Title: Acute effects of essential amino acid gel-based and whey protein supplements on appetite and energy intake in older women

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Abstract

Deficiencies in protein and energy intakes are partly responsible for age-related sarcopenia. We investigated the effects of supplements matched in essential amino acid (EAA) content (7.5 g) on energy intake and appetite. Ten women aged 69.2 ± 2.7 years, completed three trials in a randomised, crossover design. Composite appetite scores, peptide-YY (PYY), and insulin responses to a 200 ml whey protein isolate (WP, 275 kJ), a 50 ml EAA gel (GEL, 478 kJ) or nothing as the control condition (CON) were investigated over one hour, followed by an *ad libitum* breakfast. Energy intake at breakfast (CON 1957 ± 713, WP 1413 ± 623, GEL 1963 ± 611 kJ) was higher in CON and GEL than in WP (both $P = 0.006$). After accounting for supplement energy content, energy intake in GEL was higher than in CON ($P = 0.0006$) and WP ($P = 0.0008$). Time-averaged area under the curve for composite appetite scores (CON 74 ± 20, WP 50 ± 22, GEL 60 ± 16 mm) was higher in CON than WP ($P = 0.015$). Time-averaged area under the curve for PYY (CON 87 ± 13, WP 119 ± 27, GEL 97 ± 22 $pg \cdot mL^{-1}$) was higher in WP than CON ($P = 0.009$) and GEL ($P = 0.012$). In conclusion, supplementation with WP facilitated an increase in protein intake, whereas supplementation with GEL increases in both energy and protein intakes, when consumed before an *ad libitum* breakfast. Such findings, highlight potential gel-based EAA supplementation intake for addressing age-related sarcopenia.

Key words: Sarcopenia; Undernutrition; Malnutrition; Leucine; Ageing; Protein

Introduction

Age-related sarcopenia, characterised by a decline in muscle mass and function or strength (Cruz-Jentoft et al. 2010), contributes to poor health in older people (Janssen et al. 2004b). As identified in a recent review by Naseeb and Volep (2017), dietary protein intake and physical activity play a key role in the management of sarcopenia. Similarly to protein, optimal energy intake is crucial for the maintenance of muscle mass and health (Dahany et al. 2014; Thalacker-Mercer et al. 2014; Baum et al. 2016). Nevertheless, older people have reduced appetite and energy intake compared to the young (Giezenaar et al. 2016), whilst deficiencies in energy and protein intakes are contributing factors to frailty (Beasley et al. 2010; Bauer et al. 2013; Bonnefoy et al. 2015).

Older women do not achieve the current Recommended Daily Allowance (RDA) for protein intake (Kerstetter et al. 2003; Morley et al. 2010; Pasiakos et al. 2015; Farsijani et al. 2016). Given that a lack of muscle responsiveness can be overcome with larger doses than the current RDA for protein (Hulmi et al. 2010; Cramer et al. 2016; Loenneke et al. 2016), an increase in protein intake may be a viable strategy for managing sarcopenia (Janssen et al. 2004a; Clark et al. 2010; Lang et al. 2010; Lieffers et al. 2012). Indeed, evidence supports an increase in daily protein intake from the current RDA (0.8 $g \cdot kg^{-1} \cdot d^{-1}$) to 1.0-1.6 $g \cdot kg^{-1} \cdot d^{-1}$ (Bauer et al. 2013; Deutz et al. 2014; Loenneke et al. 2016; Traylor et al. 2018). Consumption of at least 0.4 $g \cdot kg^{-1} \cdot$BM of high quality protein per meal (Moore et al. 2014; Phillips 2015; Lancha Jr et al. 2016) is also recommended. This is primarily due to their high essential amino acid (EAA) content which optimises muscle protein synthesis (Breen et al. 2011; Churchward-Venne et al. 2014; Paddon-Jones et al. 2014; Xu et al. 2015; Murphy et al. 2016; Phillips 2016; Hamarsland et al. 2017). However, current evidence is limited regarding the beneficial impact of protein or EAA supplementation alone on the muscle mass and strength of predominantly healthy (Tieland et al. 2017) or clinical older populations

(Ferrando et al. 2010; Cramer et al. 2016). Potential explanations for these discrepancies may include a compensatory caloric redistribution (Fiatarone Singh et al. 2000) or partial caloric redistribution (Cramer et al. 2016) due to appetite suppression.

Ageing is associated with a progressive decrease in appetite and fluctuations in appetite regulating hormones (Benelam 2009; Ahmed et al. 2010; Akimoto et al. 2010; Donini et al. 2013; Giezenaar et al. 2016). Taking into account that dietary and whey proteins enhance satiety (Veldhorst et al. 2008; Mollahosseini et al. 2017), and in a dose response manner (Veldhorst et al. 2009; Paddon-Jones et al. 2014), the appetite of older people may be compromised further. A full investigation of the satiety control mechanisms, which have been discussed elsewhere (Sukkar et al. 2013; Tremblay et al. 2015), is beyond the scope of this paper. However, a postprandial increase in peptide YY (PYY), probably due to an increase in amino acids in the gastrointestinal tract (Moran et al. 2011) following consumption of dietary protein, plays a key role for reductions in energy intake (Leidy et al. 2015; Phillips et al. 2016). Appetite of older women may be suppressed further since postprandial PYY increases to greater extent than in younger women (Hickson et al. 2016). Recently, we have shown that ingestion of EAA gel-based nutritional prototypes providing 7.5 g of EAA by older women prior to consumption of an *ad libitum* breakfast (ALB) did neither result in an increase in PYY nor a decrease in energy intake compared to a control condition (Ispoglou et al. 2017). Whey proteins may be considered the highest quality proteins (Hoffman et al. 2004; Hulmi et al. 2010) however they reduce appetite partly due to their high amino acid content. We therefore hypothesised that a gel containing the same total amount of EAAs as in approximately 15 g of whey protein would affect appetite and appetite hormone responses to a lesser degree than the whey protein supplement, and this in turn would facilitate an increase in both protein and energy intake when taken before an ALB.

Materials and methods

This investigation was conducted in accordance with the guidelines laid down in the Declaration of Helsinki. All procedures were approved by the University Faculty Research Ethics Committee and written informed consent was obtained from all participants. Study participants were independently living female older adults aged between 65 and 75 years, free from vascular and metabolic disease, and of good health. Participants were excluded if they smoked, had used estrogens within the previous three months, or were lactose intolerant. Participants were asked to avoid alcohol and intensive physical activity during the 24 hours prior to experimental trials. All trials commenced between 07:30 am and 09:00 am after an overnight fast of at least 10 hours. Participants exerted themselves minimally when travelling to the laboratory, using motorised transport where possible.

Preliminary screening and anthropometry

The first visit to the laboratory involved an initial briefing and screening process. Participants were provided with information on the study procedures and given a detailed overview before each trial. Baseline stature (to the nearest cm) and body mass (to the nearest kg) were recorded by a stadiometer (Seca 220, Hamburg, Germany) and scales (Seca 220, Hamburg, Germany). Resting heart rate, systolic and diastolic blood pressure were measured alongside these variables, using an automatic sphygmomanometer (Omron Healthcare Ltd, Kyoto, Japan).

Experimental protocol

Older women (n=10) (see Table 1 for anthropometric characteristics) completed three trials each separated by a minimum of three days in a randomised, crossover design. Participants recorded food and fluid consumed in the 24 h prior to the first experimental trial and replicated this for all subsequent trials. They were also asked to avoid intensive physical activity during the same time period. All trials commenced between 07:30 am and 09:00 am after an overnight fast of at least 10 h. Participants exerted themselves minimally when travelling to the laboratory, using motorised transport where possible. Verbal confirmation of the dietary and exercise standardisation was obtained at the beginning of each experimental trial. For screening purposes during the initial visit, all participants had their blood pressure measured using a manual sphygmomanometer (Accoson Greenlight 300, Accoson, United Kingdom). Fingertip capillary blood samples were also analysed for fasting levels of total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides and glucose using a Cholestech LDX (Alere San Diego, Inc., San Diego, CA) analyser. For an outline of tests and testing procedures, see Figure 1.

Upon arrival, participants rested in a supine position for 15 minutes. Appetite sensations, plasma insulin and PYY responses to a 200 ml WP isolate (275 kJ) made with 178 ml water and 21.2 g of WP powder , a 50 ml GEL (478 kJ), and a CON were investigated over the course of one hour, followed by an ALB. During the CON trial, nothing was consumed by participants before the ALB. The inclusion of an isocaloric placebo and matching for volume were dismissed as a means to enhance the ecological validity of the study. In addition, it has been shown that 375 ml water pre-load 30 min before a meal does not negatively affect energy intake in older women (Walleghen et al. 2007). The WP powder was purchased from MyProtein (key nutritional information per 100g was: energy 1558 kJ, fat 0.5 g, carbohydrate 4.1 g, protein 86 g and salt 0.5 g). The EAA for the

GEL were purchased from Fagron UK Ltd. The GEL was developed in a collaboration between the university and a product developer to the food industry based at Askham Bryan College (UK). The key nutritional information per 100 g was: energy 967 kJ, fat 0.0 g, carbohydrate 44.7 g, protein 15 g, salt 0.2 g. The GEL and WP were matched by total EAA content with both supplements providing 7.5 g of EAA (Hulmi et al. 2010; Ispoglou et al. 2016), and therefore not energy-matched. In order to consume 7.5 g of EAA during the WP experimental trial, participants had to ingest 15.2 g of whey protein which was obtained through consumption of 21.2 g of the WP powder. Thus, the WP supplement supplied participants with 1.3 g, 15.2 g, and 1.6 g of carbohydrate, protein, and fat respectively. The corresponding macronutrient composition for each 50 ml GEL was 22 g, 7.5 g, and 0 g of carbohydrate, protein (all EAA), and fat respectively. The ratio of EAA in the GEL was the same as the 40% L-leucine formulation previously described by Ispoglou et al. (2016). Please see Table 2 for essential amino acid profiling in the GEL and WP supplements.

Baseline appetite perceptions and a baseline blood sample were collected five minutes prior to each condition, with participants instructed to consume each supplement within a five-minute period. Once the breakfast was consumed, participants were asked to provide their final appetite perceptions.

Body composition assessment

Total-body fat mass, lean tissue mass, bone mineral content and percentage tissue fat mass (%TFM) values were measured by a total-body dual-energy X-ray absorptiometry (DXA) scan (GE Lunar iDXA, GE Healthcare, Madison, WI). Participants were scanned in a fasted, euhydrated state as per established guidelines (Sawka et al. 2007; Nana et al. 2012). Participants removed shoes and jewellery before receiving the scan, whilst adopting a supine position with arms to the

side in the semi-prone position and ankles supported with the Lunar ankle strap (0.5 cm space between the ankles). The values for the body composition outcomes were determined from the ratio of soft tissue attenuation of two X-ray energy beams for each pixel containing a minimal amount of soft tissue but no significant bone (Mazess et al. 1990). In our laboratory, the in-vivo short-term precision (%CV) for total-body composition variables are 0.82% for fat mass, 0.51% for lean mass, 0.86% for percentage body fat and 0.60% for bone mineral content (Hind et al. 2011). The machine was checked and calibrated on a daily basis in line with the manufacturer's recommendations. All scanning and analysis procedures were performed by the same trained operator using the Lunar enCORE software package (version 15.0).

Ad Libitum Breakfast

The ALB was identical in each trial, with an energy density of 4.9 kJ/g and macronutrient composition of 59% carbohydrate, 18% protein and 23% fat. Meal preparation involved mixing of 54 g of porridge oats (Oatso Simple Original, Quaker Oats) with 292 ml semi-skimmed milk. The mixture was then cooked in a microwave for two and a half minutes at 700 W. All participants were habitual breakfast users and accustomed to eating porridge. Participants consumed the breakfast in isolation to avoid any social influence on food intake. A bowl of the aforementioned meal was provided by an investigator and participants were instructed to eat until 'comfortably full', with no time limit set for eating. This bowl was replaced before the participant had emptied it, with minimal interaction and this process continued until the participant was comfortably full. Food intake was calculated as the weighted difference in food before and after eating (Deighton et al. 2016).

Appetite assessment

Appetite perceptions (hunger, satisfaction, fullness and prospective food consumption) were measured using 100 mm visual analogue scales with descriptors anchored at each end (Flint et al. 2000). Using these scales, a composite appetite score (CAS)(0 – 100) was calculated using the following formula: CAS = ([hunger + prospective food consumption + (100 – fullness) + (100 – satisfaction)] / 4) (Stubbs et al. 2000).

Blood sampling and biochemical analysis

Participants rested in a semi-supine position for a minimum of five minutes before a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein by a trained phlebotomist. Blood samples were obtained at baseline (five min prior to each condition) and at five, 30, 60 min after supplement ingestion for the determination of plasma concentrations of insulin and PYY. At each time-point, samples were drawn into two pre-chilled 4.9 ml K3 ethylenediaminetetraacetic acid vacutainers (Becton Dickinson, USA). These vacutainers were spun at 1000 x g for 10 min at 4 °C. The plasma supernatant was then pipetted into Eppendorf tubes then stored −80 °C for subsequent analysis.

Commercially available enzyme-linked immunosorbent assay kits were used to determine plasma concentrations of PYY (Millipore, Watford, UK), and insulin (IBL International GmbH, Germany). A decision was made to measure PYY and insulin since they are both raised in older people and are known to suppress appetite (Fraze et al. 1987; Parker et al. 2004; Hickson et al.

2016). To eliminate interassay variation, samples from each participant were analysed in the same run. The within-batch coefficients of variation for each assay were 5.3% and 9.3%, respectively.

Statistical analyses

Data were analysed using SPSS for Windows (Version 22.0, IBM Corp., Armonk, NY). Normality was assessed using the Shapiro-Wilk test. Time-averaged area under the curve (AUC) values were calculated using the trapezoidal method. One-way repeated measures ANOVA was used to examine trial-based differences in energy intake as well as baseline and AUC values for appetite perceptions and plasma analytes. Where significant effects were found, post-hoc analyses using the Holm-Bonferroni correction for multiple comparisons were performed. The sample sizes employed within this study were deemed sufficient to detect a significant difference in energy intake between trials as the primary outcome measure. Calculations were performed using G*Power with a meaningful difference in energy intake established as 500 kJ according to previous research, achieving 80% power with 10 participants, and based on the standard deviation for a similar *ad libitum* meal to that used within the present study. Results in the text and tables are shown as mean ± standard deviation (SD). In addition to significance testing and for all comparisons, Cohen's *d* effect sizes were calculated and interpreted using the following thresholds: 0 – 0.2 (trivial), 0.2 – 0.5 (small), 0.5 – 0.8 (moderate), 0.8 – 1.2 (large), > 1.2 (very large) (Cohen, 1988). Cohen's *d* expresses the mean difference between two groups in standard deviation units. For example, if groups do not differ by 0.2 SD, the difference would be trivial even if significant. For calculation of all effect sizes, the largest sample mean of the conditions compared was placed first in the relevant equation. For all effects sizes, 95% confidence intervals (CI) were also determined. Graphical representations of the results are depicted as mean ± standard

error of the mean (SEM) to avoid distortion of the figures. Statistical significance was accepted as $P \leq 0.05$.

Results

Energy and macronutrient intakes

Energy intake at the ALB was significantly different between trials (CON 1957 ± 713, WP 1413 ± 623, GEL 1963 ± 611 kJ; $P < 0.0005$), with both CON ($P = 0.006$; $d = 0.81$, 95% CI [0.30,1.32]) and GEL higher than WP ($P = 0.006$; $d = 0.89$, 95% CI [0.33, 1.45]). CON and GEL were not significantly different ($P = 0.942$; $d = 0.00$, 95% CI [-0.27, 0.27]). After accounting for the energy content of the supplement, total energy intake was also significantly different between trials ($P < 0.0005$; Figure 2). Total energy intake for the GEL was significantly higher than both CON ($P = 0.0006$; $d = 0.73$, 95% CI [0.41, 1.05]) and WP ($P = 0.0006$; $d = 1.10$, 95% CI [0.60, 1.60]). CON and WP were not significantly different ($P = 0.132$; $d = 0.32$, 95% CI [-0.11 – 0.75]). The macronutrient contribution to energy intakes for the ALB and supplements singularly and combined are presented in Table 3.

Appetite

CAS did not differ between conditions at baseline (CON 73 ± 20, WP 69 ± 21, GEL 77 ± 18 mm, $P = 0.120$). A significant time-averaged appetite AUC effect was observed (CON 74 ± 20, WP 50

± 22, GEL 60 ± 16 mm, $P = 0.002$; Figure 3). Post-hoc analysis revealed that appetite ratings were significantly higher in CON vs WP ($P = 0.015$; $d = 0.20$, 95% CI [0.05, 0.35]). No significant differences were found for CON vs GEL ($P = 0.076$; $d = 0.21$, 95% CI [-0.03, 0.45]) and WP vs GEL ($P = 0.076$; $d = 0.41$, 95% CI [-0.05, 0.87]).

Plasma PYY and insulin concentrations

Baseline PYY and insulin values (Figures 4a and 4b) were not significantly different between conditions ($P = 0.262$ and $P=0.452$ for PYY and insulin respectively). Time-averaged AUC for PYY was significantly different between trials (CON 87 ± 13, WP 119 ± 27, GEL 97 ± 22 $pg \cdot mL^{-1}$; $P = 0.001$; Figure 4a), with WP higher than CON ($P = 0.009$; $d = 1.51$, 95% CI [0.51, 2.51]) and GEL ($P = 0.012$; $d = 0.89$, 95% CI [0.25 – 1.53]). CON and GEL were not significantly different ($P = 0.171$; $d = 0.55$, 95% CI [-0.29 – 0.84]). Time-averaged AUC for insulin was not significantly different between trials (CON 20 ± 12, WP 31 ± 14, GEL 33 ± 17 $\mu IU \cdot mL^{-1}$; $P = 0.095$; Figure 4b).

Discussion

This randomised cross over trial examined the acute effects of two protein-based oral nutritional supplements on appetite, selected appetite hormones and energy intake of older women. Administration of 7.5 g of EAA, the equivalent of approximately 15.2 g of WP isolate, was achieved via consumption of either a WP isolate beverage or an EAA gel, which were both given to participants one hour before the ALB. Our results demonstrated that when taking into account the energy and protein content of the supplements, the GEL facilitated an increase in both energy and protein intakes of older women during the ALB whilst WP facilitated an increase in protein intake alone. Consumption of the GEL did not affect appetite ratings or the concentration of the satiety hormone PYY, compared to the CON condition. Consumption of the WP isolate resulted in suppression of appetite as evidenced by significant reduction in CAS and increase in PYY. Thus, our data suggest that the current GEL formulation-based nutritional prototype may be more appropriate than WP for older women who would benefit from an increase in both energy and protein intakes per meal, without concomitant reductions in appetite.

A significant decrease in energy intake at the ALB was observed in the WP condition alone compared to CON and GEL. After adjusting for the energy content of the supplements, no significant differences were observed between the WP and CON despite a reduction in total energy intake in the WP compared to the CON condition. The GEL resulted in significant increases in energy intake than both the CON and the WP conditions. These findings are relevant to the care of older adults who fail to meet both protein and energy recommendations (Bonnefoy et al. 2015; Sanson et al. 2018), and specifically older women who may be less likely to meet protein recommendations than men (Kerstetter et al. 2003; Morley et al. 2010; Farsijani et al. 2016).

Therefore, supplements provided in gel form as described in this study, may contribute to the management of age-related sarcopenia (Janssen et al. 2004a; Clark et al. 2010; Lang et al. 2010) since achieving optimal protein and energy intake is crucial for the maintenance of muscle mass and strength (Dahany et al. 2014; Thalacker-Mercer et al. 2014; Baum et al. 2016). This would bear relevance for both institutionalised older adults (Sullivan et al. 1999; Elia 2006; Kaiser et al. 2010; Elia 2015; Sanson et al. 2018) and community-dwelling populations (Rist et al. 2012; Geurden et al. 2015) since reports of malnutrition or risk of malnutrition exist for both populations.

Taking into account that the protein content of the breakfast meal was 18%, participants in the CON condition consumed on average 21 g of protein at the ALB (Table 3). Notably, previous research suggests that protein intakes are significantly reduced at the breakfast meal with intakes of ~10 g (Tieland et al. 2012a). These lower levels of protein are not considered optimum for maximisation of muscle protein synthesis for our study population, who should be receiving at least 25-30 g of protein per meal (Moore et al. 2014; Phillips 2015; Lancha Jr et al. 2016) based on their body mass. Thirty grams of high quality protein per meal would in turn provide approximately 15 g of EAAs (Hulmi et al. 2010). Therefore, an alternative means to optimise muscle synthesis protein rates could be potentially achieved through administration of the amount of EAAs in approximately 30 g of protein rather than a larger bolus of dietary protein source, which will also provide non-essential amino acids that are not necessary for stimulation of muscle protein synthesis (Tipton et al. 1999). Ingestion of either the WP isolate or the GEL one hour before the ALB helped participants reach per meal protein intake recommendations however in the case of WP, this appeared to negatively affect energy intake. It could be argued that ingestion of the supplements immediately before the ALB may have also supressed appetite in the GEL. However, we have previously shown that the current EAA gel-based formulation, apart from being

palatable, is equally effective at facilitating an increase in both protein and energy intakes of older women when given either one hour or immediately before an ALB (Ispoglou et al. 2017).

Both WP and GEL supplements had a high EAA content (7.5 g); a prerequisite for optimisation of muscle protein synthesis (Phillips et al. 2009; Breen et al. 2011; Churchward-Venne et al. 2014; Xu et al. 2015; Murphy et al. 2016; Phillips 2016; Hamarsland et al. 2017). Increased muscle protein synthesis rates are credited to a large extent to the higher leucine content in high quality proteins, which contributes to the regulation of muscle protein synthesis (Hamarsland et al. 2017). The WP condition received additional non-essential amino acids compared to the EAA gel, however our key objective was that both supplements were matched in total EAA content. Furthermore, the ratio of EAAs in the GEL was specifically optimised (i.e. higher leucine content) for older people (Katsanos et al. 2006; Xu et al. 2015; Murphy et al. 2016; Phillips 2016). Therefore, another advantage of the GEL was that it also facilitated a further increase in leucine content compared to the WP. Our previous work has demonstrated that ingestion of the current GEL formulation results in peak plasma concentration of amino acids within 30-60 minutes from ingestion, highlighting the efficient digestion and absorption rates of the GEL (Ispoglou et al. 2017).

It is generally agreed that protein-based oral nutritional supplements can be an effective means for improving functional capacity and body composition in older people (Dillon et al. 2009; Ferrando et al. 2010; Zhu et al. 2011; Tieland et al. 2012b; Bauer et al. 2015; Cramer et al. 2016; Ispoglou et al. 2016). Nevertheless, there is limited evidence of their beneficial impact on muscle mass and strength of predominantly healthy older people (Tieland et al. 2017). Similarly, studies in sarcopenic or clinical populations are not always associated with beneficial changes in muscle mass (Ferrando et al. 2010; Cramer et al. 2016). Compensatory caloric redistribution may explain

observed discrepancies since food protein sources and protein-based supplements have been reported to increase satiety and consequently reduce energy intake (Mollahosseini et al. 2017). In one of the largest and most comprehensive studies in malnourished men and women (Cramer et al. 2016), daily supplementation with nutritional supplements containing 660 kcal and 40 g of protein, resulted in an increase in habitual energy intake from 1600 kcal·day^{-1} to approximately 1800 kcal·day^{-1}. Despite the positive increase in both energy and protein intakes, this study provides evidence of a partial caloric redistribution and potential appetite suppression since the average total energy intake should have exceeded 2200 kcal·day^{-1} should participants had maintained their habitual baseline energy intake. One of the key objectives of our study therefore was to investigate the impact of different forms of protein-based oral nutritional supplements matched in EAA content on appetite in older women. As a means to enhance the ecological validity of the current study, we intentionally avoided to match the beverages for volume. Water pre-loads have been shown to suppress appetite in subsequent meals only when intakes are large (~500 ml or above), and when beverages are ingested in closer proximity to a meal (i.e. <30 min) (Gray et al. 2003; Walleghen et al. 2007; Davy et al. 2008; Corney et al. 2016). Therefore, the small volume difference (150 ml) in our study between the GEL (50 ml) and WP (200 ml) is unlikely to have had an additional impact on either appetite or energy intake. In addition, it has been previously demonstrated that increasing the energy density of preload beverages is more influential at increasing energy intakes at follow-up *ad libitum* meals when beverage volume remains constant (i.e. 450 ml) (Gray et al. 2003). Nevertheless, energy intake at the ALB in the GEL condition was not compromised, despite the higher energy density compared to WP. This further emphasises the significance of our findings. Future research is advised to investigate the impact of nutritional supplementation on habitual protein intake in older populations since according to the Protein

Leverage Hypothesis dietary protein is more tightly regulated (Simpson et al. 2005; Martinez-Cordero et al. 2012).

Using a validated breakfast meal (Deighton et al. 2016), and in line with previous research (Ispoglou et al. 2017), our findings confirm that the GEL facilitated an increase in both protein and energy intakes, and did not suppress appetite compared to a powder-based WP supplement. Potential explanations for the suppression of appetite, as indicated by CAS and hormonal data, are longer digestion rates (Tremblay et al. 2015) and higher amino acid content (Moran et al. 2011) in the WP condition. Significant increases in PYY concentration, likely due to higher amino acid content in the WP, corroborate that the consequent reductions in energy intake are due to a satiating effect of whey protein (Mollahosseini et al. 2017) or dietary protein (Leidy et al. 2015; Phillips et al. 2016). Based on our findings, we suggest that the post-prandial increase in PYY may be the main reason for a suppression in subjectively reported appetite, which subsequently negatively affected energy intake at the ALB. Baseline PYY values are in alignment with previous literature (Hickson et al. 2016), demonstrating that post-prandial PYY levels are greater in older women compared to younger in response to a standard meal containing 2781 kJ and 27.5 g of protein. In our study, the highest post-prandial PYY values in response to the whey protein supplement were slightly lower than those for the latter study, however this was anticipated since the protein and energy content in our WP condition were lower than the corresponding values for Hickson et al. (2016). Insulin also acts as an appetite suppressant, whilst fasting values tend to be higher in older people (Fraze et al. 1987; Parker et al. 2004). Indeed, fasting insulin values in our study were typical of those expected for older people. As expected, insulin concentration increased following ingestion of both supplements, primarily in response to the carbohydrate and leucine content (Fraze et al. 1987; Greiwe et al. 2001; Miller et al. 2003; Leenders et al. 2011; Chowdhury et al.

2015) in WP and GEL. Nevertheless, no significant differences were observed between these two conditions. Therefore, it is unlikely that insulin played a role in appetite suppression under the current circumstances. We acknowledge that varying the time between ingestion of the supplements and consumption of a subsequent meal may result in different outcomes. However, we have previously demonstrated that the current gel results in similar responses in appetite and energy intakes regardless of whether it is taken alongside or an hour before an *ad libitum* breakfast (Ispoglou et al. 2017).

In conclusion, the current GEL formulation brought synergistic benefits to both energy and protein intakes in older women and therefore may have advantages over a WP isolate when taken before a breakfast meal. Thus, such a supplement formulation may comprise an effective dietary strategy to address undernutrition and malnutrition in older women. Further research is required to confirm the generalisability and reproducibility of our findings in older clinical and non-clinical populations. There is a specific need to investigate whether acute or long-term daily nutritional intakes of free-living older people are affected when EAA-based nutritional supplements are taken alongside other main meals of varied composition.

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Tables

Variable	*n* = 10
Age (years)	69.2 ± 2.7
Height (cm)	163.1 ± 3
Body mass (kg)	60.8 ± 7.1
BMI ($kg \cdot m^2$)	22.8 ± 2.4
Lean mass (kg)	37.7 ± 2.3
Fat mass (kg)	20.4 ± 5.7
Bone mineral content (kg)	2.2 ± 0.3
Percentage body fat (%)	33.1 ± 5.8
Systolic blood pressure (mmHg)	124 ± 7.0
Diastolic blood pressure (mmHg)	80.7 ± 4.3
Total cholesterol (TC) ($mmol \cdot L^{-1}$)	5.42 ± 0.9
High density lipoprotein (HDL)($mmol \cdot L^{-1}$)	1.8 ± 0.4
Low density lipoprotein ($mmol \cdot L^{-1}$)	3.4 ± 1.1
Triglycerides ($mmol \cdot L^{-1}$)	1.3 ± 0.5
Ratio of TC/HDL	3.6 ± 0.9
Fasting blood glucose ($mmol \cdot L^{-1}$)	5.2 ± 0.9

Table 1: Descriptive characteristics for the study population. Data presented as mean ± SD.

Table 2: Essential amino acid (EAA) profile in gel (GEL) and whey protein (WP) supplements. Adapted from Ispoglou et al. (2016) and Hulmi et al (2010). The GEL and WP were matched for total EAA content (~7.5 g each).

Amino Acids	**GEL**	**WP**	**GEL**	**WP**
	(g/100g)		**(g/finished product)**	
Leucine	40.0	12.2	3.0	1.9
Isoleucine	11.0	6.1	0.8	0.9
Valine	12.0	5.9	0.9	0.9
Lysine	12.0	10.2	0.9	1.6
Histidine	5.0	*Tr*	0.4	*Tr*
Methionine	2.0	3.3	0.2	0.5
Phenylalanine	7.0	3.0	0.5	0.5
Threonine	11.0	5.5	0.8	0.8
Tryptophan	0	1.8	0.0	0.3

Table 3: Macronutrient content (in g) from *ad libitum* breakfast (ALB), and from ALB combined with supplement (SUPPL) for each condition.

Dietary Variable	CON	WP	GEL	WP	GEL	*CON versus WP*	*CON versus GEL*	*GEL versus WP*	*CON versus WP*	*CON versus GEL*	*GEL versus WP*
	ALB *g*			**ALB + SUPPL** *g*		*ALB* *ES (95% CI)*			*ALB + SUPPL* *ES (95% CI)*		
CHO	68.7 (25.1)	49.7 (21.9) *§	68.9 (21.5) §	50.9 (21.9) *§	90.9 (21.5) *§	**0.8** (-41.1, 3.1)	**0.0** (-21.8, 22.1)	**0.9** (-1.1, 39.6)	**0.8** (-39.1, 4.3)	**0.8** (-3.5, 43.5)	**1.6** (15.8, 59.8)
PR	21.0 (7.6)	15.2 (6.7) *§	21.0 (6.6) §	30.4 (6.7) *	28.5 (6.6) *	**0.8** (-12.6, 0.8)	**0.0** (-6.7, 6.7)	**0.9** (-0.3, 12.1)	**1.3** (2.6, 16.0)	**1.1** (0.8, 14.2)	**0.3** (-8.0, 4.5)
FAT	11.9 (4.3)	8.6 (3.8) *§	11.9 (3.7) §	10.2 (3.8) *§	11.9 (3.7) *§	**0.8** (-7.1, 0.5)	**0.0** (-3.7, 3.8)	**0.9** (-0.2, 6.8)	**0.4** (-5.5, 2.1)	**0.0** (-3.7, 3.7)	**0.5** (-1.8, 5.2)

Control (**CON**), whey protein (**WP**), essential amino acid based gel (**GEL**), carbohydrate (**CHO**), protein (**PRO**), and fat (**FAT**).
95% **CI**: 95% confidence interval of the mean difference between conditions.
ES: Cohen's *d* effect sizes. The effect sizes were calculated and interpreted using the following thresholds: 0 – 0.2 (trivial), 0.2 – 0.5 (small), 0.5 – 0.8 (moderate), 0.8 – 1.2 (large), > 1.2 (very large).
Macronutrient content in each supplement: **GEL** (CHO = 22.0 g; PRO = 7.5 g (all essential amino acids); FAT = 0 g) and WP (CHO = 1.3 g; PRO = 15.2 g; FAT = 1.6 g)
An asterisk (*) denotes significantly different from **CON** ($P < 0.05$).
A section sign (§) significantly different between **WP** and **GEL** ($P < 0.05$).
Data presented as mean ± SD.

Figures

Figure 1: Schematic representation of the design of the study. Whey protein (WP), essential amino acid gel (GEL), control (CON), *ad libitum* breakfast (ALB). Black arrows = appetite rating assessment; syringe picture = blood samples.

Figure 2: Energy intake including energy from supplements and the breakfast. Whey protein (WP), essential amino acid gel (GEL), control (CON), *ad libitum* breakfast (ALB), shaded area represents energy from supplements (SUPPL). Data are displayed as individual responses (a) and mean (SEM) (b), *n=10*.

Figure 3: Composite appetite ratings for CON (▼), WP (●) and GEL (○).Values are mean (SEM), *n=10*.

Figure 4: PYY (a) and insulin (b) concentrations over the 60-minute period CON (▼), WP (●) and GEL (○). Values are mean (SEM), *n=10*.