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OLDER ADULTS HAVE DELAYED AMINO ACID ABSORPTION AFTER A HIGH PROTEIN MIXED BREAKFAST MEAL

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Abstract: *Objectives:* To measure the postprandial plasma amino acid appearance in younger and older adults following a high protein mixed meal. *Design:* Cross-sectional study. *Setting:* Clinical research setting. *Participants:* Healthy men and women aged 60-75 (n=15) years, and young controls aged 20-25 years (n=15) matched for body mass index and insulin sensitivity based on the homeostatic model assessment of insulin resistance. *Intervention:* High protein mixed meal of complete food products. *Measurements:* Circulating amino acid concentrations were determined hourly before and for 5 hours after meal ingestion. *Results:* There was no difference between cohorts in postprandial appearance of non-essential amino acids, or area under the curve of any individual amino acid or amino acid class. However, older adults had higher baseline concentrations of aspartic acid, glutamic acid, glycine, ornithine, threonine and tyrosine and lower baseline concentrations of hydroxyproline, isoleucine, leucine, methionine and valine compared to younger adults. Younger adults showed peak essential (EAA) and branched-chain amino acid (BCAA) concentrations at 1 hour post meal while older adults' peak EAA and BCAA concentration was at 3 hours. Similarly, peak total amino acid concentrations were at 3 hours in older adults. *Conclusion:* Older adults digested and absorbed the protein within a mixed meal more slowly than younger adults. Delayed absorption of AA following a mixed meal of complete food products may suppress or delay protein synthesis in senescent muscle.

Key words: Ageing, sarcopenia, protein digestion, mixed meal.

Introduction

The ageing of the world's population, most notably within developed economies, presents many health challenges. Ageing affects body composition, with a characteristic loss of lean muscle mass, ultimately resulting in impaired skeletal muscle function and the onset of sarcopenia (1). The aetiology of sarcopenia is complex; however, lifestyle factors including the habitual diet are a significant determinant (2). Dietary protein supports the maintenance of muscle mass (3), and daily intake may be insufficient in older adults (4). Beyond the changes in habitual diet, the digestive responses and metabolic fate of ingested protein differ with advancing age. It has been shown that protein digestion rate (5) and circulating amino acid (AA) availability (6) contribute to the muscle protein synthetic response to feeding, a response known to be impaired with ageing (7). Older adults may have altered protein digestion which could contribute to changes in postprandial amino acid availability.

Age-related differences in digestive factors have been previously reported. Older adults may have decreased chewing capacity (8), gastric acid secretion (9), reduced gastric peristalsis (10) or delayed gastric emptying (11), all of which could impair older adults' ability to digest and absorb protein (12). Older adults have lower efficiency of protein utilisation (13) and show reduced protein synthesis in the fed state, possibly due to differences in protein absorption and splanchnic use (14). Ageing is associated with higher rates of splanchnic uptake and use of AAs such as leucine (15, 16), phenylalanine (17) and glutamate (18). Increased intestinal demands for AAs (19) likely result in reduced AA appearance (15) and availability of AA for protein synthesis in ageing (14).

Older adults also have different metabolic responses to protein ingestion compared to younger adults. While in young adults, slowly digested proteins, such as casein (5), and 'spread' feeding patterns (protein fed throughout the day) enhance whole body protein balance and utilisation, the opposite is seen in elderly subjects. Fast proteins, i.e. whey, induce better postprandial whole body leucine balance in older subjects (20, 21) and elderly women experience better protein retention after pulse feeding (22). This shows that the kinetics of AA availability differs between young and old subjects and impacts postprandial muscle protein synthesis. However, at the level of the muscle, fast protein and bolus feeding patterns result in greater muscle protein synthesis in both young and older adults (23-26).

Protein digestion rates influence postprandial AA availability and may differ with meal composition; intact proteins such as those found in milk (casein and whey) (27) or steak (28) are more slowly digested when in these food matrices than whey alone or minced beef respectively. Additionally, carbohydrate ingested with protein delays postprandial AA appearance (29). These studies suggest that just as meal composition and food physical structure affect digestion, absorption, and subsequent appearance of carbohydrates (30) and fats (31), protein digestion is equally

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dependent on meal structure. As such, meal structure and composition may be important considerations for assessing amino acid availability and ultimately muscle protein synthesis in response to protein ingestion. Surprisingly, no studies have yet reported age-related differences in amino acid appearance in the context of a whole food mixed meal.

Therefore, we aimed to demonstrate the effects of ageing on protein digestion in the context of a whole food mixed meal; we examined the postprandial AA appearance in healthy older and younger adults after ingestion of a high protein mixed meal consisting of whole food products. We hypothesised that older adults would have impaired digestion of dietary protein in a mixed meal.

Methods

Subjects

Thirty healthy, community-dwelling subjects (n=7 young women, n=8 young men, n=9 older women, n=6 older men) from the Auckland region were recruited through newspaper advertisements and from the university community to participate in the study. Eligible subjects were required to have a body mass index (BMI) between 18 and 30 kg/m^2 and be between the ages of 20-25 years or 60-75 years. Individuals with a history of cardiovascular or metabolic disease/ conditions, or taking medications that may interfere with study endpoints (i.e. anti-inflammatory drugs, statin drugs) were not eligible for participation. All subjects gave written informed consent and the study was approved by the University of Auckland Human Participants and Ethics Committee (Ref # 8026). This study was registered prospectively at Australian New Zealand Clinical Trials Registry at anzctr.org.au (ID: ACTRN12612000515897).

Study Procedures

This cross-sectional trial was conducted at the Maurice and Agnes Paykel Clinical Research Unit (MAPCRU) at the Liggins Institute, University of Auckland, Auckland, New Zealand. The mixed breakfast meal (Table 1) was formulated to follow the Australian Guide to Healthy Eating, while maintaining a low fat load (16.6 g), and high protein (49.8 g) and carbohydrate (77.4 g) loads with a total energy content of 2790 kJ. The meal was prepared onsite at the MAPCRU.

Subjects were required to abstain from vigorous physical activity, high fat foods, anti-inflammatory medications, and dietary supplements the day prior to their visit. Subjects arrived fasted at the MAPCRU; anthropometric data were collected before a catheter was inserted into an antecubital vein and a baseline sample (time 0) was taken followed by consumption of the breakfast. Blood samples were collected hourly up to 5 hours post meal from resting subjects into blood collection tubes (BD, Mt Wellington, New Zealand) for serum and EDTA plasma. Serum tubes were allowed to clot for 15 minutes at room temperature before serum and plasma tubes were centrifuged at 1500 × g for 15 minutes at 4°C and the supernatants collected in pyrogen-free microtubes and stored at -20°C until analysis.

Serum free amino acid concentrations and plasma measures

Free amino acids were assayed from 20μl of serum with 15μM L-Nor-Valine as internal standard extracted with 20μl 10% sodium tungstate and 160μl of 0.04M sulphuric acid. The mixture was incubated on ice for 3 minutes then centrifuged at 12000 × g for 10 minutes at 4°C. 70μl of 0.2M borate buffer (pH 8.8) was added to the supernatant before adding 10μl of AccQ-tag reagent (2.8mg/ml in acetonitrile). In a sealed vial, the mixture was heated at 55°C for 10 minutes before being subjected to ultra performance liquid chromatography (UPLC). The UPLC system used a Thermo Scientific Dionex Ultimate 3000 pump, autosampler (maintained at 10ºC), column oven and fluorescence detector (set at Ex 250 nm, Em 395 nm) (Thermo Scientific, Dornierstrasse, Germany), and a Kinetex 1.7μm C18 100A 100 x 2.1 mm column, preceded by a Krudkatcher inline filter (Phenomenex, Auckland, New Zealand) at 45ºC. Mobile phase buffer, (80 mM sodium acetate, 3 mM triethylamine, 2.67μM disodium calcium ethylenediaminetetraacetic acid) at pH 6.43 (obtained by addition of orthophosphoric acid), run with a complex gradient of acetonitrile from 2 to 17% (balance, water) over 24 minutes. Data was directly captured by computer with Chromeleon 7.1 software (Thermo Scientific). AA concentrations were calculated from standard curves generated for each AA from the standard injections. The internal standard (L-Nor-Valine) signal in each chromatogram was used for data normalisation for analyte recovery and quantification.

Plasma glucose was measured using a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) by enzymatic colorimetric assay (Roche, Mannheim, Germany). Plasma insulin was measured using an Abbott AxSYM system (Abbott Laboratories, Abbott Park, USA) by microparticle enzyme immunoassay.

Calculations

Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin concentrations using the equation from Matthews, et al. (1985, 32). Serum free amino acids were pooled for mathematical analysis into total amino acids (all proteogenic amino acids), essential amino acid (EAA), branched-chain amino acids (BCAA) and non-essential amino acids (NEAA). Although arginine, proline, glutamine, glycine, taurine and tyrosine are considered conditionally essential, these were considered nonessential as the study criteria excluded persons with critical illness or malnutrition.

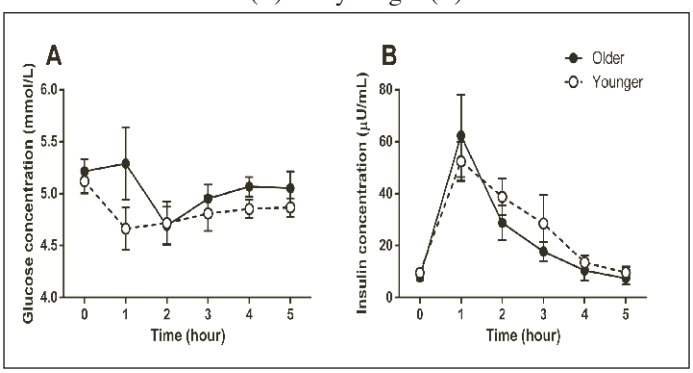
Table 1
Macronutrient composition of breakfast meal. Values presented are based on available nutrient panel data for individual products

Item name	Weight (g)	Carbohydrates (g)	Fat (g)	Protein (g)	Energy (kJ)
Rolled oats	37.0	20.8	1.9	5.0	495
1% cottage cheese	167.0	4.5	1.0	19.7	450
Mixed grain bread	41.5	11.2	2.2	5.1	390
Reduced fat peanut butter, smooth	25.0	8.4	9.4	4.4	575
Fresh peach	154.0	14.6	0.3	1.4	250
Trim milk	365.0	17.9	1.8	14.2	630
Total		77.4	16.6	49.8	2790

Statistical analyses

Statistical analyses were conducted with SPSS. Data are represented as means ± SEMs. Incremental area under the curve (AUC) was calculated after subtracting fasting values. Baseline subject characteristics, amino acids, AUC, and maximum peak concentrations were compared using Student's t test. Two-factor (age and time) repeated-measure ANOVA followed by Sidak post hoc test was used for all multiple comparisons between different groups. Alpha was set at $P<0.05$. The heatmap representation of postprandial amino acids concentration as a percent change relative to younger fasting values was created with R software.

Figure 1
Postprandial plasma glucose (A) and insulin (B) concentrations in older (●) and younger (○) adults



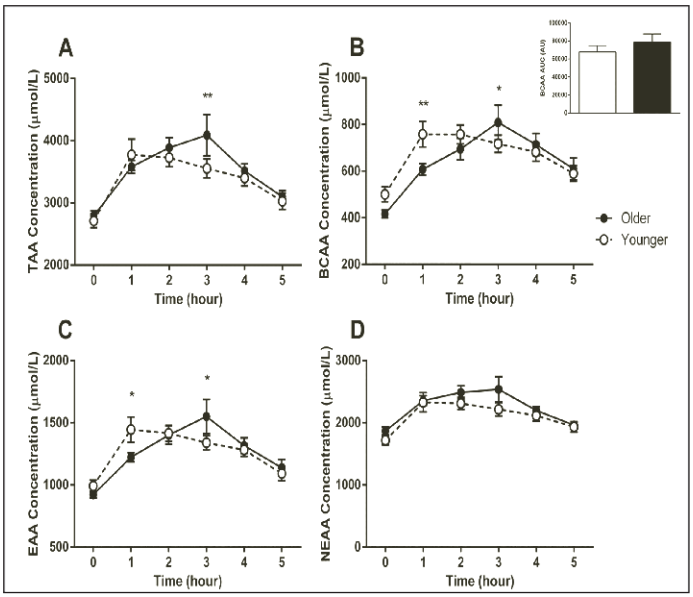
Values represent means ± SEM in mmol/L and μU/mL respectively. No main effects of age or time were identified by two-factor repeated-measures ANOVA

Results

Subject characteristics

A total of 30 subjects completed the study. Subject characteristics are shown in Table 2. There were no age differences in BMI, fasting measurements of glucose, or HOMA-IR. Glucose and insulin response to the meal was not different between age groups (Figure 1).

Figure 2
Postprandial plasma concentrations of total amino acids (TAA) (A), BCAA (B), BCAA AUC (B inset), EAA (C), and NEAA (D)



Values represent means ± SEM in μmol/L. AUC presented as arbitrary units. There were significant differences over time in the TAA, BCAA and EAA responses between older (●) and younger (○) adults (age × time interactions of $P<0.05$, $P<0.001$, and $P<0.01$ respectively, two-factor repeated-measures ANOVA). There were no differences in NEAA response or BCAA AUC between older and younger adults. *$P<0.05$, **$P<0.01$ older vs. younger at a given time-point (Sidak)

Older adults have altered basal amino acid profiles

Baseline glutamine concentrations were 15% lower in older adults compared to younger adults ($P=0.042$) while glutamic acid was 193% greater ($P<0.001$, Table 2). Older adults also had higher baseline concentrations of aspartic acid, glycine, ornithine, threonine, and tyrosine than younger adults, and lower concentrations of hydroxyproline, isoleucine, leucine, methionine, and valine (Table 2).

Table 2
Baseline subject characteristics and serum amino acid profile of older and younger adults

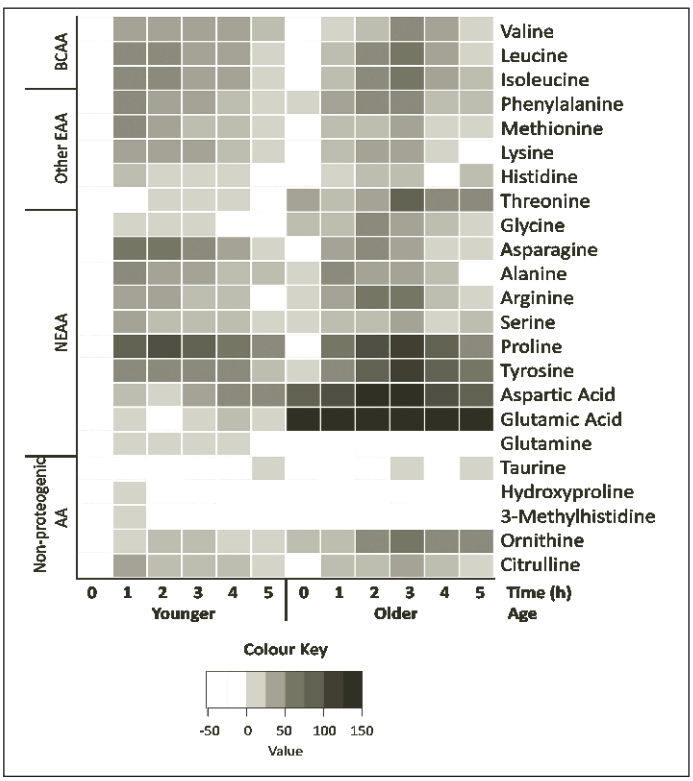
	Younger adults (n=15; 8 men, 7 women)	Older adults (n=15; 6 men, 9 women)
Subject characteristics:		
Age (years)	22.7 ± 0.4	67.3 ± 1.5***
BMI (kg/m3)	23.8 ± 0.8	24.4 ± 1.0
Fasting Plasma Glucose (mmol/L)	5.1 ± 0.1	5.2 ± 0.1
HOMA-IR	2.2 ± 0.3	2.1 ± 0.3
Branched-Chain Amino Acids:		
Isoleucine	84.7 ± 6.5	65.3 ± 3**
Leucine	146.5 ± 8.5	123.8 ± 5*
Valine	269.2 ± 17.8	226.9 ± 9.7*
All Other Essential Amino Acids:		
Histidine	78.8 ± 5.2	74.4 ± 3.7
Lysine	158.5 ± 12	150.6 ± 8
Methionine	46.9 ± 3.1	38.2 ± 1.9*
Phenylalanine	72.7 ± 4.5	73.6 ± 3.5
Threonine	58.6 ± 2.5	84.9 ± 5.4***
Non-Essential Amino Acids:		
Alanine	401.0 ± 24.3	411.8 ± 21.3
Arginine	76.2 ± 6	84 ± 4.9
Asparagine	52 ± 4.7	44.2 ± 2.1
Aspartic Acid	6.7 ± 1.2	12.4 ± 1.3**
Glutamic Acid	71.8 ± 16.6	210.4 ± 17***
Glutamine	556.7 ± 49.2	475.2 ± 30.2*
Glycine	236.6 ± 15.2	311.6 ± 26.4*
Proline	192.6 ± 11.9	189.4 ± 11.5
Serine	120.6 ± 8.3	125.3 ± 21.6
Tyrosine	80.9 ± 7.5	88.4 ± 20.5*
Non-proteogenic Amino Acids:		
3-Methylhistidine	11.2 ± 2	7.1 ± 1.2
Citrulline	66.3 ± 4.4	64.6 ± 1.9
Hydroxyproline	15.6 ± 1.9	10.2 ± 0.8*
Ornithine	52.6 ± 3.4	65.8 ± 5.7*
Taurine	109.7 ± 10.8	107.3 ± 8.3

Values represent means ± SEM. Amino acid values measured in μmol/L. HOMA-IR: Homeostatic model assessment of insulin resistance. Significance was determined by Student's t-test. *P<0.05, **P<0.01, ***P<0.001 compared with younger adults

Older adults have delayed postprandial appearance of amino acids

Older adults showed a delayed increase in serum postprandial concentrations of TAAs, BCAAs, and EAAs (Figure 2). No such differences were apparent in NEAA appearance (Figure 2). Younger adults had higher concentrations of BCAA (Figure 2 B) and EAA (Figure 2 C) at 1 hour after the meal when compared to older adults. In older adults, serum concentrations of TAAs, BCAAs, and EAAs peaked at 3 hours post meal, at which time circulating concentrations were significantly higher compared to younger adults. The AUC for isoleucine tended to be greater in older adults compared to younger adults (P=0.067). AUC did not differ between any other individual AA or class of AA. Maximal AA concentration did not differ between younger and older adults for AAs that did not show previous baseline differences, maintaining that total postprandial plasma availability was not different between age groups. A heatmap displaying mean percentage change in all detected individual serum amino acids from fasting serum concentrations in younger adult is presented (Figure 3).

Figure 3
Heatmap of postprandial changes in amino acid concentrations of older and younger adults



Values are presented as mean percent changes relative to younger adult time 0 concentration for each amino acid (i.e. the lightest grey represents a 0% change from younger baseline). White represents negative percentage changes; darker hues represent positive percentage changes up to 150%; BCAA: branched-chain amino acids; EAA: essential amino acids; NEAA: non-essential amino acids

Discussion

For the first time we have shown that older adults digest and absorb amino acids more slowly total than younger adults after a mixed meal high in protein. Older adults had similar overall

protein appearance by AUC after a mixed meal, but delayed appearance of EAA and BCAA.

Previous postprandial protein kinetic research in older adults has generally investigated the response to a variety of independent macronutrient components such as individual amino acid supplements (17, 33-35), protein fractions (hydrolysed (36, 37) or intact (14, 20, 21)), and protein (28, 38) or carbohydrate (39) independently. A few studies have tried to emulate the macronutrient composition of whole foods through formulations with protein isolates, carbohydrate in the form of dextrin, and fats such as vegetable oils (15, 27, 29, 40-43). Our study is the first to look at the differential aminoacidaemic response in older adults to protein ingestion in the physiological context of feeding a real food mixed meal.

Our study population was well matched for BMI, insulin sensitivity, and fasting plasma glucose. Nevertheless, baseline amino acid profiles appeared to differ between older and younger adults. Older adults displayed lower fasting concentrations of serum BCAAs as has been reported in some (44, 45) but not all (46) previous studies. Furthermore, significant baseline differences in other individual plasma EAAs, NEAAs, and non-proteogenic amino acids were observed. Importantly, we found that higher baseline concentrations for some amino acids in older adults only impacted postprandial peak concentrations, not overall total serum amino acid response as determined by postprandial AUC. Nevertheless, differences in basal concentrations of certain AAs may contribute to absolute postprandial AA concentrations in older adults.

There is already clear evidence that in older adults, slower availability of amino acids after protein feeding in isolation results in poorer postprandial protein balance, in contrast with young adults. Slowly digested proteins like casein (20) or spread feeding patterns (22) result in lower postprandial leucine balance and nitrogen balance respectively in older adults. In the present study, we have shown that in older adults, ingesting protein in the form of a mixed meal results in slower amino acid appearance when compared to younger adults. Although not measured in the current study, this would potentially result in suboptimal postprandial protein balance and an impaired muscle protein synthesis.

Meal composition and structure are known to impact digestion and absorption in older adults; minced beef enhanced protein digestion and absorption in elderly men when compared to beef steak (28). Similarly, casein in a hydrolysed state accelerated protein digestion in elderly men when compared to intact casein (36), showing that older adults have slower protein digestion and absorption with more intact ingested protein. This supports our finding that combined and intact casein and whey were more slowly digested and absorbed by older adults when studied in the context of a mixed meal. As previous studies have shown no impact of age on casein (14) or whey (37) digestion when ingested independently, our data highlight the importance of assessing protein metabolism in the context of intact proteins in representative whole ingested foods.

The anabolic resistance to food in older adults (47) is demonstrated through reduced muscle protein synthetic responses to EAA ingestion (34) which may reflect delayed or prolonged postprandial availability of EAAs and BCAAs as suggested by Condino, et al. (2013, 33). It is conceivable that delayed availability of EAAs in older adults may contribute to reduced postprandial muscle protein synthesis and anabolic resistance in ageing. Adequate circulating concentrations of EAAs are primarily responsible for the stimulation of muscle protein synthesis (6), particularly in healthy elderly adults (35), while NEAAs seem to be less important (48). Furthermore, the leucine trigger hypothesis suggests that a threshold concentration of leucine is required to adequately stimulate muscle protein synthesis (49), a threshold that may not necessarily be reached following delayed protein absorption. It appears that adequate EAAs must be present in circulation at the same time as the leucine peak (50), suggesting that a lag in total AA availability caused by delayed digestion and absorption in ageing could impact on muscle protein synthesis. This is supported by evidence that older adults show no difference in muscle protein synthesis rate when studied throughout the later postprandial period, which may suggest a delay in early feeding induced muscle protein synthesis activation (51), possibly attributable to delayed EAA digestion and absorption.

A possible explanation for the delayed postprandial appearance of EAAs in older adults is increased intestinal use of many EAAs. Increased splanchnic leucine extraction in elderly men has already been demonstrated (15); however, Koopman, et al. (2009, 14) showed no significant effect of age on splanchnic extraction of dietary phenylalanine. As our data do not show decreased postprandial AUC of any EAAs in older adults, splanchnic use of EAAs is not a likely explanation for delayed EAA appearance after a mixed meal. Furthermore, our data show no different postprandial changes in dietary glutamine, glutamic acid or aspartic acid, which are nearly completely used by the intestines (52), implying no difference in postprandial intestinal use of dietary amino acids between older and younger subjects.

Co-ingestion of carbohydrates with protein in elderly adults results in slower amino acid plasma appearance just as in younger adults (29), but this may in fact accelerate dietary amino acid incorporation into elderly muscle, although this effect may not be sustained in the later postprandial period (41). Previous studies in older adults have shown that ingestion of isolated EAAs results in greater postprandial aminoacidaemia (33) and that casein ingestion elicits a greater AUC appearance of BCAAs (14) compared to younger adults. Our data show no AUC differences in EAAs or BCAAs suggesting that in a mixed meal, intact protein ingested with carbohydrates may negate a greater AUC protein appearance in older adults.

Despite delayed amino acid appearance, carbohydrate co-ingestion does not impact on muscle protein synthesis

in young (39, 53) or elderly (29) adults. In young adults, the slower appearance of casein from mixed dairy ingestion resulted in more sustained systemic amino acid delivery to the skeletal muscle (27). Our data suggest that this delay in amino acid appearance after mixed dairy ingestion may be even further delayed in older adults; however, this delay may not predict an effect on skeletal muscle delivery.

Conclusions

This study shows that older adults digest and absorb proteins from a mixed meal more slowly than younger adults. Furthermore, protein ingestion in a mixed meal is a relevant concern when assessing amino acid appearance and use after a meal since these age-related delays in protein availability have not been previously reported. This slower rate of protein digestion and absorption in ageing after a mixed meal may provide some explanation for the observed decreases in postprandial muscle protein synthesis reported previously, and should be an important consideration when applying protein ingestion strategies for nutritional recommendations in older adults. As such, future investigations into dietary effects on muscle protein synthesis in ageing should explore these questions in the context of real foods, representative of a normal and realistic diet.

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Ethical Standards: The experiments in this study comply with the current laws of the country in which they were performed.

Conflict of interest: AMM, RFD, SP, CAP, MPGB, JFM, DCS and CJM have no conflicts of interest to declare.

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References

1. Koopman R, van Loon LJC. Aging, exercise, and muscle protein metabolism, 2009.
2. Paddon-Jones D, Rasmussen BB. Dietary protein recommendations and the prevention of sarcopenia. Curr Opin Clin Nutr Metab Care 2009;12(1):86-90.
3. Paddon-Jones D, Leidy H. Dietary protein and muscle in older persons. Curr Opin Clin Nutr Metab Care 2014;17(1):5-11.
4. Volpi E, Campbell WW, Dwyer JT, Johnson MA, Jensen GL, Morley JE, Wolfe RR. Is the optimal level of protein intake for older adults greater than the recommended dietary allowance? Journals of Gerontology - Series A Biological Sciences and Medical Sciences 2013;68(6):677-681.
5. Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, Ballèvre O, Beaufrère B. The digestion rate of protein is an independent regulating factor of postprandial protein retention. Am J Physiol Endocrinol Metab 2011;280(2):E340-E348.
6. Bohé J, Low A, Wolfe RR, Rennie MJ. Human Muscle Protein Synthesis is Modulated by Extracellular, Not Intramuscular Amino Acid Availability: A Dose-Response Study. J Physiol 2003;552(1):315-324.
7. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, Rennie MJ. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2005;19(3):422-424.
8. Rémond D, Machebeuf M, Yven C, Buffière C, Mioche L, Mosoni L, Mirand PP. Postprandial whole-body protein metabolism after a meat meal is influenced by chewing efficiency in elderly subjects. Am J Clin Nutr 2007;85(5):1286-1292.
9. Feldman M, Cryer B, McArthur KE, Huet BA, Lee E. Effects of aging and gastritis on gastric acid and pepsin secretion in humans: A prospective study. Gastroenterology 1996;110(4):1043-1052.
10. Shimamoto C, Hirata I, Hiraike Y, Takeuchi N, Nomura T, Katsu Ki. Evaluation of Gastric Motor Activity in the Elderly by Electrogastrography and the C-Acetate Breath Test. Gerontology 2002;48(6):381-386.
11. Brogna A, Ferrara R, Bucceri AM, Lanteri E, Catalano F. Influence of aging on gastrointestinal transit time. An ultrasonographic and radiologic study. Invest Radiol 1999;34(5):357-359.
12. Grassi M, Petraccia L, Mennuni G, Fontana M, Scarno A, Sabetta S, Fraioli A. Changes, functional disorders, and diseases in the gastrointestinal tract of elderly. Nutr Hosp 2011;26(4):659-668.
13. Fereday A, Gibson NR, Cox M, Pacy PJ, Millward DJ. Protein requirements and ageing: Metabolic demand and efficiency of utilization. Br J Nutr 1997;77(5):685-702.
14. Koopman R, Walrand S, Beelen M, Gijsen AP, Kies AK, Boirie Y, Saris WHM, Van Loon LJC. Dietary protein digestion and absorption rates and the subsequent postprandial muscle protein synthetic response do not differ between young and elderly men. J Nutr 2009;139(9):1707-1713.
15. Boirie Y, Gachon P, Beaufrère B. Splanchnic and whole-body leucine kinetics in young and elderly men. Am J Clin Nutr 1997;65(2):489-495.
16. Jourdan M, Deutz N, Cynober L, Aussel C (2011) Features, causes and consequences of splanchnic sequestration of amino acid in old rats. PLoS One 2011;6(11):e27002.
17. Volpi E, Mittendorfer B, Wolf SE, Wolfe RR. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. Am J Phisiol Endocrinol Metab 1999;277(40):E513-E520.
18. Rutten EPA, Engelen MPKJ, Castro CLN, Wouters EFM, Schols AMWJ, Deutz NEP. Decreased whole-body and splanchnic glutamate metabolism in healthy elderly men and patients with chronic obstructive pulmonary disease in the postabsorptive state and in response to feeding. J Nutr 2005;135(9):2166-2170.
19. Ten Have GA, Engelen MP, Luiking YC, Deutz NE. Absorption kinetics of amino acids, peptides, and intact proteins. Int J Sport Nutr Exerc Metab 2007;17(Suppl):S23-36.
20. Dangin M, Boirie Y, Guillet C, Beaufrere B. Influence of the protein digestion rate on protein turnover in young and elderly subjects. J Nutr 2002;132(Suppl):3228S–3233S.
21. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrère B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci U S A 1997;94(26):14930-14935.
22. Arnal M-A, Mosoni L, Boirie Y, Houlier M-L, Morin L, Verdier E, Ritz P, Antoine J-M, Prugnaud J, Beaufrère B, Mirand PP. Protein pulse feeding improves protein retention in elderly women. Am J Clin Nutr 1999;69(6):1202-1208.
23. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol 2009;107(3):987-992.
24. Pennings B, Boirie Y, Senden JM, Gijsen AP, Kuipers H, van Loon LJ. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. The American journal of clinical nutrition 2011;93(5):997-1005.
25. Areta JL, Burke LM, Ross ML, Camera DM, West DW, Broad EM, Jeacocke NA, Moore DR, Stellingwerff T, Phillips SM, Hawley JA, Coffey VG. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. The Journal of physiology 2013;591(Pt 9):2319-2331.
26. Mamerow MM, Mettler JA, English KL, Casperson SL, Arentson-Lantz E, Sheffield-Moore M, Layman DK, Paddon-Jones D. Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults. The Journal of nutrition 2014;144(6):876-880.
27. Soop M, Nehra V, Henderson GC, Boirie Y, Ford GC, Nair KS. Coingestion of whey protein and casein in a mixed meal: demonstration of a more sustained anabolic effect of casein. Am J Physiol Endocrinol Metab 2012;303(1):E152-E162.
28. Pennings B, Groen BBL, Van Dijk JW, De Lange A, Kiskini A, Kuklinski M, Senden JMG, Van Loon LJC. 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(1):121-128.
29. Gorissen SHM, Burd NA, Hamer HM, Gijsen AP, Groen BB, van Loon LJC. Carbohydrate Coingestion Delays Dietary Protein Digestion and Absorption but Does Not Modulate Postprandial Muscle Protein Accretion. J Clin Endocrinol Metab 2014;99(6):2250-2258.
30. Singh J, Dartois A, Kaur L. Starch digestibility in food matrix: a review. Trends Food Sci Technol 2010;21(4):168-180.
31. Gallier S, Singh H. The physical and chemical structure of lipids in relation to digestion and absorption. Lipid Technology 2012;24(12):271-273.
32. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis

model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-419.
33. Condino AM, Aquilani R, Pasini E, Iadarola P, Viglio S, Verri M, D'Agostino L, Boschi F. Plasma kinetic of ingested essential amino acids in healthy elderly people. Aging Clin Exp Res 2013;25(6):711-714.
34. 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(5):1065-1073.
35. 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(2):250-258.
36. Koopman R, Crombach N, Gijsen AP, Walrand S, Fauquant J, Kies AK, Lemosquet S, Saris WHM, Boirie Y, van Loon LJC. Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. Am J Clin Nutr 2009;90(1):106-115.
37. 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(5):997-1005.
38. Luiking Y, Deutz NEP, Memelink RG, Verlaan S, Wolfe RR. Postprandial muscle protein synthesis is higher after a high whey protein, leucine-enriched supplement than after a dairy-like product in healthy older people: a randomized controlled trial. Nutr J 2014;13(9):E1-14.
39. Børsheim E, Cree MG, Tipton KD, Elliott TA, Aarsland A, Wolfe RR. Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. 2004;96(674-678.
40. Dangin M, Guillet C, Garcia-Rodenas C, Gachon P, Bouteloup-Demange C, Reiffers-Magnani K, Fauquant J, Ballèvre O, Beaufrère B. The rate of protein digestion affects protein gain differently during aging in humans. J Physiol 2003;549(2):635-644.
41. Hamer HM, Wall B, Kiskini A, de Lange A, Groen BB, Bakker JA, Gijsen AP, Verdijk LB, van Loon LJ. Carbohydrate co-ingestion with protein does not further augment post-prandial muscle protein accretion in older men. Nutr Metab (Lond) 2013;10(1):15.
42. Smith GI, P A, Villareal DT, Frimel TN, Rankin D, Rennie MJ, Mittendorfer B. Differences in muscle protein synthesis and anabolic signaling in the postabsorptive state and in response to food in 65-80 year old men and women. PLoS One 2008;3(3):e1875.
43. Churchward-Venne TA, Breen L, Di Donato DM, Hector AJ, Mitchell CJ, Moore DR, Stellingwerff T, Breuille D, Offord EA, Baker SK, Phillips SM. Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial. The American journal of clinical nutrition 2014;99(2):276-286.
44. Rudman D, Mattson DE, Feller AG, Cotter R, Johnson RC. Fasting plasma amino acids in elderly men. Am J Clin Nutr 1989;49(3):559-566.
45. Polge A, Bancel E, Bellet H, Strubel D, Poirey S, Peray P, Carlet C, Magnan de Bornier B. Plasma amino acid concentrations in elderly patients with protein energy malnutrition. Age Ageing 1997;26(6):457-462.
46. Caballero B, Gleason R, Wurtman R. Plasma amino acid concentrations in healthy elderly men and women. Am J Clin Nutr 1991;53(5):1249-1252.
47. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, Rennie MJ. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB J 2004;19(3):422-424.
48. Churchward-Venne TA, Burd NA, Mitchell CJ, West DWD, Philp A, Marcotte GR, Baker SK, Baar K, Phillips SM. Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. J Physiol 2012;590(11):2751-2765.
49. Breen L, Churchward-Venne TA. Leucine: a nutrient 'trigger' for muscle anabolism, but what more? J Physiol 2012;590(9):2065-2066.
50. Magne H, Savary-Auzeloux I, Migné C, Peyron M-A, Combaret L, Rémond D, Dardevet D. Contrarily to whey and high protein diets, dietary free leucine supplementation cannot reverse the lack of recovery of muscle mass after prolonged immobilization during ageing. J Physiol 2012;590(8):2035-2049.
51. Drummond MJ, Dreyer HC, Pennings B, Fry CS, Dhanani S, Dillon EL, Sheffield-Moore M, Volpi E, Rasmussen BB. Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging. J Appl Physiol 2008;104(5):1452-1461.
52. Stoll B, Burrin DG. Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers. J Anim Sci 2006;84(E Suppl):E60-E72.
53. Staples AW, Burd N, West DWD, Currie KD, Atherton PJ, Moore DR, Rennie MJ, Macdonald MJ, Baker SK, Phillips SM. Carbohydrate does not augment exercise-induced protein accretion versus protein alone. Sci Sports Exerc 2011;43(7):1154-1161.