

## Short Communication

# Effect of food form on postprandial plasma amino acid concentrations in older adults

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(Received 11 October 2010 – Revised 10 January 2011 – Accepted 14 January 2011 – First published online 4 May 2011)

### Abstract

To assess the effect of food form (FF) on postprandial (PP) plasma amino acid (AA) concentrations, ten older adults (five men and five women, age 72 (SEM 2) years, BMI 26.0 (SEM 0.9) kg/m<sup>2</sup>) consumed, on separate days, energy and macronutrient-matched test meal replacement products (MRP) (approximately 25% of the subject's daily energy need; approximately 54% carbohydrate, 21% protein, 25% fat) in beverage and solid form. Blood samples were taken during fasting and throughout the 4 h PP period; plasma AA concentrations were assessed using HPLC. Consumption of each MRP led to an increase in total AA, branched-chain AA (BCAA), essential AA (EAA), non-essential AA (NEAA) and leucine concentrations (4 h area under the curve, AUC) (time effect;  $P < 0.05$ ). The beverage MRP resulted in a greater initial (i.e. 30 min) and sustained (4 h AUC) increase in total AA, BCAA, EAA, NEAA and leucine concentrations compared with the solid MRP (each effect of FF;  $P < 0.05$ ). Although there was no effect of FF on PP insulin response, glucose concentration was greater 1 and 2 h after the solid MRP was consumed (FF × time interaction;  $P < 0.05$ ). For all PP time points combined, total AA concentration was positively associated with plasma insulin ( $r 0.25$ ) and glucose ( $r 0.24$ ) concentrations for the solid MRP but not for the beverage MRP. In conclusion, older adults can achieve higher plasma AA concentrations when a protein-containing MRP is ingested in beverage form. The implications of the higher AA availability on anabolic processes warrant investigation.

**Key words:** Dietary protein: Food rheology: Ageing

Ageing is associated with sarcopenia, the progressive loss of skeletal muscle often accompanied by the loss of strength and functional capacity<sup>(1)</sup>. Sarcopenia has multiple aetiologies, including reduced physical activity and metabolic abnormalities. An age-related impairment in muscle anabolism following consumption of dietary protein may also exist and limit protein accretion in the postprandial (PP) state<sup>(2)</sup>. These age-related changes in protein metabolism might alter the utilisation and incorporation of dietary amino acids (AA) into skeletal muscle<sup>(3)</sup>. Ageing may also reduce AA availability resulting from a decrease in the efficiency of protein digestion, decreased absorption of AA and increased AA extraction by

the splanchnic organs<sup>(1)</sup>. This state of impaired protein anabolism and reduced AA availability with ageing may necessitate nutritional interventions that provide an increase in circulating AA to maximise substrate availability to muscle.

Commercial meal replacement products (MRP) in the form of bars and beverages are readily accessible sources of dietary protein as well as other macronutrients<sup>(4)</sup>. MRP are recommended for older adults to promote weight management and increase dietary protein intake<sup>(5)</sup> irrespective of food form (FF). Yet, the FF of MRP is documented to influence PP appetite and hormonal responses in older adults<sup>(6)</sup>. These differential responses between FF prompted our

**Abbreviations:** AA, amino acids; AUC, area under the curve; BCAA, branched-chain amino acids; EAA, essential amino acids; FF, food form; MRP, meal replacement product; NEAA, non-essential amino acids; PP, postprandial.

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interest to investigate the effects of FF on plasma AA concentrations. The primary aim of the present study was to compare the PP AA concentrations of older adults following consumption of a higher-protein beverage or solid MRP matched for macronutrient composition, fibre and energy. In light of previous literature identifying protein digestibility as an important factor having an impact on AA content, we hypothesised that the beverage MRP would provide more rapidly digestible protein, resulting in a more rapid and robust AA response compared with the solid MRP<sup>(7)</sup>. A secondary goal of the present study was to retrospectively examine the relationship between PP hormones and PP AA concentration. We hypothesised that AA concentration would be positively associated with insulin concentration following consumption of both MRP.

## Methods and procedures

### Subjects

A total of twenty-five healthy, older adults (eleven men and fourteen women) aged 70 (SEM 1; range 65–84) years were recruited from the greater Lafayette area through newspaper advertisements based on the following criteria: (1) men and women  $\geq 65$  years; (2) BMI 20–29 kg/m<sup>2</sup>; (3) not dieting and no weight loss or gain (>2 kg) within the past 6 months; (4) non-smoking; (5) not diabetic; (6) clinically normal blood profiles (normal liver and kidney function, normal complete blood count, fasting blood glucose < 1100 mg/dl); (7) maintained a free-living (non-institutionalised) lifestyle. All subjects were found to have markers of kidney and liver functions within 10% of clinical normalcy. The present study analysed plasma samples from ten subjects (five men and five women, aged 72 (SEM 2; range 65–84) years for AA content. Whole-body density was determined using air displacement plethysmography (Bod Pod; Life Measurement, Inc., Concord, CA, USA), and fat mass and fat-free mass were estimated using the two-compartment Siri equation<sup>(8)</sup>. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Purdue University Biomedical Institutional Review Board. Each subject was informed of the procedures and potential risks involved with the study before they signed an informed consent form and received a monetary stipend. The study is registered in the ClinicalTrials.gov registry (no. 0509003024).

### Experimental design

Plasma AA data are a secondary outcome from a previously published research project utilising a randomised, cross-over design to assess the effect of FF (within subject) on appetite and hormones in older adults<sup>(6)</sup>. For the AA analysis, ten subjects were randomly selected from the original study population. Subjects consumed, on separate days, energy and macronutrient-matched MRP in beverage and solid form. Subjects arrived at the laboratory following an overnight fast and were placed in a supine position. A catheter was placed

in an antecubital vein of the non-dominant arm and a baseline (fasting) blood sample was taken at time 0 (approximately 08.00 hours) before the subjects consumed a MRP in beverage or solid form along with 88.7 ml (3 oz) of water. Subjects had 15 min to completely consume the MRP, and blood samples were taken at +15, +30, +60, +90, +120, +150, +180 and +240 min during a 4 h period.

### Meal replacement products

Test MRP were in the form of a 'shake' (beverage) and a 'nutrition bar' (solid), manufactured by the Solae Company (St Louis, MO, USA), and contained the same protein source (Supro<sup>®</sup>, soya-based protein isolate). The AA composition of Supro<sup>®</sup> is provided in Table S1 of the supplementary material (available online at [www.journals.cambridge.org/bjn](http://www.journals.cambridge.org/bjn)). Energy and macronutrient compositions were equivalent between the beverage and solid forms (approximately 25% of the subject's daily energy need: approximately 54% carbohydrate, 21% protein, 25% fat; Table 1), as described previously<sup>(6)</sup>. For manufacturing purposes, the formulations could not be exactly matched, which resulted in slight variations in total sugar content. Daily energy requirements were estimated from the sex-specific Harris Benedict equation  $\times 1.5$  activity factor<sup>(9)</sup>.

### Amino acid analysis

Free AA concentrations for total AA, branched-chain amino acids (BCAA; leucine, isoleucine and valine), essential amino acids (EAA; isoleucine, lysine, methionine, phenylalanine, threonine, tyrosine, valine, leucine, arginine and histidine), non-essential amino acids (NEAA; alanine, asparagine, cysteine, glutamic acid, glycine, proline and serine) and leucine concentrations were measured using a Beckman HPLC (126 pump, 166 Detector, 507 autosampler, and 'System Gold' data system) equipped with a Waters AccQTag AA analysis column and buffers (Waters, Inc., Milford, MA, USA). Plasma samples were deproteinised with sulphosalicylic

**Table 1.** Test meal characteristics  
(Mean values with their standard errors)

	Solid		Beverage	
	Mean	SEM	Mean	SEM
Energy per test meal				
kcal	548	22	548	22
kJ	2292	92	2292	92
Total mass (g/test meal)	132	4	634	21
Macronutrient composition				
Carbohydrate (g/test meal)	73	2	73	2
Sugar (g/test meal)	39	1	48	1
Fibre (g/test meal)	0		0	
Protein (g/test meal)	27	2	27	2
Fat (g/test meal)	16	1	16	1
Palatability (au)*	7	1	6	1
Viscosity, beverage (cP)	NA		21.5	
Hardness, solid (g)	1013		NA	

au, Arbitrary units; cP, centipoises; NA, not applicable.

\*Palatability expressed as au (scales 1–9).

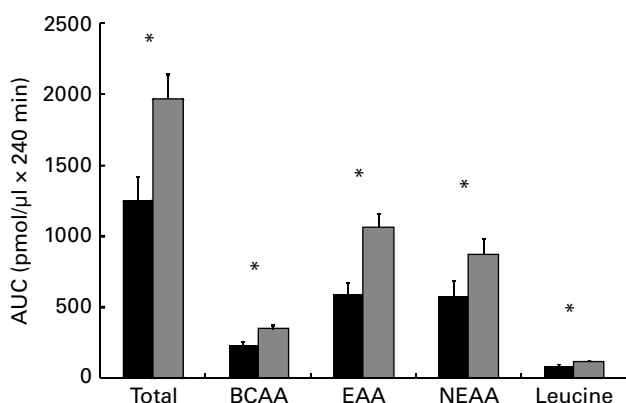
acid and centrifuged using a method adapted from Galibois *et al.*<sup>(10)</sup>. Before HPLC analysis, 200 pmol of norleucine standard were added to each sample before being dried. Samples were derivatised according to the vendor's recommendations (Waters, Inc.), using 20 mM-HCl, borate buffer and AQC (aminoquinolyl-*N*-hydroxysuccinimidyl carbamate) reagent in acetonitrile (1:3:1, by vol.).

### Hormone analysis

Plasma glucose concentration was measured by enzymatic colorimetry, using an oxidase method on a COBAS Integra 400 analyser (Roche Diagnostic Systems, Indianapolis, IN, USA). Plasma insulin concentration was measured by an electrochemiluminescence immunoassay method using the Elecsys 2010 analyser (Roche Diagnostic Systems).

### Statistical analyses

All datasets were checked for normality and logarithmic transformation was performed on some AA data. The area under the curve (AUC) was calculated using the trapezoidal rule<sup>(11)</sup>. For both 4 h AUC (Fig. 1) and time-course analyses, repeated-measures ANCOVA was used to evaluate the effects of time on AA and hormone data, with total sugar content controlled as the covariate. *Post hoc* analyses using Dunnett's test were used to evaluate the time-course analysis, comparing each time point (15–240 min) with baseline values within FF and to determine the difference between FF at each hour (1–4 h AUC) (see Figs. S1–S5 of the supplementary material, available online at [www.journals.cambridge.org/bjn](http://www.journals.cambridge.org/bjn)). Observed power was greater than 80% for the effect of FF on AA concentrations. All measurements are expressed as means with their standard errors. FF-specific relationships between AA and hormone concentrations were evaluated using all PP time points via Pearson's correlation coefficient.



**Fig. 1.** Postprandial amino acid area under the curve (AUC) data during the 4 h period following consumption of the beverage (■) and solid (▒) meal replacement products. Postprandial total amino acid AUC (pmol/μl × 240 min) was lower following consumption of solid *v.* beverage for total amino acid and for each amino acid subfraction (4 h AUC) ( $P < 0.05$ ). Values are means, with standard errors represented by vertical bars. \*Mean values were significantly different for the effect of food form (beverage *v.* solid,  $P < 0.05$ ). BCAA, branched-chain amino acid; EAA, essential amino acid; NEAA, non-essential amino acid.

The effect of sex was not significant for any dependent variable and was excluded from the statistical model. An  $\alpha$ -level of  $P < 0.05$ , two-tailed, was considered to be statistically significant. All statistical analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC, USA).

## Results

### Subject characteristics

The subject characteristics were as follows: body weight (75.8 (SEM 3.2) kg), BMI (26.0 (SEM 0.9) kg/m<sup>2</sup>), fat-free mass (50.4 (SEM 3.3) kg), body fat percentage (34.1 (SEM 2.8) %) and age (72 (SEM 2) years).

### Amino acids

Fasting total AA, BCAA, EAA, NEAA and leucine concentrations were not different between testing days (see Figs. S1–S5 of the supplementary material, available online at [www.journals.cambridge.org/bjn](http://www.journals.cambridge.org/bjn)). The PP increases in total AA, BCAA, EAA, NEAA and leucine concentrations became significant earlier for the beverage MRP (15–30 min) *v.* solid MRP (30–60 min), and remained elevated longer (240 *v.* 180 min, respectively). The peak concentration of these AA concentrations occurred at 60 min for both the beverage and solid MRP. Over the 4 h testing period, the composite PP concentrations of all AA subfractions were greater for the beverage *v.* the solid MRP. The 4 h composite results are presented in Fig. 1 and the time point-specific concentrations and hourly composite results are presented in Figs. S1–S5 of the supplementary material (available online at [www.journals.cambridge.org/bjn](http://www.journals.cambridge.org/bjn)). The beverage MRP resulted in higher concentrations for total AA at 1, 2 and 3 h; BCAA at 1, 2 and 4 h; EAA at 1, 2 and 3 h; NEAA at 1 and 2 h; and leucine at 1, 2 and 4 h.

### Hormone response

Fasting glucose (870 *v.* 910 mg/l) and insulin (40.3 *v.* 38.2 μU/ml) concentrations were not different between the acute feeding trials. A significant effect of time was observed for glucose and insulin (see Figs. S6 and S7 of the supplementary material, available online at [www.journals.cambridge.org/bjn](http://www.journals.cambridge.org/bjn)). The PP glucose response was greater for the solid *v.* beverage MRP at 1 and 2 h, but not at 3 and 4 h (time × FF interaction,  $P < 0.01$ ; see Figs. S6 and S7 of the supplementary material, available online at [www.journals.cambridge.org/bjn](http://www.journals.cambridge.org/bjn)). The PP insulin responses were not different between the beverage and solid MRP (see Figs. S6 and S7 of the supplementary material, available online at [www.journals.cambridge.org/bjn](http://www.journals.cambridge.org/bjn)).

### Association between amino acids and hormone data

Following the consumption of the solid MRP, the 4 h AUC of AA subfractions were positively associated with the 4 h AUC of glucose (total AA,  $r = 0.24$ ,  $P < 0.02$ ; BCAA,  $r = 0.49$ ,  $P < 0.0001$ ; EAA,  $r = 0.36$ ,  $P < 0.0002$ ; leucine,  $r = 0.57$ ,

$P < 0.0001$ ) and insulin (total AA,  $r 0.25$ ,  $P < 0.02$ ; BCAA,  $r 0.39$ ,  $P < 0.0001$ ; EAA,  $r 0.22$ ,  $P < 0.0001$ ; leucine,  $r 0.53$ ,  $P < 0.0001$ ; NEAA,  $r 0.23$ ,  $P < 0.03$ ). No significant correlations were observed between the 4 h AUC of AA and those of glucose and insulin following consumption of the beverage MRP. The 4 h AUC of glucose and insulin were positively correlated for the beverage ( $r 0.65$ ,  $P < 0.05$ ) and solid ( $r 0.75$ ,  $P < 0.0001$ ) MRP.

## Discussion

Maximising PP AA concentrations is important for the maintenance of muscle mass in older populations, given reported impairments in skeletal muscle protein metabolism<sup>(1)</sup>. Changes in digestion and absorption parameters of acute meals can increase AA delivery and subsequent availability in plasma, which may determine the metabolic fate and anabolic stimulus of ingested protein<sup>(12)</sup>. Despite an increased emphasis on nutrition in older adults, little is known regarding the impact of FF on the PP response to an acute feeding. The aim of the present study was to examine the effect of FF on PP plasma AA concentrations in older men and women. The present results suggest that consumption of a MRP in the form of a beverage elicits a more rapid and greater increase in AA concentration compared with a solid MRP, consistent with a faster rate of digestion and absorption.

Based on previous acute studies, we predicted that the more rapidly digesting beverage would promote a greater initial increase in PP AA concentrations returning to baseline levels more quickly than the slower digesting solid MRP<sup>(7)</sup>. As expected, consumption of the beverage MRP resulted in a greater initial increase in AA levels compared with the solid MRP, with the peak AA concentration observed at 60 min following the acute meal. Contrary to our hypothesis, however, plasma concentrations of each AA subfraction remained elevated to a greater extent following the beverage *v.* solid meal during the entire 4 h period. This finding may have been affected by several factors in the present study. The inclusion of fat and carbohydrate in the MRP probably influenced the PP AA concentrations, given previous reports that consumption of a mixed meal altered PP AA concentrations compared with the consumption of protein alone<sup>(7)</sup>. In addition, a practical consequence of our efforts to quantitatively match the macronutrient content of the MRP was a slight difference in the formulation of these items, resulting in the beverage MRP containing a greater proportion of total sugar compared with the solid MRP. The differing sugar content between the MRP may be representative of numerous commercially available MRP, given the need to create palatable items with a desired hardness or viscosity. Despite differences in sugar content, the main effects of the AA analysis were not significantly altered when an ANCOVA with sugar as a covariate was performed. Future studies investigating the impact of FF on AA levels should carefully consider potential differences in the formulation of MRP as well as the inclusion of muscle-specific endpoints to evaluate the influence of AA availability on downstream anabolic targets.

Consumption of beverage meals may promote a more rapid release of PP appetitive and metabolic hormones compared with a solid feeding<sup>(5)</sup>. In the present study, there was no main effect of FF on PP hormone levels, despite significantly higher levels of plasma glucose observed 1 and 2 h following consumption of the solid MRP. The increased glucose concentration in the initial hours post-feeding might have been expected to elicit a higher PP insulin response compared with the beverage feeding. However, a high degree of between-subject variability was observed for the PP insulin responses (CV 59%), which is probably due to the age of our subject population. While not clinically diabetic, the reduction in insulin sensitivity associated with increasing age may have led to the high variability in PP insulin response. The finding that the insulin and glucose responses were related to plasma AA levels following the solid but not beverage MRP is difficult to understand and might be linked to the variable hormone responses among subjects. Caution is warranted when interpreting these findings due to the possible impact of the small sample size, inherent variability of hormone responses, size of the test meals and the potential confounding effect of differences in sugar content. Despite these potential limitations, our findings extend the results of previous protein-only feeding trials to the use of MRP which incorporate all macronutrients and provide initial insight into the effect of FF on the PP AA response.

## Conclusions

The present study revealed a more rapid and greater increase in total plasma AA availability following the consumption of beverage *v.* solid MRP matched for the quantity of energy and macronutrients in older adults. These findings could have physiological relevance due to the preferential utilisation of key AA groups, such as EAA and BCAA, in skeletal muscle anabolism<sup>(13)</sup> and represent an important preliminary step in evaluating the effect of FF on AA availability. Broader examination of the impact of FF on AA availability and anabolic processes is needed to further elucidate the physiological relevance of these findings.

## Acknowledgements

The present study was funded through the National Institutes of Health R01 AG021911 (awarded to W. W. C.) and the Solae Company<sup>®</sup>. Support for H. J. L. was provided in part by an Ingestive Behavior Research Center postdoctoral fellowship from Purdue University. Support for J. W. A. was provided in part by a Purdue University Bilsland Fellowship. The Solae Company manufactured and provided the MRP. Plasma amino acid analysis was completed by the Purdue University Proteomics Facility. The authors' responsibilities were as follows: W. W. C. and K. A. G. conceived and designed the experiment; J. W. A. and H. J. L. conducted the clinical aspects of the study; J. W. A. and H. J. L. processed and analysed the samples; E. L. conducted the statistical analysis. All authors interpreted the data and wrote the manuscript. T. B. C., J. W. A., H. J. L., E. L. and W. W. C. had no personal

or financial conflicts of interest. K. A. G. was employed by Solae during data collection.

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