Consumption of High-Leucine-Containing Protein Bar Following Breakfast Impacts Aminoacidemia and Subjective Appetite in Older Persons

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ABSTRACT

Background: Limited data are available examining dietary interventions for optimizing protein and leucine intake to stimulate muscle protein synthesis (MPS) in older humans.

Objectives: We aimed to investigate the aminoacidemia and appetite responses of older adults after consuming breakfast, a meal frequently consumed with high-carbohydrate and below-par amounts of protein and leucine for stimulating MPS.

Methods: Five men and 3 women (means ± SD; age: 74 ± 7 y, BMI: 25.7 ± 4.9 kg/m^2, fat- and bone-free mass: 63 ± 7 kg) took part in this experiment in which they consumed breakfasts with low-protein (LP = 13 ± 2 g), high-protein (HP = 32 ± 5 g), and LP followed by a protein- and leucine-enriched bar formulation 2 h later (LP + Bar = 29 ± 2 g). The LP, HP, and LP + Bar breakfast conditions contained 519 ± 86 kcal, 535 ± 83 kcal, and 739 ± 86 kcal, respectively. Blood samples were drawn for 6 h and analyzed for amino acid, insulin, and glucose concentrations. Visual analog scales were assessed for hunger, fullness, and desire to eat.

Results: The net AUC for essential amino acid (EAA) exposure was similar between the LP + Bar and HP conditions but greater in the HP condition compared with the LP condition. Peak leucinemia was higher in the LP + Bar condition compared with the HP, and both were greater than the LP condition. Net leucine exposure was similar between HP and LP + Bar, and both were greater than LP. Hunger was similarly reduced in LP + Bar and HP, and LP + Bar resulted in a greater hunger reduction than LP. Both LP + Bar and HP resulted in greater net fullness scores than LP.

Conclusions: Consuming our bar formulation increased blood leucine availability and net exposure to EAAs to a similar degree as consuming a high-protein meal. High-protein at breakfast results in a greater net exposure to EAAs and leucine, which could support MPS in older persons. This study was registered at clinicaltrials.gov as NCT03712761.   *Curr Dev Nutr* 2021;5:nzab080.

Introduction

Aging is accompanied by a decline in skeletal muscle mass, increasing the risk of physical disability (1, 2). A contributing factor to the loss of muscle mass with aging is a diminished muscle anabolic response to a given dose of protein, a phenomenon that has been termed anabolic resistance (3). Anabolic resistance is characterized by a rightward shift in the protein intake compared with muscle protein synthesis (MPS) dose–response curve (4), such that higher protein intakes of 1.2–1.6 g protein/kg bodyweight/d are needed to maximally stimulate MPS (5). An

optimal per-meal protein dose has been suggested to be at least ∼0.4–0.6 g/kg/meal (4).

Previous reports indicate that older adults often consume lower quantities of protein at breakfast (6–10), which can result in a suboptimal quantity of protein to stimulate MPS. Practical limitations to increase protein intake in older individuals at breakfast include age-related anorexia, poor dentition, and the greater energy content of foods to achieve such intakes (6, 7, 10, 11). An alternative strategy, rather than recommending consuming more protein to stimulate MPS, would be to consume a lower-protein-containing but leucine-enriched meal to

A

B

FIGURE 1 Breakfast meals. (A) Lower-protein-containing breakfast and protein- and leucine-enriched bar (red circle). (B) Higher-protein-containing breakfast without the bar.

stimulate MPS (12–15). Such a recommendation is concordant with an increased leucine requirement in older persons (16).

Ingestion of protein and the subsequent hyperaminoacidemia is required to stimulate MPS in humans (17). Of all amino acids, leucine is paramount for activating MPS (12, 13). Milk proteins have a high leucine content (18), and coingestion of milk proteins with additional free leucine would result in a rapid and sustained rise in circulating essential amino acids (EAAs), particularly leucine. Hudson et al. (19) reported that consuming 20 g whey protein 2 h after a 10-g low-protein breakfast resulted in peak and composite postprandial aminoacidemia comparable to consuming a single 30-g higher-protein breakfast.

Dietary protein has an enhanced acute postprandial effect on satiety and fullness compared with carbohydrate and fat (20). Few studies have

assessed the effects of oral leucine supplementation on satiety and fullness in humans (21, 22). The present study aimed to examine the effects of ingesting a high-leucine-containing bar containing a micellar casein, whey protein, and leucine blend on postprandial aminoacidemia and subjective hunger, fullness, and desire to eat in older men and women. We hypothesized that ingesting free leucine and a smaller amount of protein (~16 g) 2 h after a low-protein breakfast would result in postprandial plasma aminoacidemia over a 6-h postprandial period comparable to aminoacidemia observed after consuming a single higher-protein-containing breakfast. Additionally, we hypothesized that ingesting the bar would result in similar subjective ratings of hunger, fullness, and desire to eat compared with a single high-protein-containing breakfast.

Methods

Participants

Five men and 3 women (mean ± SD age: 74 ± 7 y; BMI: 25.7 ± 4.9 kg/m^2; body mass: 83 ± 17 kg; fat- and bone-free mass: 63 ± 7 kg; body fat: 21 ± 8%) provided written consent after being informed of the purpose, protocol, and risks of the study. Exclusion criteria were the regular use of analgesic or anti-inflammatory drugs, history of neuromuscular problems, musculoskeletal disease, any acute or chronic illness, tobacco use or smoking, metabolic disorders, and the use of corticosteroids. The Hamilton Integrated Research Ethics Board approved all experimental procedures (project 3390). This study was registered at clinicaltrials.gov as NCT03712761.

Experimental design

As previously described, this randomized crossover design study consisted of 3 testing days ≥1 wk apart (23). Participants undertook 3 different conditions where they ingested low-protein breakfast (LP), high-protein breakfast (HP), and low-protein breakfast followed by a protein-

TABLE 1 Nutritional profiles for each breakfast meal and bar[1]

	LP	HP	Bar
Absolute values			
Energy, kcal	519 ± 86	535 ± 83	220
Protein, g	13 ± 2	32 ± 5	16
CHO, g	87 ± 14	44 ± 10	22
Fat, g	17 ± 4	29 ± 7	11
Relative per BM, kg			
Energy, kcal	7.7 ± 0.7	8.0 ± 0.6	3.4 ± 0.4
Protein, g	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.0
CHO, g	1.3 ± 0.2	0.7 ± 0.1	0.3 ± 0.0
Fat, g	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.0
Relative per LBM, kg			
Energy, kcal	11.4 ± 1.0	11.8 ± 1.2	—
Protein, g	0.3 ± 0.0	0.7 ± 0.1	—
CHO, g	1.9 ± 0.2	1.0 ± 0.2	—
Fat, g	0.4 ± 0.1	0.7 ± 0.1	—

[1]Values are means ± SD. Participants (*n* = 8) received LP, HP, or LP followed by the bar 2 h later in randomized order. BM, body mass; CHO, carbohydrate; HP, higher-protein-containing breakfast; LBM, lean body mass; LP, lower-protein-containing breakfast.

and leucine-enriched bar supplement consumed 2 h after breakfast (LP + Bar). The evening before each visit, participants consumed a standardized dinner meal (5.5 ± 1.0 kcal/kg body mass), providing 19% of energy as fat, 24% of energy as protein, and 57% of energy as carbohydrate (Heart-to-Home Meals). On each testing day, participants reported to the laboratory after an overnight fast. A venous catheter was placed in the antecubital vein. Arterialized venous blood samples and psychometric assessments of hunger and fullness were obtained before ($t = 0$ min) and at 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 360 min after breakfast ingestion, which was consumed within 15 min.

Study meals

Each participant consumed a breakfast meal designed to provide 25% of their daily estimated energy needs to maintain energy balance, as determined by the sex-specific Harris–Benedict equation using a moderate physical activity factor. Meals were prepared the morning of the trial with HP containing a combination of egg, processed meat slices, cheese, yogurt, cookies/pastry, juice, fruit salad/fresh fruit, and LP containing a combination of white bread with butter, cookies/pastry, juice, fruit salad/fresh fruit (**Figure 1**). The total energy of the breakfast meal consumed as protein was either 10% (LP) or 25% (HP) of the total energy content of the meal. The protein- and leucine-enriched bar (total weight 57 g) contained ∼16 g of a blend of micellar casein (AMCO), whey protein (Hilmar), whey protein hydrolysate (Hilmar), 1.5 g free leucine (Ajinomoto), 22 g low-glycemic carbohydrates (Ciranda), and 11 g monounsaturated fat (Golden Barrel). The total leucine content of the bar was ∼3.0 g, which has been shown to result in a stimulation of MPS (13–15). The bars were made by Covance (Eurofins Food Integrity and Innovation), and the nutrient content of the bar was estimated using Product Solutions Research, Inc. The energy and macronutrient content of all study meals and the bar can be found in **Table 1**.

Questionnaires

Validated questionnaires to assess hunger ("How hungry do you feel?"), desire to eat ("How strong is your desire to eat?"), and fullness/satiety ("How full do you feel?") were completed throughout each of the visits (24). Questions were randomly ordered at every time point, and all responses were assessed with a visual analog scale (VAS) incorporating a 100-mm horizontal line rating. Questions were neutrally worded, such as "How full do you feel?", with anchors of "Not at all" to "Extremely."

Blood sample collection and analyses

Blood samples were collected and analyzed as previously described (23). Although it was not possible to blind the participants or investigators, all samples were analyzed and data collated in a blinded fashion until statistical analysis was complete.

Statistical analyses

For amino acid concentrations and VAS scores, a 2-factor repeated-measures ANOVA was used to identify the differences between treatments and across time, as well as any treatment-by-time interaction. Following a significant interaction, Fisher least significant difference post hoc tests were conducted to isolate the pairwise differences. To determine the differences between treatments in peak amino acid concentrations (C_{max}), time to peak concentration (T_{max}), and AUC, 1-factor ANOVAs were used, and follow-up analyses included paired t-
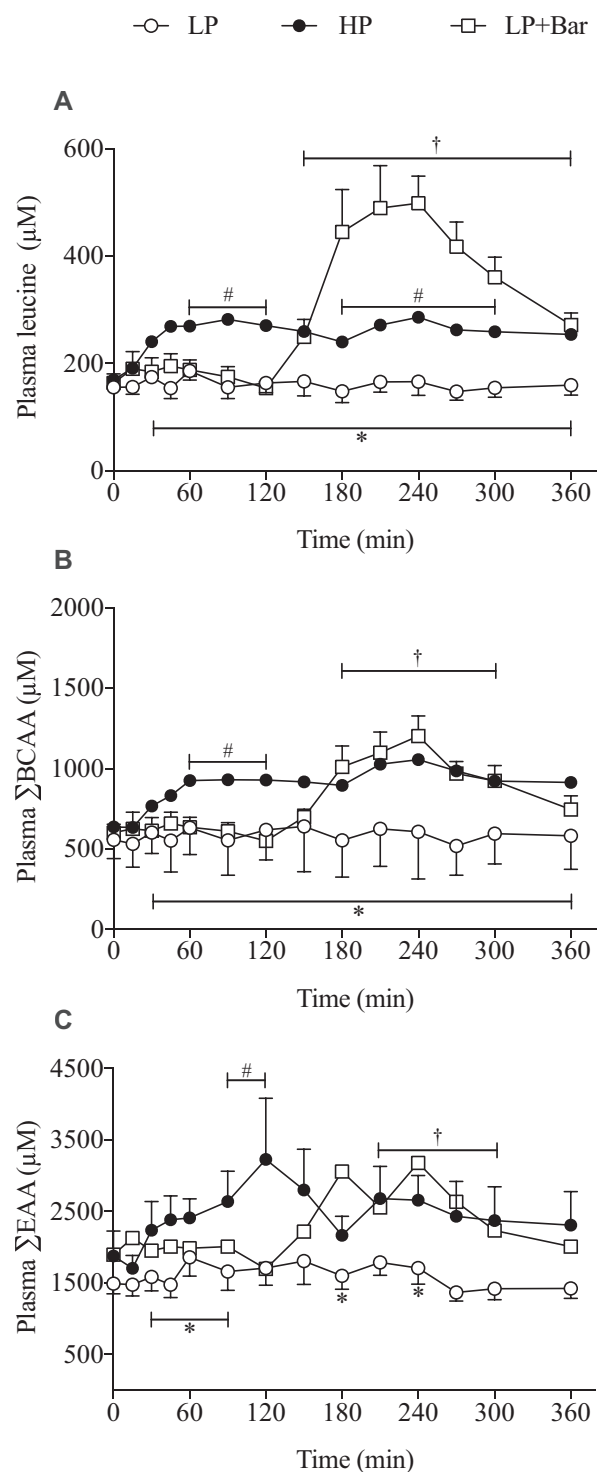
FIGURE 2 Plasma leucine (A), branched-chain amino acid (BCAA; B), and essential amino acid (EAA; C) concentrations (μM) in the fasting state and after the ingestion of a lower-protein breakfast containing 9–16 g protein (LP), higher-protein breakfast containing 23–40 g protein (HP), or a protein- and leucine-enriched bar containing ∼16 g protein ingested 2 h after consuming LP (LP + Bar) in healthy older men and women. Values are means ± SEM, $n = 8$ per treatment. *LP significantly different from HP ($P < 0.05$). †LP significantly different from LP + Bar ($P < 0.05$). #HP significantly different from LP + Bar ($P < 0.05$).

TABLE 2   Plasma amino acid, insulin, and glucose concentrations[1]

	LP	HP	LP + Bar
Leucine			
C_{max}, μM	218 ± 61[a]	336 ± 78[b]	593 ± 176[c]
T_{max}, min	116 ± 119[a]	180 ± 108[a,b]	221 ± 27[b]
AUC, μM/6 h	963 ± 280[a]	1556 ± 321[b]	1855 ± 455[b]
BCAA			
C_{max}, μM	800 ± 246[a]	1217 ± 270[b]	1305 ± 342[b]
T_{max}, min	216 ± 124	229 ± 109	229 ± 27
AUC, μM/6 h	3528 ± 1060[a]	5506 ± 1013[b]	4925 ± 947[b]
EAA			
C_{max}, μM	2176 ± 827	3684 ± 2217	3567 ± 1940
T_{max}, min	167 ± 110	148 ± 67	161 ± 94
AUC, μM/6 h	9611 ± 3065[a]	14,953 ± 6241[b]	13,854 ± 6076[a,b]
Insulin			
C_{max}, mU/L	62 ± 8[a]	50 ± 5[b]	75 ± 4[c]
T_{max}, min	75 ± 28[a]	62 ± 19[a,b]	43 ± 5[b]
AUC, mU/L/6 h	168 ± 10[a]	149 ± 5 [b]	190 ± 14[c]
Glucose			
C_{max}, mmol/L	7 ± 0	7 ± 1	7 ± 1
T_{max}, min	24 ± 8	21 ± 8	23 ± 11
AUC, mmol/L/6 h	31 ± 1	31 ± 1	31 ± 1

[1]Values are means ± SD, n = 8 per treatment. Treatments without a common letter differ, $P < 0.05$. BCAA, branched-chain amino acid; C_{max}, peak concentration; EAA, essential amino acid; HP, higher-protein-containing breakfast; LP, lower-protein-containing breakfast; LP + Bar, protein- and leucine-enriched bar ingested 2 h after consuming LP; T_{max}, time to reach peak concentration.

tests (IBM SPSS Statistics, v.25). The net AUC (subtract the area under baseline) data presented were analyzed by 1-factor ANOVA and Tukey post hoc tests for the different treatments (GraphPad Prism, Inc. Version 7.0). The significance level was set at $\alpha \leq 0.05$ for all comparisons.

Results

Plasma amino acid, insulin, and glucose concentrations
Amino acid concentrations increased after meal ingestion (**Figure 2**). Leucine, branched-chain amino acid (BCAA), and essential amino acid (EAA) concentrations increased to a greater extent after HP and LP + Bar compared with LP (time-by-treatment: $P < 0.003$; Figure 2A–C). LP + Bar resulted in greater increases in leucine, BCAA, and EAA concentrations when compared with LP.

Peak leucine concentrations were higher after LP + Bar when compared with LP and HP, and higher after HP when compared with LP ($P < 0.05$; **Table 2**). Peak BCAA concentrations were higher after LP + Bar and HP when compared with LP ($P < 0.05$). The leucine and BCAA AUCs over the entire 6-h postprandial period were higher after LP + Bar and HP compared with LP ($P < 0.05$), with no difference between HP and LP + Bar. The AUC for EAA concentrations was higher after HP when compared with LP ($P < 0.05$). For LP + Bar, the EAA AUC did not differ from both HP and LP ($P > 0.05$). Peak insulin concentrations were higher after LP + Bar when compared with LP and HP ($P < 0.05$). For HP, insulin concentrations were lower than LP ($P < 0.05$; Table 2). The AUC of insulin concentrations followed the same trend (Table 2). There were no significant differences in glucose concentrations among groups throughout the postprandial period.

Subjective ratings of desire to eat, fullness, and hunger
The perceived desire to eat and hunger transiently decreased after meal ingestion (time-by-treatment: $P < 0.041$; **Figure 3**A, B), and more rapidly returned to baseline values after LP compared with HP. After consuming the LP + Bar, desire to eat and hunger remained lowered, which is likely due to the caloric content of the bar. The AUC over the entire 6-h postprandial period for hunger was lower after LP + Bar when compared with LP ($P = 0.038$, Figure 3B, E). The AUC of perceived fullness was higher for both HP and LP + Bar when compared with LP ($P = 0.033$; Figure 3F).

Discussion

We observed that the consumption of a high-leucine-containing protein bar containing approximately half the energy of the LP meal 2 h after an LP breakfast resulted in a greater hyperaminoacidemia (particularly leucinemia) and attenuated postprandial ratings of the desire to eat and hunger, and increased ratings of fullness. In agreement with our hypothesis, EAA concentrations throughout the postprandial period and subjective appetite sensations did not differ between LP + Bar and HP, and peak leucine concentrations were highest following bar ingestion. The present study demonstrated that a protein- and leucine-enriched bar ingested 2 h after a lower-protein-containing breakfast resulted in peak leucinemia greater than after consuming a higher-protein-containing breakfast (Table 2). Our findings (peak plasma leucine ∼590 μM) are consistent with a previous study that showed peak leucine concentrations of ∼500 μM after ingesting a 20-g whey protein beverage (∼3 g leucine) (19). In comparison, a 10-g protein breakfast and 30-g protein breakfast elicited lower peak leucinemia (∼145 μM and ∼245 μM, respectively) in young males and females (19). The present study utilized a similar experimental protocol and testing procedures as Hudson et al.
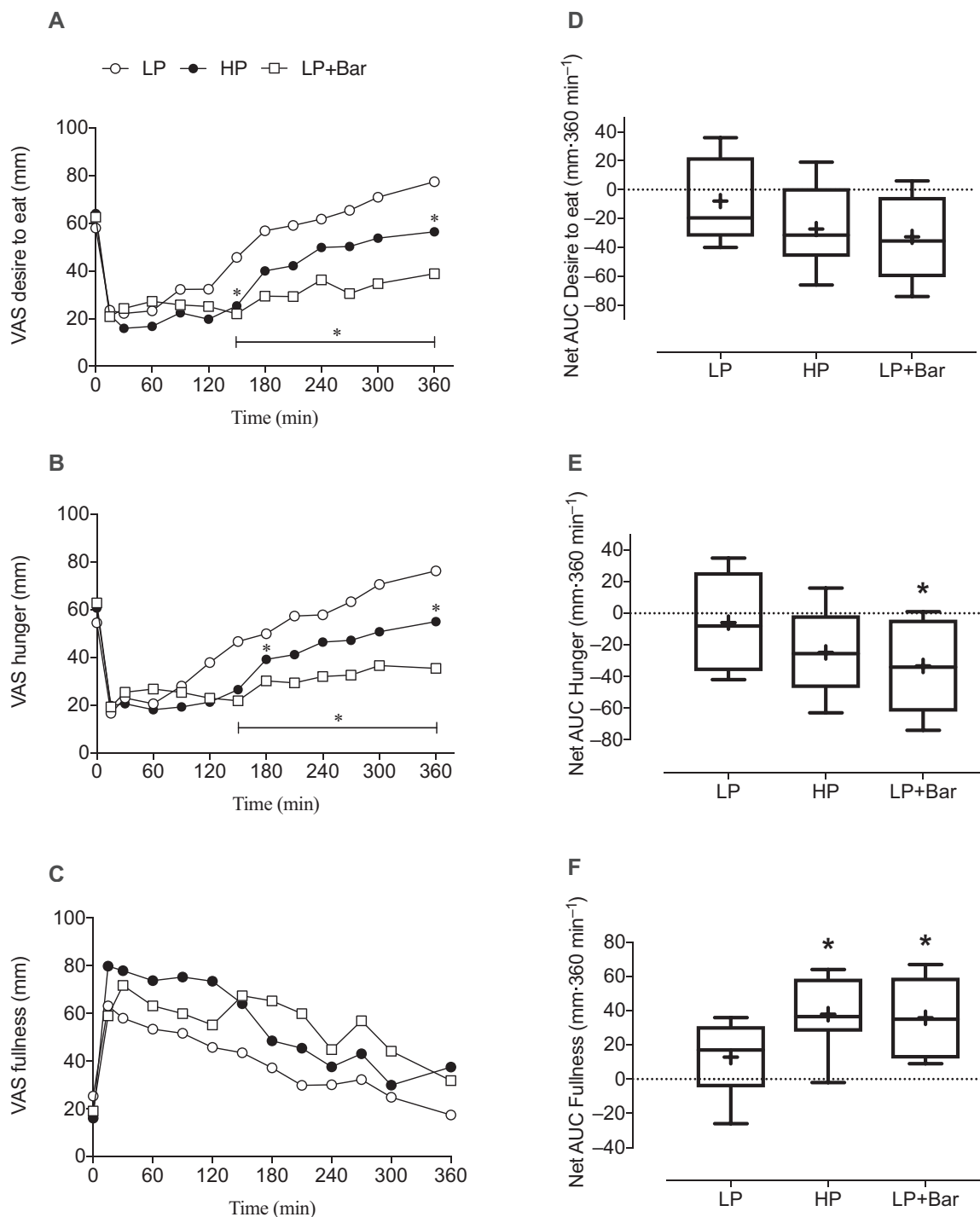
FIGURE 3 The effects of a protein-and leucine-enriched bar on the desire to eat (A and D), hunger (B and E), and fullness (C and F) [mm and mm·360 min^{-1}(6 h)] in the fasting state and after the ingestion of a lower-protein breakfast containing 9–16 g protein (LP), a higher-protein breakfast containing 23–40 g protein (HP), or a protein- and leucine-enriched bar containing ~16 g protein and 220 kcal ingested 2 h after consuming LP (LP + Bar) in healthy older men and women. VAS values are means (error bars on VAS scores are omitted for clarity), $n = 8$ per treatment. Net AUC values are presented as box-and-whisker plots with the whiskers showing maximum and minimum values, the box the IQR, the cross (+) shows the mean, and the line is the median. *Significantly different from LP ($P < 0.05$). VAS, visual analog scale.

(19), with the exception that we included older men and women and that we tested the effects of a breakfast bar containing ∼16 g protein with ∼1.5 g free leucine (as opposed to a 20-g whey protein beverage).

Ingestion of protein that results in a rapid leucinemia, along with a sustained supply of EAAs, stimulates and maintains MPS (12, 13, 25, 26). Previous studies showed that low protein intakes (e.g., ∼10 g) in a mixed meal or as isolated proteins do not robustly increase aminoacidemia or MPS in older persons (27, 28). For older adults, who typically consume less protein at the morning meal (6–10), modulating their eating patterns to increase plasma leucinemia and EAA concentrations can stimulate MPS. Our previous data demonstrated that blood leucine concentrations of ∼450 μM resulted in increased integrated MPS rates over 3 d in older males consuming low- or high-protein-containing diets (29), and the same was true for older women (12, 13). In the present study, we found that a leucine-enriched bar containing ∼16 g protein and 1.5 g free leucine resulted in peak leucinemia of ∼590 μM and maintained plasma EAA concentrations. The ingestion of the bar also resulted in similar net EAA and higher BCAA exposure than the higher-protein diet in our previous study (29), as indicated by the AUC (Table 2). The bar was formulated to take advantage of differing digestion and absorption kinetics (milk proteins being slower to digest and absorb than free leucine) and provide high concentrations of EAAs, BCAAs, and leucine, which can better elicit an initial rapid followed by a sustained hyperaminoacidemia.

The postprandial leucinemia after bar ingestion was similar to that previously reported as being sufficient to stimulate MPS following the ingestion of a single dose of leucine-enriched EAAs in young and older adults (30), a suboptimal dose of whey protein enriched with leucine in young men (14, 15), and beverages containing either 20 or 40 g whey protein in older men (27) and women (12, 13). The peak leucinemia reported in older men and women in the present study was also higher than those reported in a previous study in younger women (21). In an acute feeding study on a protein- and leucine-enriched bar containing 13 g milk protein and 3.0 g additional leucine, the magnitude of the increase in peak leucinemia (C_{max}) was ∼40% lower compared with the results of the present study (∼420 μM compared with ∼590 μM) (21). In the present study, peak leucinemia exceeded the plasma leucine response previously reported after consuming a 40-g whey protein beverage (∼320 μM) (27). The rate of dietary protein digestion and amino acid absorption and the subsequent availability of dietary protein–derived amino acids in circulation depend on gastric and intestinal motility, luminal digestion, and mucosal absorption (31–33). Thus, the postprandial leucine availability was adequate using a bar delivery system that utilized lower protein amounts in older adults. Future studies should examine the present protein- and leucine-enriched bar formulation with muscle biopsies to determine if the bar enhances MPS directly when used during a resistance training program.

To our knowledge, no previous studies have examined the effects of breakfast meals and acute ingestion of protein-containing bars on subjective appetite sensations in older adults (Figure 3). In agreement with our results, Bolster et al. (21) reported that lower-protein-containing nutrition bars coingested with 2 g or 3 g leucine peptides increased postprandial fullness ratings. Ingestion of 2 g leucine peptides decreased hunger and desire to eat in younger women. Coingesting 3 g leucine peptides did not further stimulate satiety, probably because leucine transporters likely reached a saturation point (21). However, there is limited information regarding the role of plasma leucinemia in the regulation of appetite following consumption of a suboptimal protein dose containing fast and slow digestible proteins, free leucine, low glycemic index carbohydrates, and monounsaturated fat in humans (20). We achieved plasma leucine concentrations higher than after the high-protein breakfast using the protein- and leucine-enriched bar. We acknowledge that our study design resulted in an increased energy and carbohydrate intake in the LP + Bar condition due to the bar's ingestion compared with the LP and HP conditions. However, after the bar ingestion at 120 min, we observed no further increases ($T_{max} = 43 \pm 5$ min) in plasma insulin concentrations; instead, plasma insulin concentrations decreased (data not reported). This result was likely due to the bar's sweetener system, also containing low-glycemic carbohydrates, which do not cause a rapid increase in postprandial blood glucose (34). Higher carbohydrate intake might not be desirable for older individuals at risk of developing diabetes, and therefore dietary strategies should also aim at reducing carbohydrate intake. The impact of the greater energy intake, if that is what is sensed in appetite, cannot be discounted in the LP + Bar condition. We elected to study a protein- and leucine-enriched bar as a postbreakfast snack, which does not induce hyperglycemia, to more closely mimic how the bar's ingestion would affect older persons who commonly consume a lower-protein-containing breakfast.

In conclusion, consuming a protein bar containing ∼16 g milk proteins blended with 1.5 g free leucine and half the LP breakfast meal's energy content 2 h after a lower-protein, wholefood breakfast increased leucinemia and promoted satiety. Our findings and those from previous studies provide a rationale for future research in clinical settings designed to improve muscle anabolism while potentially regulating appetite.

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