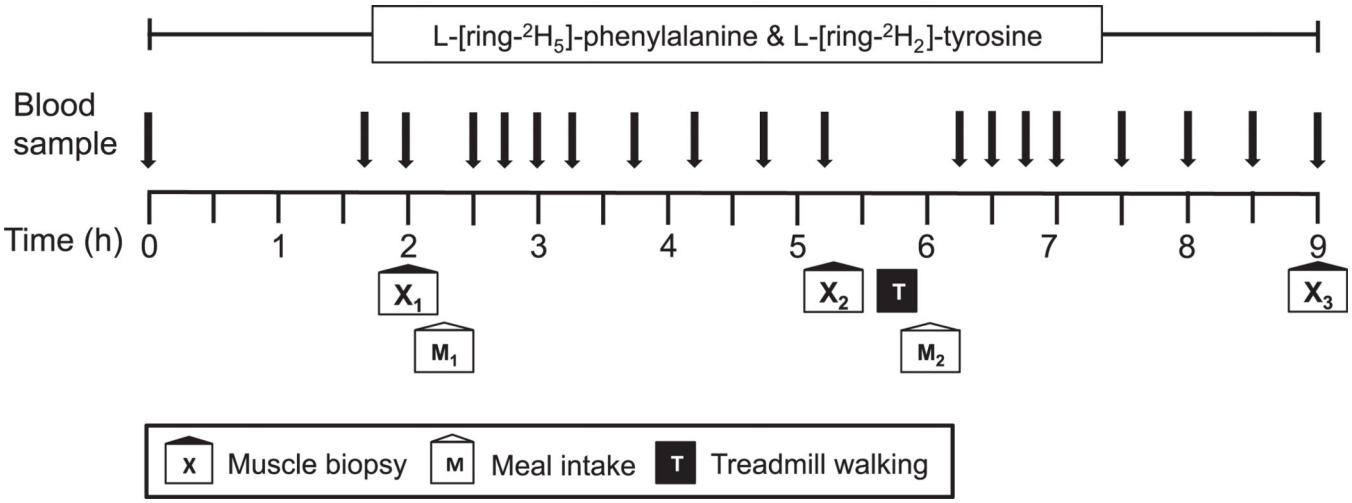

Fig. 1.
Experimental protocol.

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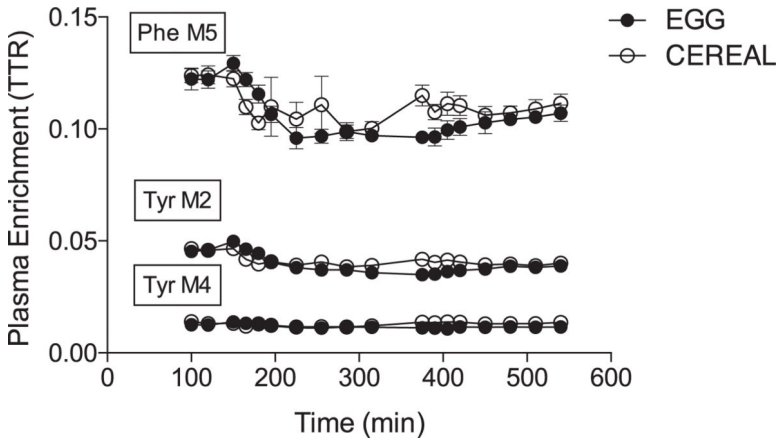

Fig. 2.

Plasma enrichments of infused tracers (Phe M5 and Tyr M2) and one derived from Phe M5 through hydroxylation (Tyr M4) before and following two successive meal intake: egg-based (EGG) or cereal-based (CEREAL) breakfast and the same standard meal. Values are expressed as mean ± SEM. Phe M5, d_5-phenylalanine; Tyr M2, d_2-tyrosine; Tyr M4, d_4-tyrosine.

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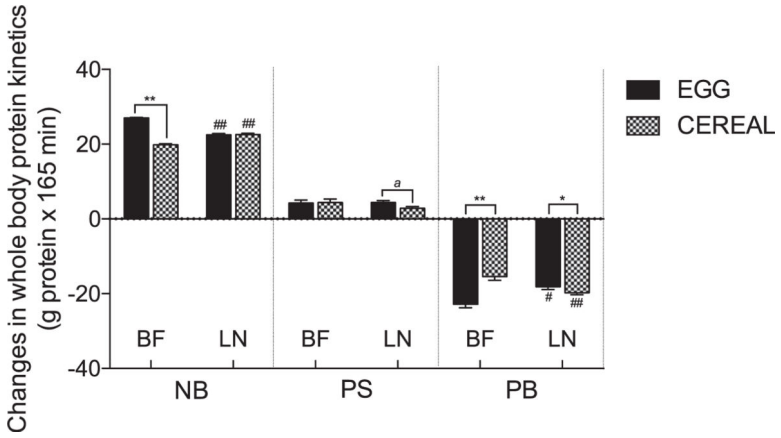

Fig. 3.

Changes in whole body protein kinetics [protein synthesis (PS), protein breakdown (PB), and net protein balance (NB)] following either EGG or CEREAL breakfast meals and then standard lunch meals. BF, breakfast; LN, lunch. Significantly different between meal types, *$p < 0.05$; **$p < 0.001$. Significantly different between breakfast and lunch responses, $^{\#}p < 0.005$; $^{\#\#}p < 0.001$. [a]Paired t-test indicates a significant difference between meals, $p = 0.005$.

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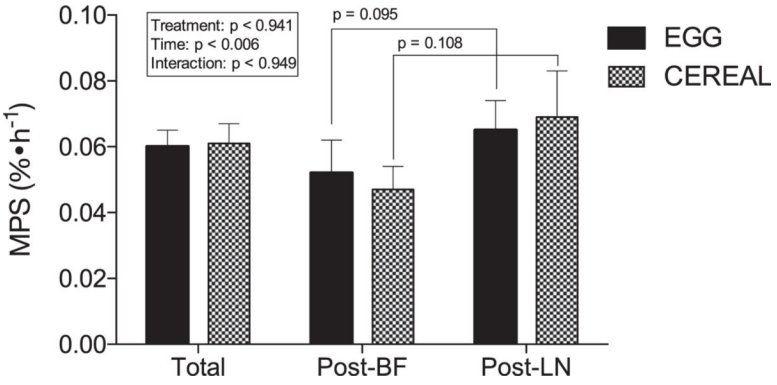

Fig. 4.

Muscle protein fractional synthetic responses (MPS) following breakfast and lunch and the integrated total FSR response: EGG or CEREAL breakfast meals and standard lunch meals. Post-BF, post-breakfast; Post-LN, post-lunch; Total, entire postmeal period.

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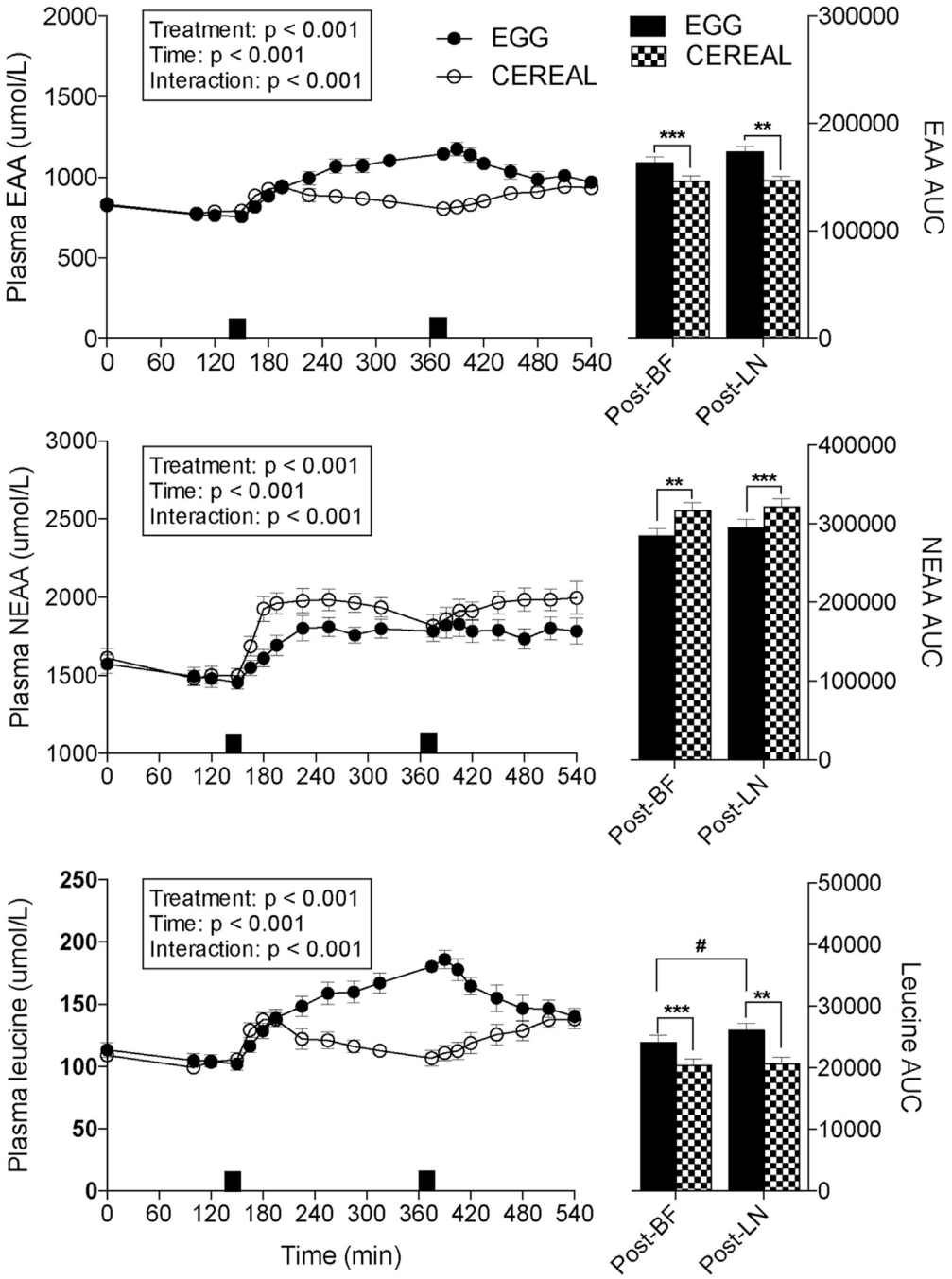

Fig. 5.

Time course responses and area under the curves of plasma essential amino acid (EAA), nonessential amino acids (NEAA), leucine after two successive meals (breakfast and lunch): EGG or CEREAL breakfast meals and standard lunch meals. Rectangular bars indicate the meal intake at 130 min (for breakfast) and at 360 min (for lunch). Post-BF, post-breakfast; Post-LN, post-lunch. Significantly different between meals, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Significantly different between breakfast and lunch responses, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$.

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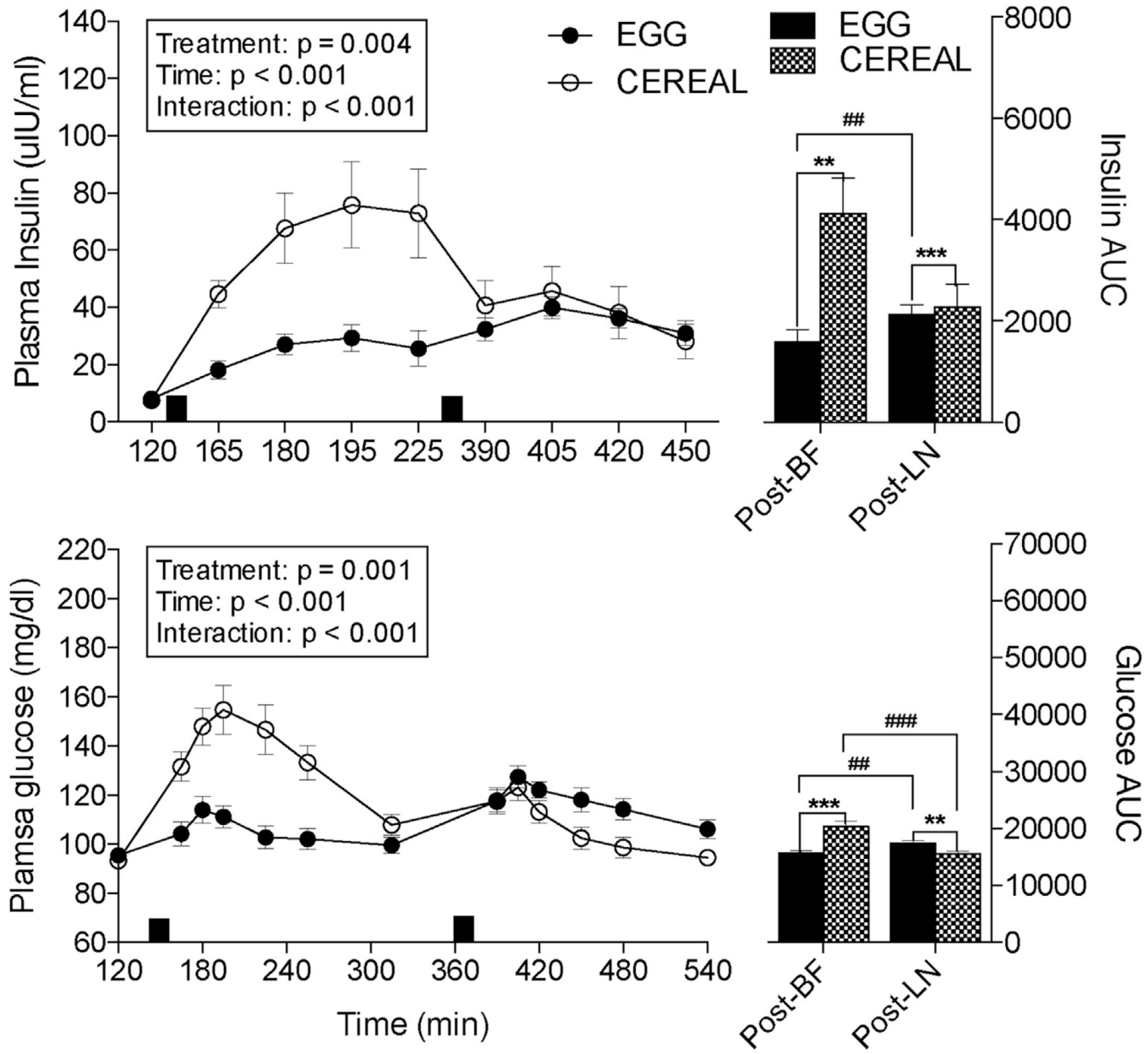

Fig. 6.

Time course responses and area under the curves of plasma insulin and glucose after two successive meals (breakfast and lunch): EGG or CEREAL breakfast and standard lunch meals. Rectangular bars indicate the meal intake at 130 min (for breakfast) and at 360 min (for lunch). Significantly different between meals, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Significantly different between breakfast and lunch responses, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$.

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Table 1

Subject characteristics. Values are mean ± SEM; M, no. of male; F, no of female; LBM, lean body mass; BMI, body mass index.

Subject (M/F)	**12 (6/6)**
Age, yrs	65 ± 1.7
Weight, kg	78.8 ± 3.0
LBM, kg	49 ± 2.9
BMI, kg/m^2	26.4 ± 0.6
Body fat, %	32.6 ± 2.3

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Table 2

Macronutrients of 2-day run-in on day 1-2 and metabolic infusion study on day 3. Values are expressed as means ± SD.

Treatment	Energy	Protein		EAA		Fat		Omega fatty acids (g/meal)		Carbohydrate	
	Kcal	g	%	g	% of protein	g	%	Omega 3	Omega 6	g	%
Macronutrients of run-in on day 1–2											
EGG	2242 ± 300	85.4 ± 3.0	15.1 ± 1.11	28.4 ± 3.9	33.3 ± 0.72	86.5 ± 12.1	34.2 ± 0.56	1.96 ± 0.11	16.87 ± 0.91	288.1 ± 40.2	50.7 ± 0.89
CEREAL	2247 ± 295	85.6 ± 3.0	15.1 ± 1.15	28.5 ± 3.9	33.3 ± 0.76	86.7 ± 12.7	34.2 ± 1.07	2.02 ± 0.11	17.50 ± 0.97	288.9 ± 38.3	50.7 ± 0.99
Macronutrients during the metabolic in infusion study on day 3											
Interventional breakfast meals											
EGG	489 ± 0.0 *	26.0 ± 0.0 *	21.3 ± 0.00 *	10.1 ± 0.0 *	38.8 ± 0.00 *	27.9 ± 0.0 *	51.3 ± 0.00 *	0.31 ± 0.00 *	4.55 ± 0.00 *	31.9 ± 0.0 *	26.1 ± 0.00 *
CEREAL	501 ± 13	25.5 ± 0.0	20.4 ± 0.53	7.1 ± 0.0	27.7 ± 0.00	9.8 ± 1.4	17.6 ± 2.26	0.16 ± 0.00	1.27 ± 0.00	80.9 ± 0.0	64.7 ± 1.73
Standardized lunch meals											
EGG	465 ± 22	25.5 ± 0.7	21.9 ± 0.45	9.3 ± 3.9	36.5 ± 3.89	14.8 ± 0.2	28.6 ± 1.06	0.17 ± 0.00	1.35 ± 0.00	57.6 ± 5.1	49.5 ± 2.28
CEREAL	465 ± 22	25.5 ± 0.7	21.9 ± 0.45	9.3 ± 3.9	36.5 ± 3.89	14.8 ± 0.2	28.6 ± 1.06	0.17 ± 0.00	1.35 ± 0.00	57.6 ± 5.1	49.5 ± 2.28

* Significantly different from CEREAL, $p < 0.01$.