

1 **Appetite, food intake, and gut hormone responses to glycomacropeptide protein**
2 **ingestion in older adults: A feasibility, acceptability, and pilot study**

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29 **ABSTRACT**

30 Glycomacropeptide (GMP) has a unique amino acid profile which may make less satiating than other
31 dietary proteins. This study assessed the feasibility and likely acceptability of a leucine-enriched GMP
32 drink and determined appetite response in older adults (OA). Thirteen OA (11f; 70±4 years) were
33 recruited for sensory assessments of a leucine-enriched GMP drink when mixed with water and with
34 fruit smoothie, compared with whey protein isolate (WHEY). Participants also partook in a single focus
35 group exploring acceptability to protein and supplementation. Separately, a counterbalanced, double-
36 blind study with twelve OA (8f; 69±3 years) was conducted to determine appetite and gut hormone
37 responses. Fasting subjective appetite was recorded using visual analogue scales and a fasted venous
38 blood sample was collected (to measures acyl-ghrelin, PYY, GLP-1, and CCK) before participants
39 consumed either: GMP protein (27g + 3g leucine, 350mL water), WHEY (30g, 350mL water), or water.
40 Participants rested for 240minutes, with appetite measures and blood sampling throughout. An *ad*
41 *libitum* pasta-based meal was then consumed. Sensory testing revealed low pleasantness rating for GMP
42 in water vs. WHEY (16±14 vs 31±24, $p=0.016$). GMP addition to smoothie reduced pleasantness
43 (26±21 vs. 61±29, $p=0.009$) and worsened the aroma (46±15 vs. 69±28, $p=0.014$). The focus group
44 revealed uncertainty of protein needs and a scepticism of supplements, with preference for food. Gut
45 hormone response did not differ between GMP and WHEY (nAUC for all gut hormones $p>0.05$). There
46 was no difference between conditions for lunch *ad libitum* intake (549±171 kcal, 512±238 kcal,
47 460±199 kcal for GMP, WHEY, and water, $p=0.175$), or for subjective appetite response. Leucine-
48 enriched GMP was not less satiating than WHEY, and low palatability and scepticism of supplements
49 question the likely acceptability of GMP supplementation. Providing trusted nutritional advice and food
50 enrichment/fortification may be preferred strategies for increasing protein intake in OA.

51

52 **Key words:**

53 Hunger; satiety; energy intake; anorexia of ageing; undernutrition; ageing.

54

55 **Abbreviations:**

56 AEBSF – 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride

57 ANOVA – Analysis of variance

58 BMI – Body mass index

59 nAUC – Net area under the curve

60 CCK - Cholecystokinin

- 61 EDTA – Ethylenediaminetetraacetic acid
- 62 EIA – Enzyme immunoassay
- 63 ELISA – Enzyme-linked immunosorbent assay
- 64 GLP-1 – Glucagon-like peptide 1
- 65 GMP - Glycomacropeptide
- 66 IPAQ-SF – International Physical Activity Questionnaire Short Form
- 67 METs – Metabolic equivalents
- 68 OA – Older adults
- 69 PYY – Peptide tyrosine-tyrosine
- 70 SNAQ – Simplified Nutritional Appetite Questionnaire
- 71 TER – Total energy requirements
- 72 VAS – Visual analogue scale
- 73 WHEY – whey protein isolate
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88 INTRODUCTION

89 Malnutrition in later life is associated with increased morbidity and mortality (Söderström et al., 2017)
90 and a decline in physical capacity and quality of life (Rasheed & Wood, 2013). Such consequences lead
91 to increased healthcare utilisation, with annual health and social care costs estimated to be 3 times
92 greater for undernourished older adults, compared with those adequately nourished (Russell et al.,
93 2014). The prevalence of severe malnutrition is reported to be up to 55% in hospitalised older adults
94 (Norman et al., 2008) and up to 45% in those in residential care (Ray et al., 2014), while over one third
95 of community-dwelling older adults suffer from milder malnutrition, nutrition risk and nutritional
96 deficiencies (van den Broeke et al., 2018). With an ageing global population, malnutrition in later life
97 is an imposing challenge for current and future healthcare systems (Stratton et al., 2018).

98 Insufficient nutritional intake is underpinned by an age-related decline in appetite, termed “anorexia of
99 ageing” (Dent et al., 2019). Impaired appetite in older adults leads, particularly, to low intakes of protein
100 and overall lower energy intake (Lonnie et al., 2018). Compared with younger adults, older adults
101 typically consume 30% less energy per day (Soenen et al., 2013), while over 20% of older adults fail to
102 meet the modest dietary recommendation of $0.8\text{g}\cdot\text{kg}^{-1}$ of protein a day (Hengeveld et al., 2020). Given
103 that low energy and low protein intakes are risk factors for sarcopenia (Cho et al., 2020; Houston et al.,
104 2008), frailty (van den Broeke et al., 2018) and falls risk (Westergren et al., 2014), maintaining drive
105 to eat and prevent early decreases in food intake with ageing is vital for maintaining muscle mass,
106 strength, physical function, and health.

107 A high-protein intake of $1\text{-}1.2\text{ g}\cdot\text{kg}^{-1}$ is recommended for older adults for the maintenance of muscle
108 mass and strength (Bauer et al., 2013; Volpi et al., 2013). However, protein is highly satiating. Protein
109 induces fullness and suppresses hunger to a greater extent than isocaloric intakes of fat and carbohydrate
110 (Brennan et al., 2012; Westerterp-Plantenga et al., 1999), and high protein diets reduce *ad libitum* food
111 intake and promote weight-loss (Weigle et al., 2005). Acute protein supplementation in older adults
112 increases satiety and reduces food intake at later meals, compared with control conditions of water or
113 nothing (Ben-Harchach et al., 2021), while long-term supplementation strategies can effectively
114 increase protein intake, but energy intake is either negatively affected or unaffected by supplementation
115 (Ben-Harchach et al., 2021). This poses the challenge of trying to optimise amino acid delivery for the
116 promotion of muscle protein synthesis, while at the same time avoiding excessive satiety that impacts
117 negatively upon subsequent food intake.

118 A solution to this challenge could be the milk-derived protein glycomacropeptide (GMP). GMP’s
119 unique amino acid composition could prove well-suited to the needs of older adults. GMP has high total
120 essential amino acid content but is free from or low in amino acids shown to have appetite-suppressive
121 effects. These include cysteine, which has been shown to suppress the hunger hormone ghrelin
122 (McGavigan et al., 2015); tryptophan, which has an appetite-suppressing effect via reductions in the rate

123 of gastric emptying and stimulation of the satiety peptide cholecystokinin (CCK) (Hajishafiee et al.,
124 2021); and phenylalanine, which is a stimulator of CCK and peptide tyrosine-tyrosine (PYY)
125 (Fitzgerald et al., 2020). The appetite response to GMP protein remains unclear. It was thought that the
126 high satiating effect of whey protein was a primarily driven by the GMP fraction (Luhovyy et al., 2007;
127 Royle et al., 2008), which constitutes approximately 20% of total whey protein. However, this has not
128 been substantiated (Burton-Freeman 2009; Chungchunlam et al., 2014) and evidence for a satiating
129 effect of GMP is limited (Daly et al., 2020; Veldhorst et al., 2009; Hoefle et al., 2019). Indeed, high-
130 GMP whey protein has been shown to be less satiating than GMP-free whey protein (Lam et al., 2009).
131 However, GMP is low in leucine – the amino acid able to directly stimulate muscle protein synthesis.
132 A leucine-enriched GMP product could achieve a desirable amino acid profile for stimulating muscle
133 protein synthesis, while avoiding a high degree of satiety and so maintaining drive to eat.

134 Therefore, the aim of this study was two-fold: a) to assess the feasibility, acceptability, and palatability
135 of a leucine-enriched GMP protein supplement to inform potential feeding or supplementation strategies
136 for older adults; b) to determine the acute appetite response to a leucine-enriched, GMP protein
137 supplement in older adults. It was hypothesised that a leucine-enriched GMP protein supplement would
138 prove feasible to produce and would match a whey protein supplement for acceptability and palatability.
139 Further, we hypothesised that the leucine-enriched GMP supplement would prove less satiating than
140 the whey protein supplements, inducing a less anorexigenic gut hormone response.

141

142 **METHOD**

143 To achieve our aim, we conducted a two-part study, adopting a convergent mixed-methods
144 approach (Creswell & Clark, 2017). Part 1 assessed feasibility and palatability of a leucine-enriched
145 GMP protein drink through taste testing, and assessed likely acceptability through conducting a
146 focus group to explore attitudes towards and experiences of protein intake and supplementation.
147 Part 2 assessed appetite and gut hormone responses to the leucine-enriched GMP protein drink.

148 **Part 1**

149 ***Drink Development***

150 A vanilla-flavoured, leucine-enriched GMP protein powder was produced (Milk Specialties Global,
151 MN, USA), containing 27g GMP and 3g leucine. The powder proved suitably soluble in water,
152 fruit juices, and milk (as potential methods of ingestion). The powder remained in solution at
153 temperate (room temperature, ~20°C and cold temperatures (~4°C). A comparator whey protein
154 isolate (30g per dose) powder was also provided (Milk Specialties Global, MN, USA).

155 ***Study Design and Participants***

156 To assess palatability and likely acceptability, thirteen older adults were recruited to attend taste
157 testing and a focus group session at the Human Nutrition Suite at Newcastle University (11 female
158 and 2 male, age: 70±4 years, Simplified Nutritional Appetite Questionnaire (SNAQ) score:
159 15.4±2.8, 4/13 classified as low appetite (score < 15, Lau et al., 2020)). Participants were recruited
160 through poster distribution at institutions in the Newcastle area (e.g., libraries and community
161 centres), and via the public engagement platform VOICE Global. Participants were aged 65 years
162 or older, were free from cardiometabolic disease, and self-reported healthy senses of taste and
163 smell. Participants were reimbursed for travel expenses and offered a £20 gift voucher to thank
164 them for their participation. They also received lunch after the session.

165 *Palatability of protein supplements*

166 The sensory properties of drinks made with either GMP powder (GMP) or whey protein powder
167 (WHEY) were compared in four taste tests. Tests 1 and 2 assessed analytic and hedonic ratings of
168 GMP and WHEY mixed with water. Participants were given a 30mL sample of GMP mixed with
169 water and a 30mL sample of WHEY mixed with water in a randomised order. Drinks were prepared
170 in stock, according to manufacturer instructions (30g protein mixed with 350mL of water). Tests 3
171 and 4 assessed analytic and hedonic ratings of GMP and WHEY mixed with fruit smoothie. In a
172 randomised order, participants received three 30mL samples: a commercial smoothie (Tesco
173 ‘Pineapple Paradise’ smoothie, consisting of pineapple juice (31%), apple juice, banana puree
174 (20%), apple puree, creamed coconut (5%) and lemon juice); GMP mixed with smoothie; and
175 WHEY mixed with smoothie. The preparation of drinks was as with water (30g protein to 350mL
176 of liquid). Participants were blinded to the contents of drinks throughout.

177 The participants were asked to rate each sample for 5 properties in each test. Properties were
178 identified before the trial by three researchers (KB, AH, RS), informed by previous studies
179 investigating taste and sensory properties of food and drink products with older adults (Norton et
180 al., 2020; Tsikritzi et al., 2015) and adapted for a simplified taste and sensory profile for participants
181 untrained in taste and sensory testing. Tests 1 and 3 addressed the analytical properties, while tests
182 2 and 4 asked about hedonic properties, in a design intended to optimise the power of the final
183 (preference) question, by supporting the untrained panellists to communicate what they like and
184 dislike about each product’. The properties and questions are detailed in **Table 1**. The tests were
185 conducted with Qualtrics™XM software, using the ‘Slider’ option to create unmarked visual
186 analogue scales (VAS), approximately 12 cm long on the screen of a tablet computer. The responses
187 were recorded as the position on the scale, with the left end of the line as 0 and the right end as 100,
188 and the cursor initially placed at the midpoint (value 50).

189

190 **Table 1.** Taste test questions.

| Property | Left limit (0) | Right limit (100) |
|--|--|---|
| Analytical (how much or little), first and third test | | |
| Texture: Take a small sip of each product and give your impression of their texture/mouthfeel | <i>Creamy and smooth</i> | <i>Grainy or lumpy</i> |
| Sweetness: Give your impression of how sweet the taste is, take another sip if needed | <i>Not sweet at all</i> | <i>Extremely sweet</i> |
| Aroma/smell: Give your impression of how strong the aroma and smell are | <i>Completely bland, no smell</i> | <i>Extremely strong aroma/smell</i> |
| Savoury/cheesy taste: Assess if there is a savoury or cheesy element to the taste | <i>Not savoury at all</i> | <i>Distinctly savoury</i> |
| Bitterness and off-taste: Were there any flavours that should be avoided in a product like this? | <i>Clean taste, no off-flavours</i> | <i>Very bitter, metallic taste or other strong off-flavours</i> |
| Hedonic (how pleasant or unpleasant), second and fourth test | | |
| Texture: Take a small sip of each product and give your impression of their texture/mouthfeel | <i>Feels pleasant and smooth on tongue</i> | <i>Unpleasant texture: sticky, sandy or slimy</i> |
| Sweetness: Give your impression of how sweet the taste is, take another sip if needed | <i>Not sweet at all</i> | <i>Much too sweet</i> |
| Aroma/smell: Give your impression of how pleasant the aroma and smell are | <i>Disgusting smell and aroma</i> | <i>Extremely pleasant aroma/smell</i> |
| Savoury/cheesy taste: Assess if there is a savoury or cheesy element to the taste | <i>Too bland</i> | <i>Too 'cheesy'</i> |
| Pleasantness of product: Give your impression of your preference for each of the products | <i>I'll never have it again!</i> | <i>I'd want more of this every day!</i> |

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192

193 ***Assessing acceptability of protein supplementation***

194 One in-person focus group was carried out with all thirteen participants immediately after the taste
 195 testing procedure. A topic guide was developed through discussions within the research team. The
 196 guide was then aligned to the Theoretical Domains Framework (TDF). The TDF serves as a

197 framework in behavioural science, offering a theoretical perspective for examining the influences
198 of cognitive, affective, social, and environmental factors on behaviour (Atkins et al., 2017).

199 The focus group was facilitated by researcher RS and three other members of the research team. It
200 was audio recorded, and lasted 45 minutes. It was determined that data reached saturation (Saunders
201 et al., 2018) at around 40 minutes. Inductive thematic saturation was also identified during analysis,
202 indicating that no further concepts or themes were being identified through analytical engagement
203 with the data. Qualitative feedback was obtained from participants after the focus group, with
204 positive responses indicating that all voices were heard during the session. The audio file was
205 transcribed verbatim by an approved transcription service, anonymised, then imported into NVivo
206 (version 12) software to aid data management and retrieval. Data analysis followed the principles
207 of thematic analysis (Braun et al., 2006), providing an interpretive exploration of participant
208 attitudes and beliefs about protein supplementation. The transcript was reviewed and coded by a
209 single researcher (RS), granting them a comprehensive insight into the data. Another author
210 independently coded a section of the transcript. The team collaboratively discussed the developing
211 codes and themes. This established coding framework was then iteratively applied to the analysis
212 of the full transcript.

213

214 **Part 2**

215 ***Study Design***

216 In a double-blind, within-subject design, participants completed three experimental conditions in a
217 counter-balance order: glycomacropeptide drink (GMP), whey protein drink (WHEY), and a water
218 placebo drink (WATER). Outcome measures were subjective appetite, lunch *ad libitum* intake, and
219 circulating concentrations of acyl-ghrelin, PYY, glucagon-like peptide 1 (GLP-1) and CCK. The
220 study adhered to the ethical guidelines as outlined in the Declaration of Helsinki and gained ethical
221 approval from the Newcastle University Faculty of Medical Sciences Research Ethics Committee
222 (LREC #: 2525/30107).

223

224 ***Participants***

225 Fourteen, low-to-moderately active, older adults (OA) living without obesity were recruited
226 through poster distribution at institutions in the Newcastle area (e.g., libraries and community
227 centres), advertisement through social media sites (e.g., Facebook interest groups), and via the
228 public engagement platform VOICE Global. Inclusion criteria were an age of 65 years or older, a
229 score of < 3000 metabolic equivalent of task (MET) mins · week⁻¹ on the International Physical
230 Activity Questionnaire Short Form (IPAQ-SF; Craig et al., 2003), body mass index (BMI) of < 33

231 kg·m⁻² (Winter et al., 2014), non-smoker, not attempting to change bodyweight or composition, not
232 taking medication likely to impact on appetite, and free from metabolic disease and any known
233 food allergy. As laboratory *ad libitum* test meals can promote over-consumption (Blundell et al.,
234 2010), those who had an average consumption of > 40% of estimated total energy requirements
235 (TER; estimated using the Mifflin-St Joer equation (Mifflin et al., 1990)) across trials were
236 excluded as over-eaters. This value of >40% TER represents a substantially greater intake than the
237 typical lunch energy intake of ~27% of TER in UK mid-life adults (Pot et al., 2014).

238

239 ***Enrolment and Familiarisation***

240 Participants visited the Human Nutrition Suite, Newcastle University for enrolment and
241 familiarisation. The study was verbally explained, and the participants were afforded the
242 opportunity to ask any questions prior to informed written consent being obtained. Height and
243 weight were recorded, and body composition was assessed by bioelectrical impedance (SECA
244 mBCA 515 Body Analyser). Habitual physical activity was recorded, habitual appetite was assessed
245 using the Simplified Nutritional Appetite Questionnaire (SNAQ, Wilson et al., 2005), and
246 participants were screened for any known food allergies. Participants were then familiarised with
247 the test meals to be consumed on the experimental visit. Samples of the breakfast drinks and lunch
248 meal were provided to confirm palatability. All participants confirmed that the pasta-based lunch
249 meal was sufficiently palatable to eat until full.

250

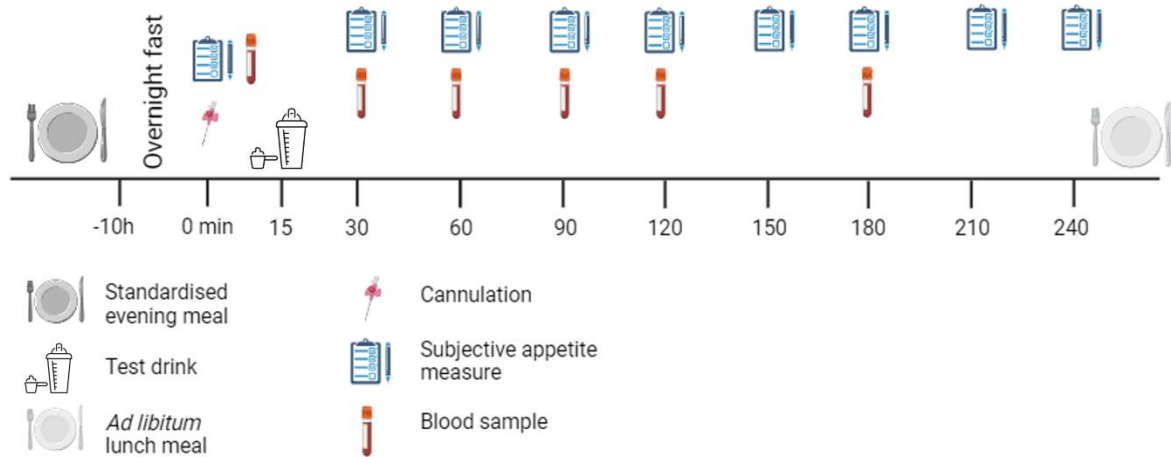
251 ***Procedures***

252 Participants arrived at the Human Nutrition Suite in a fasted state, between 08:00 and 09:00.
253 Participants had consumed a standardised, nutrient-balanced evening meal of beef hash, yoghurt
254 and orange juice (691 kcal; 47% energy from carbohydrate, 29% fat, 23% protein) a minimum of
255 12 hours prior to arrival and had drunk 300mL of water upon waking. They had also been instructed
256 to abstain from exercise, caffeine and alcohol on the day before each experimental visit. Adherence
257 to dietary and exercise controls was confirmed prior to commencing the experimental trial.

258 Subjective appetite was then assessed using the VAS method (Flint et al., 2000). A cannula was
259 inserted into the antecubital vein of one arm and a fasted, rested blood sample was obtained ten
260 minutes after insertion. Participants then consumed one of the three test breakfast drinks within
261 five minutes.

262 Upon completion of breakfast drink and 30 minutes after the cannulation, subjective appetite was
263 measured, and a second blood sample was obtained. Participants then rested for a further 210
264 minutes, with appetite measured every 30 minutes and blood samples obtained at 60, 90, 120, 180

265 and 240 minutes (see **Figure 1**). Participants remained seated and were free to read, watch
 266 television or use a laptop computer. Activity was monitored to minimise exposure to food cues in
 267 reading and viewing material. At 240 minutes, the cannula was removed and participants were
 268 provided with an *ad libitum* lunch meal. Upon completion of the meal, the trial was complete.



269

270 **Figure 1.** Schematic of study protocol

271

272 ***Test Breakfast Drinks***

273 The WHEY test drink consisted of 30g of vanilla-flavoured whey protein isolate. The GMP test
 274 drink consisted of 27g of vanilla-flavoured GMP protein, fortified with 3g of leucine. Each was
 275 made with 350mL of water and additionally flavoured with strawberry flavour drops (10 drops).
 276 The water test drink consisted of 350mL of water with 10 strawberry flavour drops and four vanilla
 277 flavour drops. Each drink was made with temperate temperature water and mixed thoroughly,
 278 before being chilled in a fridge at 4°C for 15 minutes.

279

280 ***Outcome Measures***

281 ***Ad libitum food intake***

282 Food intake was assessed using homogeneous pasta-based *ad libitum* test meal (Astbury et al.,
 283 2011; Gonzalez et al., 2013). The nutrient-balanced meal consisted of pasta (Napolina penne pasta),
 284 Bolognese sauce (Sainsbury’s Tomato & Herb), grated cheddar cheese (Sainsbury’s Grated Mature
 285 British Cheddar), and olive oil (Sainsbury’s) (energy density = 1.79 kcal·g⁻¹. 50% energy from
 286 carbohydrate, 15% protein, 35% fat). Participants were instructed to eat until they felt “satisfyingly
 287 full.” To avoid an empty bowl providing a cue to stop eating prior to satiation, the “bottomless
 288 bowl” method was adopted whereby each bowl of pasta was replaced with a fresh, full bowl before

289 the participant cleared the bowl (Deighton et al., 2016). Food was consumed in isolation, free from
290 distractions (such as a mobile phone) and food cues, and with no time limit. Each bowl was weighed
291 before and after serving, with the difference in weight indicating the mass consumed. As the energy
292 density of the meal was known, caloric content was calculated from the total mass consumed by
293 the participant.

294 *Subjective appetite*

295 Measures of subjective appetite perceptions were obtained using the 4-item VAS method, assessing
296 hunger, fullness, desire to eat and expected consumption (Flint et al., 2000). Each item was
297 answered with participants placing a vertical mark on an ungraded, 100mm horizontal line anchored
298 on both ends with extreme answers to the question posed (“How hungry are you?”, “How full are
299 you?”, “How strong is your desire to eat?” and “How much would you expect to eat right now?”).
300 A score was obtained by measuring the distance from the left-hand side anchor to the mark. A
301 single composite score was calculated from the four items as: (hunger score + (100-fullnessscore)
302 + desire to eat score + expected intake score) / 4 (Holliday & Blannin, 2017). As well as monitoring
303 appetite profile over the 240-minute experimental period, the time period from consuming the
304 breakfast drink to the point at which appetite rating returned to the baseline value was recorded. If
305 appetite rating remained lower than baseline value at 240 minutes, the time to returning to baseline
306 was estimated by extrapolating the data from the final three appetite measures.

307 *Plasma concentration of acyl-ghrelin, total PYY, GLP-1, and CCK*

308 Blood was collected in EDTA-treated blood collection tubes for the measure of total PYY, GLP-1
309 and CCK. For acyl-ghrelin, blood was collected in EDTA tubes pre-treated with AEBSF protease
310 inhibitor as a concentration of $1\text{g}\cdot\text{mL}^{-1}$ of whole blood (Deschaine & Leggio, 2020). Whole blood
311 was centrifuged at 2000g and 4°C for 15 minutes to separate plasma. Plasma was aliquoted into
312 0.5mL sample cups and stored at -80°C for later analyses. Plasma aliquots for the measure of acyl-
313 ghrelin were treated with 0.2mL of 1M hydrochloric acid.

314 Acyl-ghrelin, PYY, GLP-1, and CCK concentrations in plasma were measured by enzyme-link
315 immunosorbent assay (ELISA) using commercially available kits (Human Ghrelin (active) ELISA
316 kit, Merck Millipore; Human PYY (total) ELISA kit, Merck Millipore; Multispecies GLP-1 Total
317 ELISA kit, Merck Millipore; Cholecystokinin EIA kit, Merck). Sensitivity of acyl-ghrelin, PYY,
318 GLP-1, and CCK kits were $8\text{pg}\cdot\text{mL}^{-1}$, $1.4\text{pg}\cdot\text{mL}^{-1}$, $1.5\text{ng}\cdot\text{mL}^{-1}$, and $0.2\text{pg}\cdot\text{mL}^{-1}$ respectively.
319 Coefficients of variation were 4.45%, 4.18%, 4.82% and 9.7%.

320 The decision was made to measure total PYY, rather than the more bioactive form, PYY3-36
321 (Chelikani et al., 2005; Sloth et al., 2007). PYY3-36 accounts for ~65% of total PYY and total PYY
322 and PYY3-36 exhibit comparable responses to feeding (Batterham et al., 2006). As such, it is
323 believed that changes observed in total PYY are representative of changes in PYY3-36, while also

324 accounting for the less anorexigenic but still active PYY1-36 form (Chelikani et al., 2005; Sloth et
325 al., 2007).

326

327 *Statistical Analyses*

328 All values are presented as mean \pm SD (mean \pm SEM in figures). Differences in sensory test scores
329 of the taste test were determined using paired t-test when comparing GMP and whey in water and
330 using a repeated-measures analysis of variance (ANOVA) when comparing GMP, whey and pure
331 smoothie. To assess acyl-ghrelin, PYY, GLP-1, and CCK responses to feeding, change-from-
332 baseline concentration was calculated. Differences between conditions in gut hormone responses
333 and in subjective appetite were assessed using a factorial (condition x time) repeated measures
334 ANOVA. Net area-under-the-curve (nAUC) was calculated for each of these variables using the
335 trapezium method. Differences in nAUC values were assessed using a one-way repeated measures
336 ANOVA. Differences in lunch energy intake were also determined using a one-way repeated
337 measures ANOVA. Throughout, significant interactions and main effects were explored further
338 using Bonferroni-corrected pairwise comparisons. Partial η^2 (η_p^2) effect sizes were calculated for
339 main effects and interactions, while Cohen's *d* (*d*) effect sizes were calculated for pairwise
340 comparisons. Outliers were identified from fasted appetite scores and hormone concentrations as
341 >1.5 x interquartile range above the third quartile or below the first quartile. Statistical significance
342 was determined at an alpha level of 0.05. All statistical analyses were conducted using Statistical
343 Package for Social Sciences (SPSS, Version 29.0.1.0).

344 An *a priori* power calculation was conducted to determine the sample size required to provide
345 adequate statistical power to detect a large effect for gut hormones based on previous studies
346 determining meaningful gut hormone changes with feeding in older adults (Giezenaar et al., 2018;
347 Giezenaar et al., 2017). With statistical power of 0.8 and an alpha value of 0.05, a sample of at least
348 12 participants per group was required to detect a large difference ($d = 1.2$).

349

350 **RESULTS**

351 **Part 1**

352 *Taste Testing*

353 Analytic ratings of texture, sweetness, aroma, savoury, and bitterness, and hedonic ratings of texture,
354 sweetness, aroma, savoury, and pleasantness for test drinks are shown in **Table 2** and **Table 3**. When
355 GMP and WHEY were added to water (Table 1), pleasantness rating was significantly lower for GMP,
356 compared with WHEY ($p = 0.016$. $d = 0.763$). There were no differences in any analytical ratings, or

357 hedonic ratings of texture, sweetness of savoury taste (all $p > 0.1$), while there was a trend for a
 358 difference in rating of aroma, ($p = 0.077$).

359 When GMP and WHEY were added to a fruit smoothie (Table 2), there was a condition main effect for
 360 pleasantness ($p = 0.004$, $\eta_p^2 = 0.522$). Post hoc pairwise comparisons showed that GMP score
 361 significantly lower than pure smoothie ($p = 0.009$, $d = 1.382$). There was no difference in pleasantness
 362 score between GMP and WHEY ($p = 0.102$). There was also a condition main effect for hedonic ratings
 363 of sweetness ($p = 0.014$, $\eta_p^2 = 0.300$) and aroma ($p < 0.001$, $\eta_p^2 = 0.447$). Pairwise comparisons showed
 364 a trend for a higher hedonic sweetness score for WHEY compared with pure smoothie, indicating lower
 365 preference and a perception of it being “too sweet”. Both GMP and WHEY scored lower than pure
 366 smoothie for hedonic rating of aroma ($p = 0.012$, $d = 0.604$ and $p = 0.042$, $d = 0.332$, respectively).
 367 There was also a significant condition main effect for analytical rating of texture ($p = 0.040$, $\eta_p^2 =$
 368 0.236), with tends for higher ratings for both GMP ($p = 0.060$) and WHEY ($p = 0.092$) compared with
 369 smoothie, indicating a more grainy or lumpy texture. However, this did not affect hedonic rating of
 370 texture, where no condition main effect was observed.

371 Qualitative data showed that participants perceived the GMP, both in water and when added to
 372 smoothie, to have an “artificial” and “metallic” taste, and an “unpleasant” and “off” smell.

373

374 **Table 2.** Mean \pm SD analytical and hedonic ratings for GMP and WHEY added to water.

| <i>Analytic Assessment</i> | | | | | |
|----------------------------|----------------|------------------|--------------|----------------|---------------------|
| | Texture | Sweetness | Aroma | Savoury | Bitterness |
| GMP | 19 \pm 14 | 62 \pm 34 | 54 \pm 32 | 21 \pm 26 | 50 \pm 30 |
| WHEY | 26 \pm 21 | 60 \pm 32 | 64 \pm 30 | 20 \pm 31 | 47 \pm 33 |
| <i>p</i> | <i>0.241</i> | <i>0.681</i> | <i>0.302</i> | <i>0.853</i> | <i>0.562</i> |
| <i>Hedonic Assessment</i> | | | | | |
| | Texture | Sweetness | Aroma | Savoury | Pleasantness |
| GMP | 42 \pm 31 | 63 \pm 30 | 51 \pm 23 | 48 \pm 26 | 16 \pm 14 |
| WHEY | 33 \pm 31 | 68 \pm 23 | 61 \pm 19 | 42 \pm 23 | 31 \pm 24 |
| <i>p</i> | <i>0.229</i> | <i>0.309</i> | <i>0.077</i> | <i>0.306</i> | <i>0.016</i> |

375

376 **Table 3.** Mean \pm SD analytical and hedonic ratings for GMP and WHEY added to smoothie, and for
 377 pure smoothie.

| <i>Analytic Assessment</i> | | | | | |
|----------------------------|---------------------|------------------|--------------|----------------|-------------------|
| | Texture | Sweetness | Aroma | Savoury | Bitterness |
| GMP | 47 \pm 34 | 58 \pm 31 | 50 \pm 28 | 20 \pm 21 | 44 \pm 31 |
| WHEY | 39 \pm 36 | 65 \pm 29 | 58 \pm 19 | 33 \pm 32 | 46 \pm 26 |
| Pure smoothie | 21.8 \pm 20.3 | 52 \pm 25 | 64 \pm 17 | 20 \pm 21 | 40 \pm 38 |
| <i>p</i> | <i>0.040</i> | <i>0.159</i> | <i>0.091</i> | <i>0.161</i> | <i>0.788</i> |
| <i>Hedonic Assessment</i> | | | | | |

| | Texture | Sweetness | Aroma | Savoury | Pleasantness |
|----------------------|----------------|------------------|------------------|----------------|---------------------|
| GMP | 46±33 | 64±23 | 46±15 * | 43±19 | 26±21 * |
| WHEY | 35±25 | 67±22 | 53±19 * | 41±21 | 36±26 |
| Pure smoothie | 27±29 | 46±26 | 69±28 | 38±15 | 61±29 |
| p | <i>0.066</i> | 0.014 | <0.001 | <i>0.611</i> | 0.009 |

378 * = significantly different to pure smoothie.

379

380

381 ***Focus Group: Attitudes towards nutritional interventions and protein supplements***

382 Findings are presented across three themes:

- 383 (1) **distrust**, with sub-themes of (a) distrust of government and (b) distrust of official guidance;
- 384 (2) **confusion and unawareness**, with sub-themes of (a) uncertain of which information sources
- 385 are best and (b) unawareness of protein benefits;
- 386 (3) **‘natural is better’**, with sub-themes of (a) a traditional, whole-food diet is best and (b) protein
- 387 powders are unnatural.

388

389 *Theme 1. Distrust*

390 *Sub-theme 1.1. Distrust of government*

391 Many participants expressed their distrust of various official bodies related to diet and nutrition.

392 Scepticism about the health-related decisions that the Government make, and in turn, dietary guidance

393 produced by official bodies associated with the Government, was evident throughout the focus group

394 (See **Table 4** for supporting quotations).

395

396 *Sub-theme 1.2. Distrust of official guidance*

397 Some participants made a concerted effort to keep up to date with latest dietary advice, however advice

398 from trusted, non-governmental sources was preferred. Several highlighted websites and books from

399 individuals such as Dr Tim Spector, who they felt was more open and honest than official sources.

400 Participants were often sceptical of the claims made by food supplement (and other health related)

401 products aimed at older adults. They felt that this sector was monetized specifically to tap into, and

402 exploit, the health-related concerns of older adults. Food supplement products were seen as making

403 untrue promises of the benefits they could offer them.

404

405 *Theme 2. Confusion and unawareness*

406 *Sub-theme 2.1. Uncertain of which information sources are best.*

407 Participants expressed confusion over dietary guidance, often with exasperation. They felt that official
408 sources of dietary guidance moved the goalposts of what they were ‘meant’ to stick to so frequently,
409 that ultimately they were inclined to attend to other trusted sources instead. Protein intake guidance in
410 particular was felt to be confusing, with many unaware of current guidance, or were unsure of how to
411 personalise guidance to their own diet and living situations. The source of this confusion was ultimately
412 blamed on changing and unclear guidance, with participants feeling frustrated.

413 After being presented with research findings indicating that protein supplementation may help to stall
414 certain health issues in the future, many felt that there should be more awareness raised. Participants
415 wanted a personalised, straightforward approach which detailed what they could take and the benefits
416 of doing so.

417

418 *Sub-theme 2.2. Unawareness of protein benefits*

419 Some took supplements regularly, after reading various ‘official’ (NHS-related) and ‘unofficial’
420 sources. These primarily consisted of pre- and pro-biotics and products containing vitamins and fibre.
421 The decision to take these supplements were based on tackling current health problems, such as poor
422 sleep, rather than to help stave off any health issues in the future. Taking supplements, such as protein,
423 with a view to limit future health problems was not a particular consideration for any of the participants
424 in our sample.

425

426 *Theme 3. ‘Natural is better’*

427 *Sub-theme 3.1. A traditional, whole-food diet is ‘best’.*

428 There was a unanimous agreement amongst participants that whole food, natural sources of vitamins
429 and other food/dietary components should be preferred over anything else in the first instance. Some
430 felt that the advent of processed foods was partly to blame for the need to consider food
431 supplementation, and reflected on ‘naturally balanced’, yet sometimes restricted, diets from their
432 childhood. There was a feeling that if individuals ate sensibly and intuitively, that food supplementation
433 was unnecessary.

434 However, there was pushback from other members of the focus group who highlighted that
435 supplementation is necessary in certain cases, and that attaching value and blame to the ‘laziness’ of
436 society and their consumption of processed foods was an unhelpful stereotype. This also extended to
437 the concept of potentially using supplementation as a replacement for a naturally healthy lifestyle.

438

439 *Sub-theme 3.2. Protein powders are unnatural.*

440 The feeling that food supplementation is an unnatural and therefore undesirable method of increasing
441 protein intake was enhanced by the artificial tasting nature of many protein supplements on the market,
442 including some of the powders tested by this group in the session. The taste of the product was very
443 important to participants.

444 Ultimately, many would only take a protein powder to supplement their diet as a last resort, after
445 exploring all natural options.

446

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452 **Table 4.** Selected quotations from the focus group to support the identified themes and key themes.

| Theme and sub-theme | Selected Verbatim Quotations |
|--|--|
| Sub-theme 1.1 Distrust of government | <p><i>People in parliament who lobby against sugar content of drinks and all that stuff, and contribute to the Tories and whatever - and a big profession of parliamentary lobbyists who are there to do exactly what they're saying they're doing. [P3].</i></p> <p><i>But the government is different from large health organisations such as the WHO and maybe they're more trustworthy...[P4].</i></p> <p><i>Well, WHO is dependent on government... [P3].</i></p> |
| Sub-theme 1.2 Distrust of official guidance | <p><i>I've read Tim Spector's book about food mix, and he's a researcher at Kings College in London. He said that a lot of food research is actually funded by food companies, and if they don't like the results it would be hard to get it published. [P1]</i></p> <p><i>What you read on the packets everything tells you you're going to be a super woman or a superman having this product - but how long do you take it for? Does it really help you? Does it get into the psyche, and you think 'I'm taking this and I do feel better'? I think that's the hardest thing and there's a lot of monetising that's happening for the older generation trying to give us a quality life – a healthy life and that's what concerns me. [P7].</i></p> <p><i>The cost [of protein supplements]! I'm not convinced it's a necessary thing. I think perhaps it's just a method of making money. [P4].</i></p> <p><i>I think it's an excuse to charge more because if you eat sensibly with all of the vegetables it's already included. [P10].</i></p> |

| | |
|---|--|
| <p>Sub-theme 2.1. Uncertain of which information sources are best.</p> | <p>ny sources of information [about diet] that sometimes you just think, 'Go away!' [P3].</p> <p><i>I think loads of people are aware of how you should eat good fats and some protein. And I'm like that. So, I'll make sure I'll get some - and I've got absolutely no idea how much I take in. You know when you're talking about kilograms per body weight [to dose protein]? I really don't know! And I didn't know that elderly people or some elderly people become deficit in protein. So that's where I am. I suspect that's where a lot of people are. [P2].</i></p> <p><i>There's so much on tv, on social media, everywhere you look. Magazines, books, we've all mentioned them this morning, sources about food and nutrition. That's what we think about all day long. We're buying food, we're cooking food, we're looking at diets, whether it's to lose weight or put weight on. There's so much information out there. I think if [protein supplementation] was presented in the right way, in the right medical or research recommendations, then I think something like that could be useful. [P1].</i></p> <p><i>I'm concerned that we're bombarded with so much information it's just coming from so many different directions... [P7].</i></p> <p><i>So many contradictories as well... [P4].</i></p> <p><i>Contradictory. You really don't know. You look at a magazine, you look at Saga website or something which is supposedly for our generation and you think okay, they're talking about accredited organisations that are giving us this information then you go into women's magazines and then the newspapers and on tv. There's so much you just don't know and that's what's frightening. [P7].</i></p> |
| <p>Sub-theme 2.2. Unawareness of protein benefits Sub-theme 3.1. A traditional,</p> | <p><i>I thought, if it would help me to maintain my independence for longer, I would definitely consider doing something like that [take protein supplements]. I mean, I take probiotics and soluble fibre because it helps my problems, so I'll definitely take something that helps me to maintain what I do now. [P1].</i></p> <p><i>Maybe there's not enough information out there, saying that this is what's going to happen to older people. Older people are going to lose all this muscle but if you started to take protein earlier on it could help. [P8].</i></p> <p><i>Well, I was brought up I think like a lot of people in my generation with reasonably well balanced meals because we had breakfast, we had lunch – light lunch and then we had dinner. We didn't snack in between, so we didn't talk about proteins, carbohydrates. We</i></p> |

| | |
|--|--|
| <p>whole-food diet is 'best'.</p> | <p><i>just ate sensibly and we ate good portions so I find it now very complicated to have to listen to all these proteins, amino acids, whatever, etcetera. I think we've complicated the whole situation. If we could just go back to eating sensibly in moderation, the way we were brought up, I think it would be so much more simpler. [P7].</i></p> <p><i>I would only consider anything like that [protein supplementation] if I had some sort of illness or maybe something that would stop me from eating. I'm thinking like oesophageal cancer or something like that where it's very difficult to actually intake the food sometimes to actually keep you healthy. [P12].</i></p> <p><i>What about the other side of the thing which is exercise? Is it effective just to take the supplements and sit on your backside all day or...? [P3].</i></p> <p><i>You probably need to do both. [P10].</i></p> <p><i>Exactly, yeah that's what I was thinking. Is there any point just taking supplements if you don't change your lifestyle? ... To me, people think 'I'll take this and not change [my] lifestyle'. [P3].</i></p> |
| <p>Sub-theme 3.2. Protein powders are unnatural.</p> | <p><i>What would you think about enriching your diet with more protein? [F1]</i></p> <p><i>As long as it didn't taste like powder. [It was] horrible. [P9].</i></p> <p><i>I didn't like some of them. [P2].</i></p> <p><i>The first couple were okay and then after that they just got... [P9].</i></p> <p><i>I thought they were all rather sweet. Very sweet. [P11].</i></p> <p><i>[Agreement]</i></p> <p><i>But there's a peculiar thing that some of them have where the taste of them was alright but the smell was disgusting. [P11].</i></p> <p><i>If you published [information on protein supplementation], the first thing I would wonder is, 'can I get this protein from ordinary food sources'? Because I'd just eat more protein. And at [the age of] 40 I guess people are not having appetite problems, you know? So, I would certainly do that. [P2].</i></p> |

454 **Part 2**

455 **Participant Characteristics**

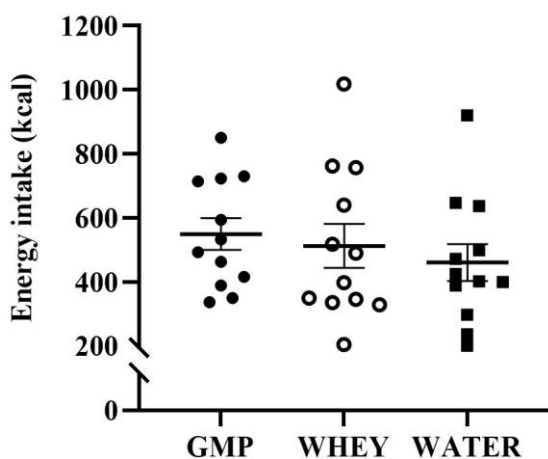
456 Of fourteen participants recruited, one withdrew due to discomfort of the cannula and one participant
457 was excluded as over-eaters (consuming 43% and 55% of TER at the *ad libitum* lunch meal). As such,
458 twelve participants completed the study and were included in the analyses (4m and 8f; age 69 ± 3 years;
459 weight 67.9 ± 13.8 kg; BMI 23.5 ± 3.7 kg·m⁻²; fat mass 24.5 ± 9.1 kg; percentage body fat $35.3 \pm 9.8\%$;
460 fat free mass 43.5 ± 9.1 kg; SNAQ score 15 ± 2 , 6/12 classified as low appetite (score < 15, Lau et al.,
461 2020); IPAQ-SF score 1804 ± 1112 METS·day⁻¹).

462 Blinding of test drinks was assessed qualitatively. No participants were able to distinguish between
463 protein conditions. Differences in texture were reported, with four participants confidently identifying
464 the water condition.

465

466 **Energy Intake**

467 Energy intake at the *ad libitum* lunch meal for the three test conditions is shown in **Figure 2**. There was
468 no difference in energy intake (549 ± 171 kcal vs. 512 ± 238 kcal vs. 460 ± 199 kcal for GMP, WHEY
469 and WATER, respectively, $p = 0.175$, $\eta_p^2 = 0.147$). Data was also analysed for a trial order effect. There
470 was a significant main effect for trial order (Trial 1 = 458 ± 209 kcal, Trial 2 = 476 ± 199 kcal, Trial 3
471 = 588 ± 190 kcal; $p = 0.019$, $\eta_p^2 = 0.366$), with post hoc pairwise comparisons revealing that intake was
472 significantly greater on trial 3 (588 ± 190 kcal) then trial 2 (467 ± 199 kcal, $p = 0.012$), with a trend of
473 a greater intake on trial 3 than trial 1 (458 ± 209 kcal, $p = 0.090$).



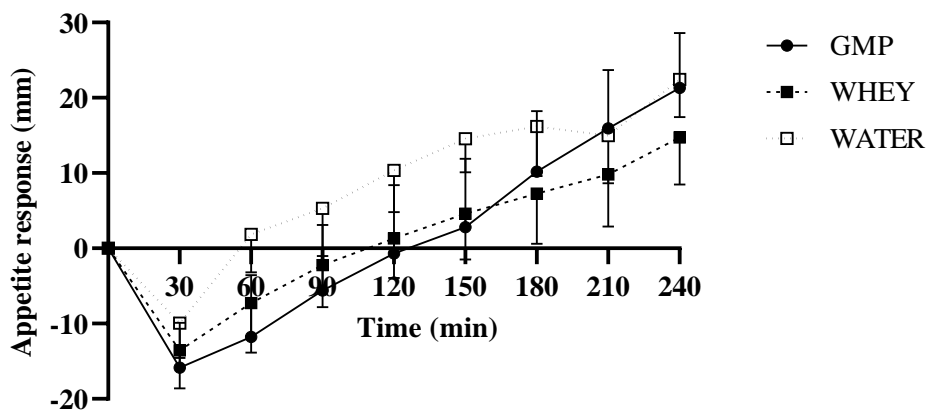
474

475 **Figure 2.** Mean \pm SEM lunch *ad libitum* energy intake for WATER, WHEY and GMP.

476

477 **Subjective Appetite**

478 The subjective appetite response to each of the three breakfast drinks are shown in **Figure 3**. There was
 479 no condition x time interaction ($p = 0.251$, $\eta_p^2 = 0.111$), nor group main effect ($p = 0.360$, $\eta_p^2 = 0.089$).
 480 Net AUC for appetite response did also not differ across conditions ($p = 0.349$, $\eta_p^2 = 0.091$). We also
 481 assessed the time period from consuming the drink until appetite rating had returned to the baseline
 482 value. There was a trend for a group main effect for time taken for appetite score to return to baseline
 483 (145 ± 93 mins vs. 118 ± 83 mins vs. 76 ± 63 mins for GMP, WHEY and WATER, respectively; $p =$
 484 0.081 , $\eta_p^2 = 0.229$). Post hoc pairwise comparison showed a trend for a difference between WHEY and
 485 WATER ($p = 0.065$, $d = 0.574$), with no difference between GMP and WHEY.



486
 487 **Figure 3.** Mean \pm SEM subjective appetite response for WATER, WHEY and GMP.

488

489 *Gut hormone responses*

490 Blood samples were not obtained for one participant due difficulties with cannulation. All hormone
 491 analyses were conducted for 11 participants. Responses of acyl-ghrelin, PYY, GLP-1, and CCK to
 492 GMP, WHEY, and WATER are shown in **Figure 4a - 4d**.

493 *Acyl-ghrelin*

494 There was no condition x time interaction ($p = 0.572$, $\eta_p^2 = 0.065$), nor condition main effects for acyl-
 495 ghrelin response ($p = 0.431$, $\eta_p^2 = 0.070$). There were also no differences in nAUC ($p = 0.417$, $\eta_p^2 =$
 496 0.074).

497 *PYY*

498 There was no condition x time interaction effect for PYY response ($p = 0.404$, $\eta_p^2 = 0.091$). There was
 499 a group main effect ($p = 0.046$, $\eta_p^2 = 0.265$); however, post hoc analyse revealed no pairwise differences
 500 (all $p > 0.1$). There was also a condition main effect for nAUC ($p = 0.047$, $\eta_p^2 = 0.263$), with no
 501 significant pairwise differences (all $p > 0.1$).

502 *GLP-1*

503 For GLP-1 response, there was a significant condition x time interaction ($p = 0.038$, $\eta_p^2 = 0.237$). Post
504 hoc analysis showed a greater increase in GLP-1 concentration in both GMP and WHEY compared
505 with WATER at 30min, 60min, 90min, and 120min (all $p < 0.05$, $d > 1.081$). A greater increase in
506 WHEY compared with WATER was also observed at 180min ($15.3 \pm 7.1 \text{ ng}\cdot\text{mL}^{-1}$ vs. $3.6 \pm 10.8 \text{ ng}\cdot\text{mL}^{-1}$,
507 $p = 0.034$, $d = 1.408$), with a trend for a difference between GMP and WATER at this time (9.2 ± 8.4
508 $\text{ng}\cdot\text{mL}^{-1}$ vs. $3.6 \pm 10.8 \text{ ng}\cdot\text{mL}^{-1}$, $p = 0.087$, $d = 0.647$). There was a group main effect ($p < 0.001$, $\eta_p^2 =$
509 0.551), with post hoc analyse identifying differences between GMP and WATER ($p = 0.006$, $d = 1.404$)
510 and between WHEY and WATER ($p = 0.006$, $d = 2.093$). There was no difference between GMP and
511 WHEY ($p = 0.382$). There was also a condition main effect for nAUC ($p < 0.001$, $\eta_p^2 = 0.544$), with
512 significantly greater nAUC value in GMP compared with WATER ($p = 0.007$, $d = 1.367$) and WHEY
513 compared with WATER ($p = 0.007$; $d = 2.050$). There was no difference between GMP and WHEY (p
514 $= 0.390$).

515 *CCK*

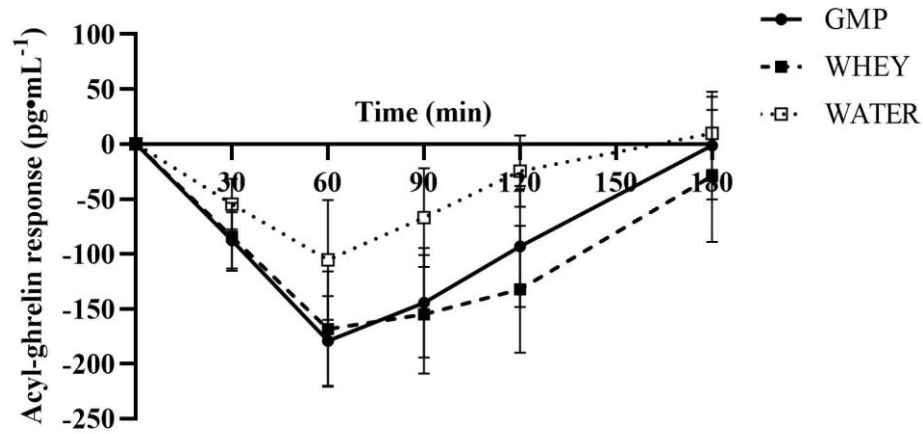
516 There was no condition x time interaction ($p = 0.438$, $\eta_p^2 = 0.084$), nor condition main effects for CCK
517 response ($p = 0.395$, $\eta_p^2 = 0.081$). There were also no differences in nAUC ($p = 0.440$, $\eta_p^2 = 0.069$).

518

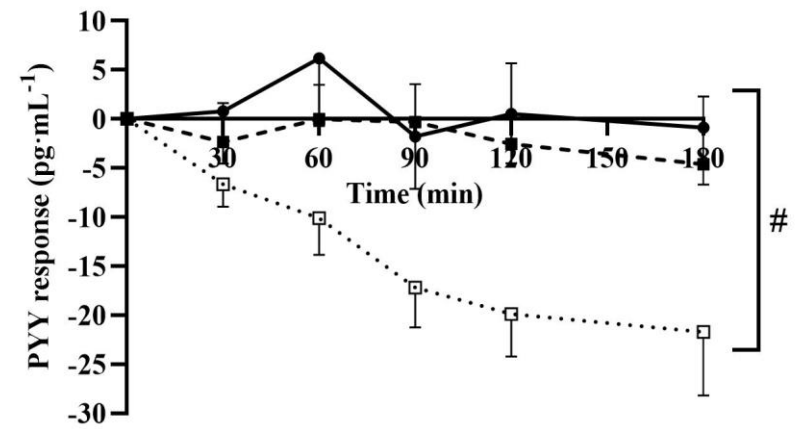
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a)



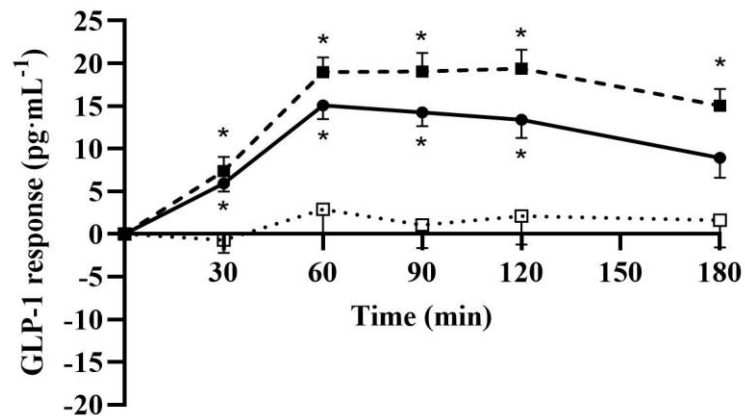
b)



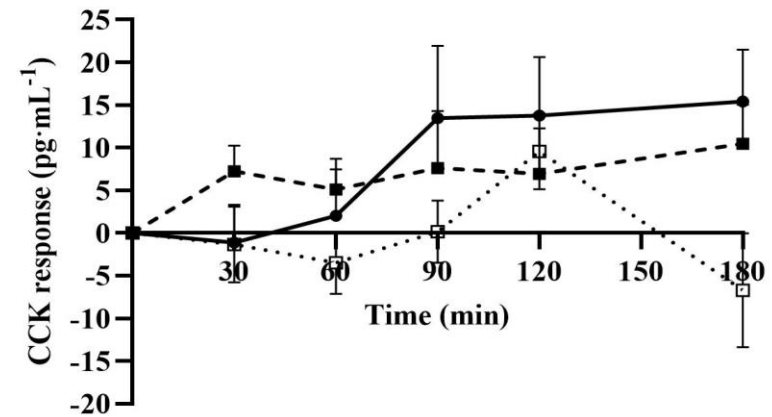
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522

c)



d)



523

524 **Figure 4** – Mean \pm SEM plasma concentrations of acyl-ghrelin (a), PYY (b), GLP-1 (c) and CCK (d) is response to water, whey protein and GMP protein525 ingestion. # significant condition main effect, $p < 0.05$. * significantly different to WATER, $p < 0.05$.

526 DISCUSSION

527 The aim of this study was to determine the feasibility and acceptability of developing a GMP-based
528 protein product to meet the needs of older adults. Our findings suggest that: a) leucine-enriched GMP
529 is not less satiating than whey protein; b) low palatability of a GMP-based supplement and negative
530 attitudes towards protein supplementation would make effective adoption of such a supplement
531 challenging in older adults; c) future study and interventions to increase protein intake in older adults
532 may benefit from providing trusted nutritional advice and opting for food enrichment/fortification
533 approaches.

534 It was hypothesised that the amino acid profile of GMP would favour a less anorexigenic hormonal
535 response. This was not the case. There was no difference between the two protein drinks for acyl-
536 ghrelin, PYY, GLP-1 or CCK response. Our rationale was informed largely by studies investigating
537 appetite and gut hormones responses to isolated amino acids. It is perhaps unsurprising that gut hormone
538 response to intact, whole protein differs to the that of individual amino acids or amino acid mixtures
539 (Gwin et al., 2020). MacLeod and colleagues (2010) showed greater ghrelin suppression after the
540 ingestion of intact GMP, compared with the ingestion of an essential amino acid mix, despite very
541 comparable amino acid profiles. Studies determining gut hormone secretion in response to GMP are
542 scarce, and this is the first study to identify responses or a range of gut hormones in older adults. Hoefle
543 et al. (2019) showed no difference in GLP-1 response after consuming a 50g dose of GMP compared
544 with 50g of whey in prediabetic adults, while Veldhorst et al. (2009) observed no difference in GLP-1
545 or ghrelin response between ingestions of whey and GMP-free whey. Keogh et al. (2010) assessed CCK
546 response to ~40g doses of different protein fractions, observing no difference in response between GMP
547 and GMP-free whey protein in males living with overweight and obesity. Our findings echo these
548 observations in younger cohort, with a modest intake of 30g of protein – one likely ecologically valid
549 for older adults. Burton-Freeman (2008) did show a lower CCK response to GMP ingestion compared
550 with GMP-free whey protein, in younger and mid-life adults. However, CCK concentration was
551 measured for only 75 minutes after ingestion; our data indicate a more rapid CCK release in response
552 to whey protein, with a delayed release after GMP. As such the data of Burton-Freeman (2008) may be
553 representative of a delayed, rather than attenuated, CCK response to GMP.

554 In line with no difference in gut hormone response, subjective appetite and *ad libitum* energy intake did
555 not differ between conditions. Subjective appetite profiles for GMP and whey protein were very
556 comparable, while the mean energy intake at the lunch meal differed by just 37 kcal. It is noted that
557 there was an order effect for energy intake. However, the counterbalancing of condition order should
558 have negated such an effect. Poppitt et al. (2014) and Keogh et al. (2010) had previously shown no
559 differences in subjective appetite and acute *ad libitum* feeding after consuming GMP and whey protein
560 drinks in lean, young men, and mid-life males living with obesity, respectively. Our data suggest a
561 similar comparative response between the two types of protein in older adults. As such these findings

562 do not support the efficacy of leucine-enriched GMP as a low-satiety alternative protein source for older
563 adults.

564 Our data also suggest low likelihood of acceptability of a GMP protein supplement. While low
565 pleasantness scores for both protein powders mixed with water indicates that participants clearly did
566 not like the taste of either, GMP was deemed less pleasant in taste than whey protein. When mixed with
567 a fruit smoothie, the addition of both proteins reduced the rating of pleasantness, with this reduction in
568 pleasantness score significant for GMP. The GMP-smoothie drink was also rated lower than pure
569 smoothie for aroma, with participants reporting an “unpleasant” or “off” smell. Condition main effects
570 also pointed towards both protein drinks being perceived as “too sweet” and of less-desirable a lumpy
571 and grainy texture.

572 It must be emphasised that most or all the participants had no direct experience with this type of product,
573 so their responses to the flavours were primarily first impressions, not affected by any post-ingestive
574 rewarding effects of the proteins (Berthoud et al., 2012). The result might have been different had the
575 participants been asked to consume the products regularly (e.g., at least once per day for a week) before
576 the taste testing took place. In particular if any of the participants habitually did consume less protein
577 (specifically less leucine) than their individual requirement (Gietzen & Aja, 2012), such familiarisation
578 could have given the post-ingestive effect of the more balanced amino acid profile of GMP opportunity
579 to overcome the flavour’s negative initial impression.

580 Previous studies have also shown low pleasantness and likeability ratings of GMP protein when mixed
581 with milk. Our data show that such effects of GMP on beverage taste persist when mixed with a more
582 flavoursome fruit smoothie and are prevalent for an older adult pallet. Studies have highlighted that
583 compliance to supplementation strategies is low amongst older adults (Jobse et al., 2015) and the
584 systematic review of Hubbard et al. (2012) found that compliance to oral nutritional supplements is
585 negatively associated with age. Further, taste and palatability are important factors influencing
586 compliance (Darmon et al., 2008; Lester et al., 2022). As such, efforts should be made to improve the
587 palatability of protein supplements, particularly if adopted for older adults suffering from or at risk of
588 undernutrition.

589 The focus group revealed other significant challenges in promoting protein supplementation among
590 older adults, beyond palatability. Findings highlighted a pervasive distrust of ‘official’ dietary guidance,
591 a widespread sense of confusion and unawareness about the benefits of protein supplementation, and a
592 strong preference for natural, whole-food diets. Participants expressed deep-rooted scepticism and
593 distrust towards ‘official’ sources of dietary guidance. This sentiment was particularly strong in relation
594 to protein supplementation. Participants' apprehensions were rooted in a perceived lack of transparency
595 and potential conflicts of interest within government and health organisations. The scepticism extends

596 to the commercialisation of health products targeted at older adults, with participants questioning the
597 validity and motivations behind the marketing of such products.

598 A significant level of confusion and unawareness regarding the benefits of protein and its
599 supplementation was evident among participants. This confusion was compounded by the perceived
600 ever-changing landscape of dietary recommendations, leading to frustration and a reliance on varied
601 and sometimes contradictory information sources. The lack of clarity in official guidelines is a critical
602 issue, as it impedes the ability of older adults to make informed decisions about their dietary needs.
603 Interestingly, even when presented with research findings about the potential benefits of protein
604 supplementation, participants expressed a desire for more straightforward, personalised advice. This
605 suggests a gap in the current dissemination of dietary information, particularly regarding protein, where
606 the personalisation and simplification of guidance could play a role in improving understanding and
607 adherence. Our data echo recent calls to provide education and increasing awareness of the benefits of
608 protein consumption amongst older adults in order to promote greater protein intake (Linschooten et
609 al., 2021; Norton et al., 2022).

610 The study participants overwhelmingly preferred natural dietary sources over supplementation. This
611 preference is not just a matter of taste or habit; it also reflects a philosophical stance about what
612 constitutes a healthy lifestyle. Preference for whole food rather than oral nutritional supplements has
613 been shown previously (Jacobs et al., 2014; Griffiths et al., 2023), with van der Zanden et al. (2014)
614 also identifying old adults' distrust of certain products they perceived to be supplements and not food.
615 Our discussion about supplementing diets with protein powders revealed a reluctance to use such
616 products unless as a last resort, with a notable emphasis on taste and naturalness. It perhaps should be
617 considered that raising the awareness of the importance of protein for health ageing may lead to a greater
618 willingness to try protein supplementation amongst older adults; but this is somewhat speculative.
619 Nonetheless, our finding suggests that nutritional interventions targeting this demographic should
620 perhaps be food-focused. Indeed, fortification of commonly-consumed foods with protein has proved
621 an effective means of increasing protein in those in residential care (Beelen et al., 2017a), those in
622 hospital (Mills et al., 2018) and those recently discharged (Beelen et al., 2017b). Our data support such
623 approaches and suggest greater likely acceptability, compared with the use of supplements.

624 The present study is not without limitations. An optimal protein source for older adults would have a
625 low satiety effect while also providing a range of amino acids, particularly essential amino acids and
626 the anabolic amino acid leucine. While we did ensure leucine content reached 3g per 30g dose of GMP,
627 a limitation of the present study was that we did not determine amino acid delivery through the measure
628 of postprandial plasma amino acid concentration. In addition, the study sample size was determined to
629 power the study to detect large differences in gut hormones. Consequently, the study may have been
630 under-powered for the measure of subjective appetite and *ad libitum* food intake. However, the
631 magnitude of difference in these two measures, particularly between the two protein conditions, were

632 sufficiently small to be confident of no type II statistical error. It is pertinent to also acknowledge the
633 order effect for *ad libitum* food intake. As mentioned earlier, the counterbalancing of the study
634 conditions should negate the order effect and as such, the current findings can be considered valid.
635 Nonetheless, the observation of a greater intake in trial 3 is not easily explained, as *ad libitum* test meals
636 have been shown to exhibit good reproducibility in younger adults (Gregersen et al., 2008; Horner et
637 al., 2014). It is possible that participants underate due to observer bias (Robinson et al., 2015) in the
638 early trials, and that this diminished with repeated trials. However, this cannot be confirmed.

639 We also acknowledge that a thorough assessment of all sensory responses to taste testing would require
640 a larger sample size. Nonetheless, it is felt that the inclusion of both the sensory tests and the focus
641 group in their present form strengthen this exploratory study by providing a mixed methods and
642 interdisciplinary insight into the likely feasibility and acceptability of a GMP-based product for older
643 adults. We are confident that our data, despite a small sample size, robustly indicates that more targeted
644 product development is needed to attain more acceptable sensory properties. Future iterations of product
645 development would require more thorough, adequately-powered, sensory assessment. Finally, we
646 acknowledge that the cohort who took part in the focus group was predominantly female. A more
647 balanced representation of men and women may have resulted in different attitudes towards supplement
648 use, as men have been shown to be more receptive of supplements than women in cohorts of younger
649 adults (Aguilar-Navarro et al., 2020)

650

651 **CONCLUSION**

652 This study would indicate that leucine-enriched GMP is unlikely to prove an effective protein source to
653 meet the protein needs and preferences of older adults. It proved no less satiating than the more
654 commonly-adopted and cost-effective whey protein. Low palatability ratings and negative attitudes
655 towards protein supplementation suggest poor likely acceptability of a GMP-based drink for
656 community-dwelling older adults. Indeed, our data is supportive of alternative means to
657 supplementation for promoting protein intake in older adults. Providing trusted education on the
658 importance of protein for healthy ageing and fortifying commonly-consumed “healthy” foods with
659 protein should be pursued.

660

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666

667 **AUTHOR CONTRIBUTIONS**

668 A.H., D.R.C., and L.O. conceived the research question. A.H., D.R.C., K.B., R.S., and E.S. designed
669 the study. A.H., K.B., R.S., and J.W. collected data. A.H., K.B., R.S., and J.W. conducted data
670 analyses. A.H., K.B., R.S., and J.W. wrote the manuscript. D.R.C., E.S., and L.O. edited the
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672

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676

677 **CONFLICT OF INTEREST**

678 L.O. is an employee of Milk Specialties Global. L.O. contributed to the conception of the research
679 question, but did not contribute to data collection, analyses, or interpretation.

680

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